Reversal of hypertension by angiotensin II type 1 receptor antisense gene therapy in the adult SHR

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Katovich, Michael J., Craig H. Gelband, Phyllis Reaves, Hong-Wei Wang, and Mohan K. Raizada. Reversal of hypertension by angiotensin II type 1 receptor antisense gene therapy in the adult SHR. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1260–H1264, 1999.—Pharmacological interruption of the renin-angiotensin system (RAS) is considered rational therapy for marked hypertension. Most notably, the spontaneous hypertensive rat (SHR; Ref. 9) has been widely used in the development and maintenance of hypertension. The renin-angiotensin system (RAS) is considered rational therapy for hypertension. Recent studies have established that viral mediated delivery of antisense to the angiotensin II type 1 receptor (LNSV-AT1R-AS) will attenuate or abolish the development of hypertension in the SHR. However, the effectiveness of this gene therapy approach to reduce high blood pressure (BP) has not been ascertained. In this study, we investigated the hypothesis that viral delivery of AT1R-AS into the adult SHR will reduce BP and reverse the vascular reactivity associated with the hypertension. Intracardiac injection of virus particles containing LNSV-AT1R-AS into adult SHR resulted in a 30- to 60-mmHg reduction in BP that was maintained for up to 36 days compared with SHR treated with virus alone (LNSV without antisense). Measurement of renal resistance arteriovascular responsiveness; blood pressure

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via daily cardiac injection in anesthetized rats (Metofane, Mallinckrodt Veterinary, Mundelein, IL). Virus particles containing an empty vector (LNSV) were used as the control.

Specific protocols. Adult male SHR and age-matched male Wistar-Kyoto rats (WKY) from Harlan Sprague Dawley (Indianapolis, IN) were used in all the studies. Rats were maintained at 25 ± 2°C with a 12:12-h light-dark cycle. The Institutional Animal Care and Use Committee approved all animal protocols and procedures. In the initial study, 75-day-old male SHR (244–268 g), and 90-day-old male SHR were used. A second study used animal protocols and procedures. In the initial study, 75-day-old male WKY were used as controls. Age-matched SHR were used in the vascular reactivity study. 70-day-old male SHR (244–268 g), and 90-day-old male SHR (230–270 g) were used. A second study used an empty vector (LNSV) were used as the control. 

Mallinckrodt Veterinary, Mundelein, IL). Virus particles containing an empty vector (LNSV) were used as the control. 

RESULTS

Initially, the effect of AT1R-AS treatment on BP was investigated in 75-day-old male WKY and SHR. Animals were administered viral particles (either LNSV or LNSV-AT1R-AS), at a concentration of 5 × 10^8 plaque-forming units (pfu) daily for 6 consecutive days. A significant reduction in systolic BP was observed as early as 3 days after delivery of the AT1R-AS treatment in SHR (AT1R-AS-SHR: 136 ± 8 mmHg, n = 5; LNSV-SHR: 170 ± 7 mmHg, n = 5). This reduction in BP was maintained for at least 10 days, at which time there was an ~30-mmHg reduction in BP in the AT1R-AS-treated SHR compared with the LNSV-treated SHR (Fig. 1A, left). At this time, direct systolic BP was 154 ± 5 (n = 3) and 188 ± 12 (n = 3) mmHg in the AT1R-AS-treated and the LNSV-treated SHR, respectively (Fig. 1A, right).

In WKY treated with AT1R-AS or LNSV, no significant difference was observed in direct BP after 10 days (120 ± 12 vs. 128 ± 6 mmHg, n = 3 for each group).

In a subsequent study, a larger dose of viral particles (1 × 10^9 pfu) containing AT1R-AS was administered in
109 pfu) could reverse cardiovascular alterations observed in age-matched adult SHR (n = 6 animals) compared with age-matched control WKY (n = 6 animals). A significant leftward shift in the KCl and PE concentration-response relationships was observed in SHR compared with WKY controls. This shift was associated with a decrease in the mean effective concentration (EC50) and an increase in the logarithmic effective concentration (−pEC50) for KCl and PE in the SHR compared with the WKY (Table 1). In the bottom panels of Fig. 2, the leftward shift in the concentration-response relationship was still observed for the LNSV-treated SHR (n = 6 animals) but was reversed back toward the WKY control values for the AT1R-AS-treated SHR (n = 6 animals). The EC50 and −pEC50 values for the AT1R-AS-treated SHR were similar to those observed for WKY (Table 1).

Endothelial dysfunction is a characteristic of hypertension in the SHR. The ACh concentration-response relationship was shifted to the left in the AT1R-AS-treated SHR (139 ± 3 mmHg, n = 6) was significantly lower than that in the LNSV-treated SHR (178 ± 6 mmHg, n = 6) and was comparable with age-matched AT1R-AS-treated WKY controls (137 ± 3 mmHg, n = 6). Figure 1B, left, shows that the systolic BP remained significantly reduced for 36 days but slowly recovered to pretreatment levels 45 days after treatment. The direct systolic BP was 146 ± 4 and 187 ± 8 mmHg in the AT1R-AS- and LNSV-treated SHR, respectively (Fig. 1B, right; n = 3). No significant difference was observed in direct BP of the WKY treated with either AT1R-AS or LNSV (132 ± 12 vs. 135 ± 6 mmHg, n = 3 for each group). In addition, no significant changes in body weight at the start (260 ± 2 and 253 ± 6 g) or at the conclusion (307 ± 12 and 309 ± 3 g) of the experiment were observed between the antisense- and the LNSV-treated SHR, respectively.

Because the most significant decrease in BP in AT1R-AS-treated SHR occurred within 2 days after the cessation of treatment, this time point was chosen in a subsequent study to investigate whether AT1R-AS (1 × 109 pfu) could reverse cardiovascular alterations observed in the SHR. Therefore, renal vascular reactivity was determined 2 days after the cessation of the 6 days of AT1R-AS treatment, when BP was 185 ± 5 mmHg in the LNSV-treated SHR and 156 ± 11 mmHg in the AT1R-AS-treated SHR. The rationale for this was based on our past observations that had established that the leftward shift in the concentration-response relationships in the SHR was associated with a decrease in the mean effective concentration (EC50) and an increase in the logarithmic effective concentration (−pEC50) for KCl and PE in the SHR compared with the WKY (Table 1). In the bottom panels of Fig. 2, the leftward shift in the concentration-response relationship was still observed for the LNSV-treated SHR (n = 6 animals) but was reversed back toward the WKY control values for the AT1R-AS-treated SHR (n = 6 animals). The EC50 and −pEC50 values for the AT1R-AS-treated SHR were similar to those observed for WKY (Table 1).

### Table 1. Effect of AT1R-AS gene delivery on alterations in vascular reactivity of adult renal resistance arterioles

<table>
<thead>
<tr>
<th>Groups</th>
<th>KCl</th>
<th>PE</th>
<th>ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC50 (EC50, nM)</td>
<td>EC50 (EC50, nM)</td>
<td>EC50 (EC50, nM)</td>
</tr>
<tr>
<td>WKY</td>
<td>1.43 ± 0.008 (36.7)</td>
<td>6.45 ± 0.02 (357.6)</td>
<td>7.23 ± 0.008 (58.8)</td>
</tr>
<tr>
<td>SHR</td>
<td>1.70 ± 0.009* (19.9)*</td>
<td>6.96 ± 0.01* (110.3)*</td>
<td>7.17 ± 0.003* (67.8)*</td>
</tr>
<tr>
<td>SHR + AT1R-AS</td>
<td>1.52 ± 0.008† (29.9)†</td>
<td>6.44 ± 0.01† (365.7)†</td>
<td>7.28 ± 0.007† (52.9)†</td>
</tr>
<tr>
<td>SHR + LNSV</td>
<td>1.71 ± 0.008* (19.6)*</td>
<td>7.03 ± 0.01* (92.3)*</td>
<td>7.18 ± 0.002* (66.5)*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 24 rings from 6 animals/group. AT1R-AS, antisense to ANG II type 1 receptor; PE, phenylephrine; −pEC50, logarithmic EC50; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; LNSV, retroviral vector. *P < 0.01 vs. WKY; †P < 0.01 vs. SHR.
curve was right shifted and the maximal relaxation was decreased in the SHR \( n = 6 \) animals) compared with the WKY (Fig. 2, top right, \( n = 6 \) animals). This shift was associated with an increase in the \( EC_{50} \) and a decrease in the \(-pEC_{50}\) values in the SHR compared with the WKY (Table 1). In the \( AT_1R-AS \)-treated SHR, the shift in the \( ACH \) concentration-response relationship was reversed and the \( EC_{50} \) and \(-pEC_{50}\) values returned to values approximating those of the WKY compared with the LNSV-treated SHR (Fig. 2, bottom right; Table 1).

Finally, the ability of \( AT_1R-AS \) to decrease the number of \( AT_1R \) in an ANG II target tissue was investigated 2 days after the cessation of treatment. This coincides with the maximum decrease in BP (see above). The left ventricles of LNSV- or \( AT_1R-AS \)-treated WKY and SHR were removed for binding studies. Table 2 shows that there was no significant difference in the maximum binding capacity (\( B_{\text{max}} \)) for \( ^{125}\text{I}-\text{ANG II} \) in the WKY treated with LNSV or \( AT_1R-AS \). However, there was a significant increase in the \( B_{\text{max}} \) for \( ^{125}\text{I}-\text{ANG II} \) in the SHR treated with LNSV. \( AT_1R-AS \)-treated SHR showed no significant difference from the control WKY.

No change in dissociation constant (\( K_d \)) was observed in any treatment group. These data demonstrate that a decrease in \( AT_1R \) coincides with the decrease in BP.

### Table 2: Effect of \( AT_1R-AS \) gene delivery on \( ^{125}\text{I}-\text{Sar}^1, \text{Ile}^8\)-ANG II binding in adult rat heart

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>( B_{\text{max}} ) (fmol/mg protein)</th>
<th>( K_d ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY + ( AT_1R-AS )</td>
<td>4</td>
<td>88 ± 11</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>WKY + LNSV</td>
<td>4</td>
<td>96 ± 12</td>
<td>0.63 ± 0.14</td>
</tr>
<tr>
<td>SHR + ( AT_1R-AS )</td>
<td>4</td>
<td>117 ± 6.7</td>
<td>0.50 ± 0.07</td>
</tr>
<tr>
<td>SHR + LNSV</td>
<td>4</td>
<td>131 ± 7.9*</td>
<td>0.84 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of hearts. \( B_{\text{max}} \), maximum binding capacity; \( K_d \), dissociation constant. *\( P < 0.05 \).
that this effect is observed at other relevant cardiovascular tissue. Additionally, the decrease in vascular responsiveness observed in the present study is consistent with the hypothesis that some vascular remodeling occurs in hypertension and may be another possible site of antisense action in the adult animal. It is also possible that the success of retroviruses in infecting poorly dividing cells is much better than previously realized. We believe that a combination of these possibilities may be responsible for our observed results.

Although pharmacological intervention is relatively effective in reducing BP, the reversal of hypertension-induced pathophysiological changes by these agents is controversial (6, 23). Previously, we observed that in SHR treated neonatally, similar AT1R-AS treatment prevented the altered vascular responses to KCl, PE, and ACh normally associated with hypertension (8, 11, 18). However, the results presented here are the first to demonstrate that AT1R-AS delivery in adult hypertensive animals effectively reduces BP toward that of controls and reverses the pathophysiological effects in the renal vasculature associated with hypertension. Thus, in addition to the antihypertensive effects and the normalization of the vascular pathophysiology, this gene therapy approach may, for the most part, reduce the “compliance” problem related to conventional therapy in hypertensive patients. Utilization of the next generation of retroviral vectors that can improve the transduction efficiency in nondividing cells should increase the effectiveness of this delivery system even further so that a more permanent reversal of BP and pathophysiological complications associated with hypertension may be forthcoming. This type of therapy therefore offers promising inroads to single-dose, long-term treatment of hypertension and its associated complications in established hypertensive patients.

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