Blood Pressure–Independent Attenuation of Cardiac Hypertrophy by AT₁R-AS Gene Therapy

Alok S. Pachori, Mohammed T. Numan, Carlos M. Ferrario, Debra M. Diz, Mohan K. Raizada, Michael J. Katovich

Abstract—Our studies have established that a single intracardiac administration of the retroviral vector containing angiotensin II type I receptor antisense gene causes prolonged antihypertensive actions in the spontaneously hypertensive rat. These results suggest that antisense gene therapy is a conceptually valid strategy for the control of hypertension at the genetic level. To evaluate whether attenuation of the pathophysiological aspects of hypertension are dependent on the blood pressure lowering actions of antisense gene therapy, we chose the renin transgenic rat as a hypertensive animal model and cardiac hypertrophy as the hypertension-associated pathophysiology. A single intracardiac administration of the retroviral vector containing angiotensin II type I receptor antisense in the neonatal rat resulted in long-term expression of the antisense transgene in various cardiovascular-relevant tissues, including the heart. This expression was associated with a significant attenuation of cardiac hypertrophy despite its failure to normalize high blood pressure. Developmental studies indicated that cardiac hypertrophy was evident as early as 16 days of age in viral vector–treated control transgenic rats, despite these animals exhibiting normal blood pressure. These observations demonstrate that, in the renin-transgenic rat, the onset of cardiac hypertrophy occurs during development and is prevented without normalization of high blood pressure. Collectively, these results provide further proof of the concept and indicate that antisense gene therapy could successfully target the local tissues’ renin-angiotensin system to produce beneficial cardiovascular outcomes. (Hypertension. 2002;39:969-975.)

Key Words: antisense elements ■ rat, transgenic ■ renin-angiotensin system ■ hypertension, essential ■ genes

Left ventricular hypertrophy (LVH) is a risk factor for cardiovascular-related mortality. Besides high blood pressure (BP), other hemodynamic factors that affect LVH include increased arterial stiffness, blood viscosity, and volume overload. However, increasing evidence has demonstrated a role for nonhemodynamic factors such as the sympathetic nervous system, the local renin-angiotensin system, aldosterone, and genetic factors in the modulation of BP-independent LVH.¹⁻⁷

Recent clinical and experimental studies have demonstrated that pharmacological agents, such as angiotensin converting enzyme (ACE) inhibitors can reduce LVH independently of a reduction in BP.⁸⁻¹⁰ This evidence implicates the role of the tissue-based renin-angiotensin system (RAS) in the development of end-organ damage and demonstrates that the pathophysiology may be independent of high BP. However, it has not been established whether the end-organ damage observed in hypertension is a result of high BP or due to an inherently hyperactive RAS.

The renin-transgenic rat (TGR mRen2) has been developed as an animal model of hypertension that exhibits fulminant hypertension and displays cardiac and vascular hypertrophy because of an overexertion of renin in various tissues.¹⁰,¹¹ However the plasma levels of renin and Ang II range from low to normal in this model. These characteristics make this animal an excellent model for studying the participation of tissue RAS in the development and maintenance of hypertension.

We have previously shown that a single intracardiac injection of a retroviral vector containing angiotensin II type I receptor antisense gene (AT₁R-AS) prevented the development of high BP and associated cardiac and vascular pathophysiology in spontaneously hypertensive rats (SHR).¹²,¹³ These observations support our view that genetic targeting of the RAS is a conceptually viable strategy for long-term control of hypertension. However, by using the SHR model, we were not able to conclude whether normalization of cardiac and vascular pathophysiology was due to a direct
that AT1 R-AS treatment in controls does not significantly affect BP used as nontransgenic controls. We have previously demonstrated were obtained, and their male offspring were treated with LNSV and vector. Similarly, SD mothers (SD females mated with SD males) LNSV-AT 1 R-AS constructs containing viral particles were adminis-

were administered to 5-day-old TGR, essentially as described in the methods. RNA was isolated from various tissues from 10- (A) and 120- (B) day-old TGR and subjected to RT-PCR analysis. The 1.2 Kb band corresponds at the AT1R-AS transcript. Positive control is from the transfectected PA317 producer cell line, which is used to package the viral particles.

Figure 1. The AT 1 R-AS expression was robust within 10 days of age. Their hearts were removed, blotted, and weighed. In addition, characteristics of LNSV-AT 1 R-AS

effect of the antisense gene on the blockade of tissue RAS or was a result of decreased BP. Thus, the present study was designed to validate the efficacy of AT,R-AS gene therapy in preventing the development of cardiac hypertrophy (CH) and BP in the TGR model of hypertension.

Methods

Treatment of TGR with the Retroviral Vector Containing AT1,R-AS

Female Sprague Dawley (SD) rats were mated with male homozygous TGR obtained from the breeding colony at the Wake Forest University (Winston-Salem, NC). Male TGR offspring were removed from their mothers 5 days after birth and divided into 3 groups: untreated TGR, LNSV-treated TGR control, and AT,R antisense cDNA (LNSV-AT 1,R-AS)-treated TGR containing LNSV vector. Similarly, SD mothers (SD females mated with SD males) were obtained, and their male offspring were treated with LNSV and used as nontransgenic controls. We have previously demonstrated that AT,R-AS treatment in controls does not significantly affect BP or cardiac events. 

Animals were lightly anesthetized with methoxyflurane (Metofane, Pitman Moore). A single bolus of 25 ìL of either physiological saline or 1x10^8 colony forming units (cfu) of LNSV or LNSV-AT 1,R-AS constructs containing viral particles were adminis-
tered as a bolus into the left ventricle as previously described. 

Following viral vector administration, offspring were returned to their respective mothers until weaning. All animal procedures were conducted with approval from our Institutional Animal Care and Use Committee (IACUC) and adhered to the guidelines for the care and use of laboratory animals.

Physiological and Pathological Measurements

Five animals from each group were euthanized at 12, 19, and 26 days of age. Their hearts were removed, blotted, and weighed. In addition, hearts from a 16-day-old group were perfusion-fixed with 4% paraformaldehyde for histology. In another group of animals, indirect BPs were monitored by the tail-cuff method starting at 3 weeks of age and continuing until 12 weeks of age.

Echocardiographic Studies

Rats of 12 to 14 weeks of age were lightly anesthetized with methoxyflurane and placed on a heating pad for the echocardiographic studies. A Hewlett Packard Sonos Model 5500 with a 12-Hz transducer was used with a focal zone of 2 cm, depth of 2 cm, and a pulse wave Doppler gate length of 0.06 cm. Measurements obtained by echocardiography include left ventricular end diastolic diameter (LVED), left ventricular posterior wall thickness (LVPW), left ventricular free wall thickness (LVFW), interventricular septum thickness during diastole, LV mass, and LV stroke volume. The shortening fraction was calculated as follows: (LVED-LVES)/LVED x 1/100. Left ventricular mass measurements were calculated from the bullet method as follows: 1.05(5/6 A 1 – (5/6 A 1 

Statistics

All results were expressed as mean±SE. Indirect BP was analyzed by repeated measures ANOVA. Values of P<0.05 were considered statistically significant. All experiments had at least 7 animals per group unless stated otherwise.

Results

Characteristics of LNSV-AT,R-AS–Treated Transgenic Rats

Single intracardial administration of LNSV-AT,R-AS in 5-day-old rats resulted in a long-term expression of AT,R-AS in various cardiovascularly relevant tissues. A representative RT-PCR analysis of AT,R-AS mRNA levels is presented in Figure 1. The AT,R-AS expression was robust within 10 days after treatment and was persistent at 120 days postadministra-
tion of LNSV-AT,R-AS. No expression was seen in tissues of rats injected with saline or LNSV control vector (data not shown).

Indirect BP was significantly elevated in the TGR compared with their age-matched SD controls at 8 to 12 weeks of age.
age (Figure 2A). At 12 weeks of age, TGR showed 90 mm Hg higher BP compared with SD controls (220±3 versus 130±3 mm Hg, n=9). Whereas LNSV treatment in the TGR showed no significant effect on BP compared with both TGR groups. After this time period, each animal was implanted with a catheter in the carotid artery to measure direct BP (B), Differences between groups were maintained, and probability values are as shown in Figure 2B.

**Effect of AT1 R-AS Treatment on Cardiac Pathophysiology**

At 12 weeks of age, control and LNSV-AT1 R-AS rats were subjected to echocardiography to characterize hypertension-induced cardiac pathophysiology and to determine the effects of AT1 R-AS expression on vascular wall thickness. LVFW thickness was significantly increased in TGR compared with SD rats (5.02±1.2 versus 2.8±0.3 mm, P<0.001). This was fully corrected in the AT1 R-AS–treated TGR (Figures 3A through 3D). Other parameters such as LVFW thickness, shortening fraction, and stroke volume were not statically different between the SD and TGR and were not affected by the AT1 R-AS treatment. Heart-weight/body-weight ratio (HW/BW) of LNSV-treated TGR controls (3.8±0.6 g/kg) was similar to that of the untreated TGR (3.3±0.13 g/kg). This was 48% higher than the SD controls (Figure 3E). LNSV-AT1 R-AS transduction significantly reduced this ratio in the TGR (2.6±0.8 g/kg) and restored the value to that observed in the control SD rats (2.3±0.1 g/kg) (Figure 3E). We also observed the presence of pericardial effusion in untreated and LNSV-treated transgenic rats, which was not present in either SD or LNSV-AT1 R-AS–treated TGR (5/6 for LNSV versus 1/6 for LNSV-AT1 R-AS).

**Development of Cardiac Hypertrophy and Effect of AT1 R-AS**

A developmental study was undertaken to determine if the beneficial outcome of the AT1 R-AS treatment on CH is independent of normalization of high BP. Five TGR and five SD rats were euthanized at 12, 19, and 26 days of age. A significant increase in HW/BW ratio was observed in untreated TGR compared with untreated SD rats at all the investigated time points (7.4±0.98 versus 4.8±0.14 g/kg, P<0.05; 5.2±0.08 versus 4.2±0.06 g/kg, P<0.01; 4.5±0.15 versus 3.7±0.03 g/kg, P<0.001; Figure 5A). At the same time there was no difference in absolute body weight between the groups (data not shown). Indirect BP was measured in 21-day-old SD and TGR (the earliest time measurements can be made with high degree of reliability) to determine whether the TGR with CH show high BP. No significant difference in elevation of BP was observed in TGR compared with the age-matched control SD rats (Figure 5B).

CH in the neonatal TGR was further evident by the marked increase in the left ventricle wall thickness in the TGR compared with the SD rat. A representative histological section shown in Figure 6A demonstrates an increased septal (SW), anterior (AW), and lateral (LW) wall thickness in LNSV-TGR (SW 0.762 mm, AW 0.873 mm, LW 1.001 mm) compared with untreated SD rat (SW 0.721 mm, AW 0.698 mm, LW 0.78 mm). This CH was significantly attenuated in the TGR expressing AT1 R-AS as early as 16 days of age (Figure 6B).

**Discussion**

The most significant findings of this study are that (1) the development of CH is independent of high BP, and (2) CH is normalized with neonatal AT1 R-AS treatment, despite the maintenance of high BP in adult, renin-transgenic rats. Thus, our data support the hypothesis that high BP may not be entirely responsible for the pathophysiological changes associated with hypertension.14,15 They underscore the importance of the AT1 R in tissue remodeling and the pathophysiology of hypertension.16
We had previously established that AT1 R-AS gene transduction prevents both cardiac and renovascular pathophysiology in the SHR, along with a profound reduction in high BP. Therefore, it was not possible to determine whether the observed tissue-protective effects of the antisense gene were mediated via a decrease in BP or by direct effects on the tissue RAS. In the present study, we have attempted to test this hypothesis by using the TGR, an established model of hypertension that exhibits an overactive RAS, despite low plasma RAS levels.

Severe LVH, measured by both echocardiography and gross measurement of HW/BW ratio, was observed in 12-week-old hypertensive TGR rats. A single intracardiac administration of AT1 R-AS blocked the development of CH with only a modest reduction in BP in adult TGR. The LV mass in the adult AT1 R-AS–treated TGR was similar to that observed in AT1 R-AS–treated TGR.

![Figure 3](image-url)
SHR.\textsuperscript{12,13} The increase in LV mass in TGR was associated with an upregulated expression of fetal and structural genes such as ANP and α-skeletal actin, the levels of which were reduced by 50% and 55%, respectively, in AT\textsuperscript{1} R-AS treated adult TGR. The observation that CH could be completely prevented in the face of only a 13% decrease in BP illustrates the point that a complete normalization of BP is not necessary for improvement in CH. Additionally, it indicates that an overactive tissue RAS, and not pressure overload, may be primarily responsible for CH in the TGR. This view is further supported by studies using pharmacological strategies.\textsuperscript{20} This study also demonstrated that our antisense treatment significantly reduced HR in the adult TGR to that observed in the control SD. The relationship between the reduction in HR and CH in this model could not be established because HR was not ascertained in the neonatal TGR.

CH was studied at various stages of growth to further confirm BP-independent development of CH in TGR. TGR, as young as 12 days of age, exhibited significantly higher HW/BW ratios than normotensive SD rats. This hypertrophy was not dependent on high BP because BPs of 21-day-old TGR were comparable with the normotensive SD rats. These findings were confirmed by histology demonstrating higher lateral, septal, and anterior left ventricle wall thickness in TGR hearts compared with age-matched normotensive SD rats. These observations are consistent with the SHR model of hypertension in which cardiac enlargement and increased DNA synthesis are seen in the newborn SHR hearts when compared with WKY rats.\textsuperscript{21,22} These results support the hypothesis that CH observed in adult hypertensive rats,
whether it is TGR or SHR, occurs during the early stages of growth and thus argue against the role of high BP in the development of hypertension-associated organ pathology.

Other lines of evidence also support a role of the intrinsic cardiac RAS in the regulation of cardiac growth and hypertrophy. These include increased expression of renin in hypertrophied SHR hearts, elevated tissue-converting enzyme activity in the SHR, and the presence of cardiac hypertrophy in mice overexpressing AT1 receptors in cardiomyocytes without any effect on BP. However, studies correlating mRNA levels of components of the RAS during different developmental stages of CH have not been performed, thus leaving to speculation the exact role of tissue RAS in BP-independent hypertrophy.

Collectively, these results demonstrate that development of CH, which is a characteristic of hypertension, can be manifested independently of high BP. We also demonstrate that the induction of CH may actually take place during the perinatal growth period, preceding the onset of high BP, and may be a result of an overactive cardiac RAS.

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References

Figure 6. Development of cardiac hypertrophy in 16-day-old renin transgene rats. Five-day-old TGR and SD rats were injected with LNSV or LNSV-AT,R-AS viral vector. After 11 days, the animals were perfusion-fixed with 4% paraformaldehyde and hearts were excised, cut into 5-mm-thick sections, and stained with hematoxylin and eosin. A, Representative sections from hearts of each group sacrificed at 16 days of age. Hearts were removed, blotted, and weighed (B). Data are shown as HW/BW ratio. **P<0.001 versus SD; *P<0.01 versus LNSV-TGR; n=5/group.
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