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 blockade of the renin-angiotensin system in both hypertensive patients and animal models such as the spontaneously hypertensive rat (SHR) effectively reduces blood pressure (BP). Recent studies have established that virally mediated delivery (vector LNSV) 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 BP once it is established in the adult 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 arteriolar reactivity demonstrated a leftward shift in the KCl and phenylephrine concentration-response relationships and an impaired endothelium-dependent relaxation to ACh in LNSV-treated SHR compared with control Wistar-Kyoto rats. These vascular alterations were reversed in the LNSV-AT1R-AS-treated SHR. Collectively, these data demonstrate that virally mediated gene delivery of AT1R-AS can effectively reduce BP and reverse renovascular pathophysiology associated with the hypertensive state when administered to the adult SHR.

PHARMACOLOGICAL INTERRUPTION of the renin-angiotensin system (RAS) is considered rational therapy for hypertension. It is well documented that the RAS is the key in the development and maintenance of hypertension in both primary hypertensive patients and in animal models of hypertension, most notably, the spontaneous hypertensive rat (SHR; Ref. 9). Thus interruption of the RAS reduces the systemic blood pressure (BP) and some of the cardiovascular complications associated with hypertensive disease (3, 13, 19). In addition, pharmacological agents that inhibit the RAS are therapeutically beneficial in patients with congestive heart failure (3, 19) myocardial infarction (2, 13), and diabetic neuropathy (14). Losartan, a newly developed selective angiotensin type 1 receptor subtype (AT1R) antagonist, has been demonstrated to be an effective antihypertensive agent without many significant side effects (2).

It has been demonstrated previously that AT1R-encoding gene polymorphism is associated with hypertension (20). Thus it has been proposed that targeting the RAS at the genetic level with an antisense gene delivery approach could provide an improved therapeutic intervention in the treatment of hypertension. Our recent observations (11, 15–18) have provided significant experimental evidence in support of this hypothesis. We have established that delivery of AT1R-antisense cDNA (AT1R-AS) by a retroviral vector system in neonatal rats attenuates the development of hypertension exclusively in the SHR (8, 11, 17, 18). The lowering of BP is similar to that observed after administration of losartan. However, the antihypertensive effect can be manifested with only a single dose of AT1R-AS treatment for >120 days, whereas the effect of a single dose of losartan is no longer apparent within 24 h (17). In addition, this antisense gene therapy approach was associated with the prevention of pathophysiological changes normally associated with hypertension in the SHR (8, 18). Despite our previous success in neonates, the effectiveness for this treatment in adult animals remains to be proven. Thus the present study was designed to determine the effectiveness of AT1R-AS treatment in lowering BP in established hypertension in the adult SHR and to determine the effectiveness of AT1R-AS treatment to reverse the vascular pathology that exists in this model of hypertension.

METHODS

Preparation of viral particles containing AT1R-AS. AT1R-AS was cloned in a retroviral vector containing long terminal repeats, a neomycin selection, and an internal simian virus 40 promoter (LNSV), prepared as previously described (8, 11, 17, 18), and delivered, for 6 consecutive days, into adult rats...
via daily cardiac injection in anesthetized rats (Metofane, Mallinkrodt 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 were used. A second study used 70-day-old male SHR (244–268 g), and 90-day-old male SHR (242–328 g) were used in the vascular reactivity study. Age-matched WKY were used as controls.

Indirect BP was monitored in nonanesthetized animals by a standard tail-cuff method at specific times before, during, and after administration of the AT1R-AS or LNSV alone (8, 11, 17, 18). Direct BP was determined in free-moving animals as previously described (8, 11, 17, 18). For the vascular smooth muscle studies, 3-mm-long segments of rat renal resistance arteries were dissected free and cleaned of fat and adventitia and mounted in isolated organ chambers (10 ml) maintained at 37°C in PSS as previously described (8, 11, 18). A resting force of 1.5 g was applied to the vessels. KCl, phenylephrine (PE), and ACh dose-response curves were performed in a cumulative manner. In the KCl contractile experiments, the bath contained propranolol (1 µmol/l) and phentolamine (1 µmol/l) to block β- and α-adrenoceptors, respectively. ANG II receptor binding was determined in cardiac tissue as previously described (11).

Statistics. Results are expressed as means ± SE. Statistical significance for the direct BP data was evaluated using ANOVA and a Fisher's post hoc test. Significance for the vascular studies and indirect BP over time was evaluated with repeated-measures ANOVA and Student's t-test for unpaired data. In the vascular studies, all rings were normalized to tissue weight and cross-sectional area. Differences were considered significant at P < 0.05.

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 × 10⁸ 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). Heart rates were similar between the two groups (337 ± 49 and 348 ± 54 beats/min). Body weights also were not altered throughout the experiment (265 ± 12 and 274 ± 13 g at the beginning and 282 ± 10 and 282 ± 9 g at the end of the injection period in LNSV- and AT1R-AS-treated groups, respectively). 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 × 10⁹ pfu) containing AT1R-AS was administered in
the same manner as before to determine whether the antihypertensive effect is dose dependent and whether the duration of the response could be prolonged. As early as 1 day after the treatment, indirect systolic BP 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 × 10^6 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 reactivity of the renal resistance arteriole to vasoactive agents is altered in hypertension (8, 18). Figure 2 and Table 1 summarize the results from the vascular reactivity studies. The top panels of Fig. 2 summarize the enhanced contractile responses to both KCl and PE, which were 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).

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

<table>
<thead>
<tr>
<th></th>
<th>KCl</th>
<th></th>
<th>PE</th>
<th></th>
<th>ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC50 (EC50, nM)</td>
<td>pEC50 (EC50, nM)</td>
<td>pEC50 (EC50, mM)</td>
<td></td>
<td></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>
<td></td>
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<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>
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</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>
<td></td>
<td></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>
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</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 EC50 and a decrease in the \(-pEC_{50}\) values in the SHR compared with the WKY (Table 1). In the AT1R-AS-treated SHR, the shift in the ACh concentration-response relationship was reversed and the EC50 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 AT1R-AS to decrease the number of AT1R in an ANG II target tissue was investigated was a significant increase in the Bmax for 125I-ANG II in the WKY treated with LNSV or AT1R-AS. However, there was no significant difference from the control WKY. SHR treated with LNSV or AT1R-AS. However, there was no significant increase in the Bmax for 125I-ANG II in the SHR treated with LNSV. AT1R-AS-treated SHR showed no significant difference from the control WKY. No change in dissociation constant (Kd) was observed in any treatment group. These data demonstrate that a decrease in AT1R coincides with the decrease in BP.

**DISCUSSION**

The results of this study are the first to demonstrate that the delivery of AT1R-AS by a retroviral gene delivery vector is effective in reducing BP in the adult SHR and in reversing some of the renal vascular pathophysiological changes associated with hypertension. The 30-mmHg reduction in BP observed with this approach is comparable to the reduction in BP observed with acute losartan treatment (17). However, antisense gene therapy offers a potential advantage in that losartan-induced decreases in BP are short-lived (17), whereas the reduction in BP induced by antisense gene therapy was maintained for >1 mo (Fig. 1). In addition, there were no visual adverse effects of the antisense treatment as judged by body weight, water intake, or any behavioral observations. Also, the antisense treatment had little effect on the normotensive WKY (Figs. 1 and 2).

It is well documented that vascular changes are associated with hypertension. Studies have demonstrated an enhancement of renal vascular tone (21), which may lead to an increase in vascular resistance, a hallmark of hypertensive disease. This increase in resistance could be caused by an enhanced contractile responsiveness to vasoactive agonists and/or an impaired endothelium-dependent relaxation (10). The results of our study demonstrate that both the impaired endothelium-dependent relaxation and the enhanced contractile responsiveness observed in the renal vasculature of the SHR are reversed in the antisense-treated animal. Our results (Fig. 2, Table 1) demonstrate an increased vascular contractile response to both KCl and PE and an impaired relaxation response to ACh in the renal vasculature of the SHR. However, within 2 days of the cessation of AT1R-AS treatment, this augmented vascular response in the renal arteries of the AT1R-AS-treated SHR was reversed and similar to that observed in the normotensive WKY controls. Thus AT1R-AS treatment completely reversed the abnormal vascular contractile responses associated with hypertension in the adult SHR.

Altered endothelial dysfunction has been reported in several vascular beds and in numerous models of hypertension, and impairment of ACh-induced vasorelaxation is one such example (7, 18, 22). It is speculated that the endothelium, and thus endothelial dysfunction, is a target for end-organ tissue damage as a complication of hypertension and not a predisposing factor contributing to the disease. Our data (Fig. 2, Table 1) establish that this impaired endothelium-dependent relaxation is completely reversed by antisense therapy. Thus, regardless of the precise mechanism, it is clear that vascular complications associated with hypertension are reversed in the SHR by this gene therapy approach.

Although the results provide relevant information for long-term control of hypertension, they raise an important issue of how a retroviral vector is able to integrate, express, and transduce alterations of physiological signals in adult animals. This is relevant in view of the strongly held belief that retroviruses have poor efficiency in infecting nondividing cells (1). The answer to this question remains largely speculative at the present time. Components of the RAS are located in numerous tissues, including vascular tissue (4, 5, 12), and the local RAS may play a key role in hypertension-induced tissue remodeling. Thus it is quite possible that this tissue remodeling is enough to allow for transduction of AT1R-AS expression by sufficient cells to produce the observed effects. This view is supported by our previous observations (11, 15–18) that showed that AT1R-AS treatment resulted in a reduction in AT1 receptors in adrenal, cardiac, and renal tissues. This effect is highly specific because the levels of AT2 receptor are not altered by this treatment (17). Further support for this knockdown hypothesis is derived from our binding experiments in the current study. AT1R-AS treatment resulted in a significant decrease in Bmax for cardiac AT1R in the SHR compared with the LNSV-treated SHR. Few, if any, significant changes were observed in Kd. Although only one tissue was assessed for ANG II receptor number in the current study, data from our previous studies (11, 15–18) would suggest

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Table 2. Effect of AT1R-AS gene delivery on 125I-[Sar1, Ile8]-ANG II binding in adult rat heart

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Bmax (fmol/mg protein)</th>
<th>Kd (nM)</th>
</tr>
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<tbody>
<tr>
<td>WKY + AT1R-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 + AT1R-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. Bmax, maximum binding capacity; Kd, 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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