Permanent Cardiovascular Protection From Hypertension by the AT1 Receptor Antisense Gene Therapy in Hypertensive Rat Offspring

Phyllis Y. Reaves,* Craig H. Gelband,* Hongwei Wang,* Hong Yang, Di Lu, Kathleen H. Berecek, Michael J. Katovich, Mohan K. Raizada

Abstract—Our previous studies have demonstrated that the introduction of angiotensin II type I receptor antisense (AT1,R-AS) cDNA by a retrovirally mediated delivery system prevents the development of hypertension in the spontaneously hypertensive rat (SHR), an animal model for primary hypertension in humans. These results have led us to propose the hypothesis that an interruption of the renin-angiotensin system (RAS) activity at a genetic level would prevent hypertension on a permanent basis. F1 and F2 generations of offspring from a retroviral vector, LNSV- and LNSV-AT,R-AS–treated SHR, were generated, and various physiological parameters indicative of hypertension were studied and compared with those of their parents to investigate this hypothesis. Both F1 and F2 generations of LNSV-AT,R-AS–treated SHR expressed a persistently lower blood pressure, decreased cardiac hypertrophy and fibrosis, decreased medial thickness, and normalization of renal artery excitation-contraction coupling, Ca2+ current, and [Ca2+]i when compared with offspring derived from the LNSV-treated SHR. In fact, the magnitude of the prevention of these pathophysiological alterations was similar to that observed in the LNSV-AT,R-AS–treated SHR parent. The prevention of cardiovascular pathophysiology and expression of normotensive phenotypes are, at least in part, a result of integration and subsequent transmission of AT1,R-AS from the SHR parents to offspring. These data demonstrate that a single intracardiac injection of LNSV-AT,R-AS causes a permanent cardiovascular protection against hypertension as a result of a genomic integration and germ line transmission of the AT1,R-AS in the SHR offspring. The full text of this article is available at http://www.circresaha.org.

Key Words: AT1 receptor antisense • gene therapy • hypertension • SHR • antisense transmission to offspring

Hypertension is a complex disease that is associated with major economic and emotional burdens to society. It is also a significant risk factor in stroke, arteriosclerosis, heart failure, coronary artery disease, and progressive renal damage.24 Decades of investigation have established that a hyperactive renin-angiotensin system (RAS) is one of the many physiological events that becomes dysfunctional in hypertension.2–5 This fact is further supported by the observation that pharmacological intervention in the activity of the RAS has proved to be highly successful in the treatment and management of hypertension in a significant population of hypertensive patients.6,7 In spite of this success, the traditional pharmacological therapy targeted to inhibit specific components of the RAS suffers from many significant disadvantages. One is patient compliance. This is particularly important in view of the fact that chronic administration of drugs is almost always necessary for a persistent, long-term antihypertensive effect, and prolonged therapy with certain antihypertensive agents can lead to significant side effects such as coughing, angioedema, hypotension, renal dysfunction, and hyperkalemia.8–10 Additionally, although a traditional pharmacological strategy can be successful in the control and the management of high blood pressure (BP), its effectiveness in the prevention and/or reversal of other associated pathophysiological alterations such as tissue remodeling leading to end-organ damage remains to be proven.11 In fact, disproportionate reversal of some alterations, such as left ventricular hypertrophy and peripheral resistance, has proven to be unfavorable in the successful management of this disease.12,13

In view of the success of pharmacological agents targeted toward the inhibition of the RAS, and the recent rapid advances in gene delivery, we decided to investigate whether antisense gene therapy could be a superior treatment for hypertension. We used a retrovirally mediated delivery system to administer AT1 receptor antisense (AT1,R-AS) cDNA.
in vitro. These studies established that AT1R-AS cDNA could be incorporated into the genome and that the transcript could be expressed on a long-term basis. This expression was associated with a significant alteration of the AT1R-mediated cellular action of angiotensin II (Ang II) indicating that such an approach was feasible. Animal experiments were highly successful and demonstrated that intracardiac delivery of AT1R-AS in the neonatal spontaneously hypertensive rat (SHR) prevented the development of hypertension, renal, and cardiovascular pathophysiological changes on a long-term basis. The antihypertensive effect was associated with a robust long-term expression of AT1R-AS transcript. These studies led us to hypothesize that the interruption in the activity of the RAS during development by the AT1R-AS would attenuate hypertension on a permanent basis. The present study was designed to support or refute this hypothesis.

**Materials and Methods**

**Preparation of Viral Particles Containing AT1R-AS**

AT1R-AS was cloned in a retroviral vector containing long terminal repeats, neomycin selection, and simian virus promoter (LNSV) as described previously. Media-containing viral particles from the PA317 cells were collected and concentrated to provide \( 1 \times 10^{10} \) cfu/mL as described previously. Viral particles that did not contain AT1R-AS (LNSV) were also prepared by the same protocol and used in control experiments.

**Administration of LNSV- and LNSV-AT1R-AS–Containing Viral Particles in Rats**

Five-day-old Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats were divided into two groups; virus-along control (LNSV) or virus-containing AT1R-AS (LNSV-AT1R-AS). Animals were injected with a bolus of \( 1 \times 10^6 \) cfu of viral particles in 10 µL physiological saline intracardially, weaned, and raised as described previously. Indirect BP was monitored throughout development. Two sets of parents of LNSV- or LNSV-AT1R-AS–treated WKY and SHR were bred at 100 days of age to generate F1 offspring. Similar to their parents, they were weaned, raised, and monitored for BP indirectly by the tail-cuff method. At 100 days of age, two sets of F1 offspring of LNSV- and LNSV-AT1R-AS–treated WKY and SHR were bred to generate F2 offspring. One hundred-day-old parents and F1 and F2 offspring were used for all biochemical and physiological experiments.

**Biochemical Experiments**

Binding of \(^{125}\text{I}\)-Sar\(^{1-8}\)-Ang II to membrane AT1 receptors was carried out as described elsewhere. Computer-assisted Scatchard analysis was done to determine the \( B_0 \) and \( K_D \) values. Polymerase chain reaction (PCR) followed by a Southern analysis was carried out to determine the genomic integration of the AT1R-AS essentially as described elsewhere. The expression of AT1R-AS transcript in various Ang II target tissues was carried out by a semiquantitative reverse transcriptase (RT)-PCR method as we described previously.

**Physiological Protocols**

Indirect BP was monitored in nonanesthetized animals by a standard tail-cuff method. Direct BP was determined in free-moving animals as previously described. For vascular smooth muscle studies, 3-mm-long segments of rat renal resistance arterioles were used. Contractile responses to KCl, phenylephrine, and acetylcholine were evaluated as described previously. [Ca\(^{2+}\)], in dissociated renal resistance arterial cells in response to KCl and Ang II was measured exactly as we described previously. Current recordings were carried out by voltage-clamping single cells.

**Pathophysiological Parameters**

Heart weight-to–body weight ratio, cardiac fibrosis, and collagen volume in both endocardium and epicardium were determined by our previously published protocols. Animals were killed and perfused with fixative without applying additional pressure, according to a previously published protocol. Morphometric analysis of thoracic aorta to determine the wall thickness and media/lumen ratio was carried out by an established protocol.

**Statistics**

Results are expressed as mean\( \pm \)SE. Statistical significance was evaluated with repeated measures, ANOVA, and Student’s t test for unpaired data. Differences were considered significant at \( P<0.05 \). For vascular studies, all rings were normalized to tissue weights and cross-sectional area.

**Results**

Five-day-old WKY rats and SHR were administered \( 1 \times 10^9 \) cfu of LNSV (control virus) or LNSV-AT1R-AS (LNSV virus containing AT1R-AS cDNA) via an intracardiac injection. Animals were allowed to grow and were subjected to routine indirect BP monitoring. Two sets of LNSV-treated WKY rats (mean BP 129±6.0 mm Hg) and SHR (mean BP 198±18 mm Hg) and LNSV-AT1R-AS–treated WKY and SHR (mean BP 120±6.0 and 153±9 mm Hg, respectively) were used as parents for breeding and an F1 generation of rats was produced. Offspring from LNSV-AT1R-AS–treated SHR expressed significantly lower BP as early as 80 days of age compared with their LNSV-treated SHR control. By 120 days, the average BP of the AT1R-AS–treated SHR offspring was of 45±9 mm Hg lower than that observed in the LNSV-treated SHR offspring (150±10 versus 185±11 mm Hg, Figure 1). No significant effect on BP was observed between offspring of AT1R-AS– and LNSV-treated WKY rats. The LNSV-AT1R-AS–treated WKY rat group was not used further in our exper-
Figure 2. A, Representative Southern blot demonstrating the presence of LNSV-AT1-AS in the SHR. Five-day-old SHR were injected with $1 \times 10^9$ cfu of LNSV-AT1R-AS viral particles. Animals were allowed to grow for 120 days, and F1 generation offspring was produced. F2 offspring was generated by breeding 120-day-old F1 parents. Genomic DNA was isolated and subjected to PCR with the use of a set of LNSV-specific primers that contained AT-R-AS (sense primer: 5'-GCC TCT GAG CTA TTC CAG AAG TAG-3'; antisense primer: 5'-GAG CCT GGA CCA CTG AT-3'). The resulting product was subjected to Southern blotting essentially as described. AT1-AS generated by ClaI and SacI restriction enzyme treatment of the LNSV-AT-R-AS vector was used as a probe. The fragment (~1 kb) was randomly labeled with $^{32}$P-dATP and used for hybridization by the standard protocol.

Lane 1, PA317 cells; lane 2, liver; lane 3, heart; lane 4, adrenal. Tissues from rats that were not injected with the LNSV-AT1-R-AS were found to be negative. B, Representative RT-PCR demonstrating the expression of AT1-AS transcript in various tissues of LNSV-AT1R-AS–treated SHR parents and F2 offspring. Total RNA was subjected to RT-PCR with the use of specific primers to detect AT1R-AS.

Figure 3. Effect of losartan on direct blood pressure in F1 and F2 generation offspring of LNSV-AT1-R-AS–treated SHR. F1 and F2 generations of offspring from control LNSV- and LNSV-AT1R-AS–treated SHR parents were produced. SHR parents were treated with physiological saline (Ctrl), LNSV (open column), or LNSV-AT1-R-AS (solid column) and allowed to grow. Parents were bred to produce LNSV (open column) or LNSV-AT1R-AS (solid column) F1 and F2 offspring. Losartan was injected at a dose of 10 mg/kg IV and direct mean BP was measured 2 hours later. Data are mean ± SE (n=8). *Significantly different (P<0.01) from saline or control LNSV-treated parents, as well as F1 and F2 LNSV offspring.

The effect of losartan, an AT1 receptor–specific antagonist and well-established antihypertensive drug, on offspring derived from AT1R-AS–treated SHR was also studied. Treatment of control SHR by losartan resulted in a 29±6 mm Hg decrease in BP (Figure 3). A comparable decrease in BP was observed in LNSV-treated SHR. In contrast, no significant decrease in BP was observed in the LNSV-AT1-R-AS–treated SHR. Similar to AT1R-AS–treated parents, their F1 and F2 offspring showed little lowering of BP by losartan whereas offspring from LNSV-treated parents experienced a 25 to 29 mm Hg decrease in BP (Figure 3). These observations confirm that the AT1-R-AS treatment of parents produced antihypertensive effects in both parents and offspring through an AT1 receptor–mediated mechanism and that the antihypertensive effect is as effective as the AT1 receptor antagonist therapy. The conclusion that antisense gene therapy influences BP in the SHR by affecting the levels of AT1 receptors is demonstrated by comparing the cardiac AT1 receptors in the F1 generation of LNSV- and AT1-R-AS–treated SHR. Total numbers of AT1 receptors (Bmax) in the ventricles of the F1 offspring derived from AT1-R-AS–treated SHR were decreased by 36% compared with offspring from parents of LNSV-treated SHR (Table 1).

Tissue remodeling and associated ultrastructural changes in tissues relevant to cardiovascular functions such as heart, kidney, and arteries are major contributing factors in the morbidity and mortality associated with hypertension. For example, left ventricular hypertrophy, a compensatory response of the heart to an increase in peripheral vascular resistance is an important pathophysiological manifestation of hypertension. We determined whether AT1-R-AS treatment influences cardiac pathophysiology, and, if so, could this effect be maintained in their offspring. Heart weights of untreated SHR were 68% higher than those of WKY rats (Table 1). AT1-R-AS treatment significantly prevented this cardiac hypertrophy in parents. Similarly, heart weights of the F1 generation of AT1-R-AS–treated SHR were 26% lower than the F1 generation of LNSV-treated SHR (Table 1).

Cardiac hypertrophy was significantly prevented in the F2 generation of AT1-R-AS–treated SHR as well. Multifocal areas of fibrosis in the myocardium are another characteristic of hypertension in this model. Figure 4 provides an example
of sections taken from the left ventricular subendomyocardium of F2 offspring derived from LNSV- and AT1R-AS–treated SHR. Multiple areas of fibrosis were clearly evident in the offspring of LNSV-treated SHR (Figure 4b) but were rarely observed in the offspring of AT1R-AS–treated animals (Figure 4c) or in the control WKY rat (Figure 4a). Collagen volume in both endocardium and epicardium, a measure of cardiac fibrosis, was <90% decreased in the offspring of LNSV-AT1R-AS SHR (Table 1), confirming the morphological detection of fibrosis.

Ultrastructural examination of the thoracic aorta of the F2 offspring of parents treated with LNSV-AT1R-AS revealed a significant decrease in the wall and medial thickness compared with offspring of LNSV-treated SHR parents (Table 1). The lumen area was increased in this group of SHR offspring. For example, wall thickness in the SHR and LNSV-treated SHR demonstrated a 34% decrease in wall thickness, reflecting in the EC50 for KCl and phenylephrine, was significantly lower in the offspring of LNSV-AT1R-AS–treated SHR. Data for the F2 generation were comparable to those in the WKY rat. In contrast to the result with KCl and phenylephrine were shifted rightward as a result of a leftward shift of the concentration-response curve reflecting in the EC50 for KCl and phenylephrine, was attenuated in AT1R-AS–treated SHR. In the present study, the renal vascular response in offspring of both F1 and F2 generations of parents treated with LNSV-AT1R-AS was examined. In the parents, the vascular contractile responses to KCl and phenylephrine were shifted rightward as a result of an increase in EC50 values in both F1 and F2 generation of offspring from LNSV-AT1R-AS–treated SHR. Data for the F2 generation are presented in Table 2 as an example. As a result, the EC50 values for F1 and F2 generations from the LNSV-AT1R-AS–treated SHR parents were comparable to those in the WKY rat. In contrast to the result with KCl and phenylephrine when compared with WKY, the untreated and LNSV-treated SHR showed a shift to the right in the acetylcholine-induced vasorelaxation of preconstricted renal arteriole as reflected by an increase in the EC50 as well as a decrease in the maximal effect. This effect was significantly improved in the offspring of parents treated with LNSV-AT1R-AS. For example, the EC50 response of F2 offspring of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY Rat*</th>
<th>SHR+</th>
<th>LNSV-SHR</th>
<th>LNSV-AT1R-AS-SHR</th>
</tr>
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<tbody>
<tr>
<td>Heart weight/body weight†, mg/g</td>
<td>2.9±0.7</td>
<td>4.9±0.8</td>
<td>4.2±0.0</td>
<td>3.1±0.8</td>
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<tr>
<td>Bmax for AT1 receptor‡, fmol/mg protein</td>
<td>46±5</td>
<td>131±8</td>
<td>130±8</td>
<td>83±6</td>
</tr>
<tr>
<td>Cardiac fibrosis§</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare</td>
</tr>
<tr>
<td>Collagen volume§, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>4.0±0.02</td>
<td>NA</td>
<td>4.8±0.4</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>Epicardium</td>
<td>0.3±0.02</td>
<td>NA</td>
<td>7.8±1.4</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>Thoracic aorta§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness, mm²</td>
<td>0.11±0.04</td>
<td>0.25±0.03</td>
<td>0.21±0.008</td>
<td>0.14±0.005</td>
</tr>
<tr>
<td>Medial area, mm²</td>
<td>0.61±0.04</td>
<td>1.30±0.09</td>
<td>1.52±0.008</td>
<td>0.82±0.010</td>
</tr>
<tr>
<td>Lumenal area, mm²</td>
<td>4.10±0.10</td>
<td>3.20±0.04</td>
<td>3.00±0.300</td>
<td>4.40±0.120</td>
</tr>
<tr>
<td>Media/lumen (ratio)</td>
<td>0.28±0.01</td>
<td>0.37±0.02</td>
<td>0.35±0.010</td>
<td>0.20±0.030</td>
</tr>
</tbody>
</table>

*Data taken from Martens et al. 18 and Gelband et al. 23
†Data are mean±SE, n=12.
‡AT1 receptor Bmax was determined essentially as described previously. 14,15 F1 generation offspring were used for
§Morphometric data were collected and analyzed as described previously from the F2 generation of offspring. 24
¶Values are significantly different (P=0.01) from the LNSV-SHR and SHR.
\Values are significantly different (P<0.05; n=3) from the LNSV-SHR.
NA indicates not analyzed.
LNSV-AT1R-AS–treated SHR was 77% lower when compared with the LNSV-treated SHR and was similar to that of the WKY rat. Similarly, the efficacy was 2.2-fold higher and comparable to the WKY rat (Table 2). These data demonstrate that endothelial dysfunction associated with hypertension is prevented by AT1R-AS gene therapy on a permanent basis.

We also studied L-type Ca\textsuperscript{2+} current in F\textsubscript{1} and F\textsubscript{2} generations from LNSV- and LNSV-AT1R-AS–treated SHR parents. The rationale for this experiment was based on our previous observations that demonstrated that L-type Ca\textsuperscript{2+} current is increased in VSMCs of the renal arterioles of the SHR.\textsuperscript{23} Ca\textsuperscript{2+} current was significantly decreased in both F\textsubscript{1} and F\textsubscript{2} generations of LNSV-AT1R-AS–treated SHR parents compared with that from the LNSV-treated SHR parent. The mean I-V relationship demonstrating this conclusion is presented in Figure 5A for the F\textsubscript{2} generation of offspring. Differences in the peak Ca\textsuperscript{2+} current are shown in Table 2.

**Figure 4.** Morphological changes in myocardium and renal artery in F\textsubscript{2} offspring of LNSV-AT1R-AS–treated SHR parents. Hearts of F\textsubscript{2} generation offspring of WKY (a), LNSV-treated SHR (b), and LNSV-AT1R-AS–treated SHR (c) parents were used to determine fibrosis essentially as described elsewhere.\textsuperscript{18} Bar=12 mm. Arrows show multifocal areas of fibrosis. F\textsubscript{2} rats were killed, and renal arteries were dissected from the main aorta and fixed in 3% paraformaldehyde.\textsuperscript{24} Sections from 3 mm distal from the aorta were compared in WKY (d), LNSV-treated SHR (e), and LNSV-AT1R-AS–treated SHR parents (f). Bar=1 mm.

### Table 2. Pathophysiological Parameters of Renal Arteriole in Offspring of Parents Treated With LNSV-AT1R-AS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY Rat\textsuperscript{*}</th>
<th>SHR\textsuperscript{*}</th>
<th>LNSV-SHR</th>
<th>LNSV-AT1R-AS-SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl constriction (EC\textsubscript{50}, nmol/L)\textsuperscript{*}</td>
<td>35±2\textsuperscript{†}</td>
<td>24±1</td>
<td>19±3</td>
<td>42±6\textsuperscript{†}</td>
</tr>
<tr>
<td>Phenylephrine constriction (EC\textsubscript{50}, nmol/L)\textsuperscript{*}</td>
<td>380±10\textsuperscript{†}</td>
<td>202±13</td>
<td>138±7</td>
<td>483±10\textsuperscript{†}</td>
</tr>
<tr>
<td>Acetylcholine vasorelaxation (EC\textsubscript{50}, nmol/L)\textsuperscript{*}</td>
<td>30.7±5\textsuperscript{†}</td>
<td>143±9</td>
<td>168±11</td>
<td>38±6\textsuperscript{†}</td>
</tr>
<tr>
<td>Acetylcholine vasorelaxation (efficacy percent)\textsuperscript{*}</td>
<td>100\textsuperscript{†}</td>
<td>40±3</td>
<td>42±4</td>
<td>100\textsuperscript{†}</td>
</tr>
<tr>
<td>Peak Ca\textsuperscript{2+} current [Ca\textsuperscript{2+}], pA/pF</td>
<td>1.1±0.01\textsuperscript{†}</td>
<td>2.2±0.02</td>
<td>2.4±0.01</td>
<td>0.95±0.01</td>
</tr>
<tr>
<td>% Increase in [Ca\textsuperscript{2+}], %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>118±7\textsuperscript{†}</td>
<td>228±13</td>
<td>240±11</td>
<td>122±8\textsuperscript{†}</td>
</tr>
<tr>
<td>Ang II</td>
<td>192±6\textsuperscript{†}</td>
<td>306±4</td>
<td>312±12</td>
<td>188±11\textsuperscript{†}</td>
</tr>
</tbody>
</table>

Data are mean±SE (n=24) and taken from F\textsubscript{2} generation of offspring.\n
\textsuperscript{*}Data taken from Martens et al\textsuperscript{18} and Gelband et al\textsuperscript{23} for comparison.

\textsuperscript{†}Values are significantly different (\textit{P}<0.01) from LNSV-SHR and SHR.
Finally, we investigated the effects of KCl and Ang II on [Ca\(^{2+}\)] in renal arteriolar VSMCs. Our previous studies have established that KCl- and Ang II–induced [Ca\(^{2+}\)] were significantly elevated in the SHR compared with the WKY rat. Data in Figure 5B show that KCl- or Ang II–induced [Ca\(^{2+}\)], increases in F1 offspring from LNSV-AT1R-AS–treated SHR parents were significantly attenuated when compared with the KCl and Ang II responses in the offspring from LNSV-treated SHR parents. Similar data were obtained in the F2 generation. These findings provide additional evidence that alterations in the [Ca\(^{2+}\)] homeostasis by the SHR renal arteriolar cells are permanently prevented by AT1R-AS gene therapy.

**Discussion**

The observations presented in this study demonstrate that a single intracardiac administration of a retroviral vector containing AT1R-AS causes a permanent protection against hypertension in this animal model of human primary hypertension. Thus, it suggests that an antisense strategy to inhibit the RAS at a genetic level is a conceptually novel approach for the prevention of hypertension. The reduction in BP is consistent with previous reports demonstrating the integration of retroviral vector and its germ-line transmission in other systems. However, this study is unique because it shows that the transmission and accompanied expression of the AT1R-AS is associated with profound antihypertensive physiological changes in the offspring. Although we know little about the efficiency of this transduction, it must be high enough to influence the expression of antihypertensive phenotypes, an end point that is of ultimate relevance to hypertension. The possibility that lack of a blood-gonadal barrier and the presence of significant numbers of undifferentiated germ cells in the neonatal rat cannot be ruled out. Thus, a critical age of the rat at which the viral administration was carried out may be the key for such a high efficiency of transduction in the offspring.

In spite of our evidence in favor of AT1R-AS transmission, other possibilities to explain this prolonged antihypertensive effect should not be ruled out at the present time. For example, studies have shown that parental environment is critical in the development of hypertension. Thus, it quite possible that the exposure of an antihypertensive environment by the AT1R-AS treatment of parents induces normotensive phenotype in the offspring. Cross studies with the SHR would support this review. In addition, the possibility that the AT1R-AS expression at a critical stage of SHR development may irreversibly prevent the parents and offspring from developing hypertension. This would be consistent with previous suggestions. Finally, it is also quite possible that a combination of these mechanisms may ultimately be responsible for such a dramatic protection against hypertension.

Finally, is antisense gene therapy that targets the RAS a therapeutic step forward? On the basis of our data, the answer has to be affirmative. In our model, a single injection of retroviral vector containing AT1R-AS offers permanent prevention of hypertension. It not only minimizes side effects but also resolves the compliance issue observed in traditional pharmacological therapy. However, caution must be taken in use of this vector for any long-term therapeutics because of some concern as to an unknown insertion site of the retroviral vector in the genome. Another caveat of this study is that its success depends on the identification of the genetic determinants of hypertension at the prehypertensive stage before the therapy can be considered for human use. Angiotensin I–converting enzyme (ACE) may be one such determinant. It is well-established that ACE gene polymorphism cosegregates with hypertension, and that mutations at key places in ACE are associated with the development of high BP. Thus, targeting ACE by such an antisense strategy may be important. Would gene therapy reverse hypertension in the adult animal? A pilot study demonstrates a relatively long-term reversal of high BP and other renal pathophysiological changes induced by hypertension in adult SHR. In conclusion,...
sion, our observation provides an initial step forward toward the use of gene therapy for a permanent benefit to the cardiovascular system in the control of hypertension.

Acknowledgments

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References

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