Full-length article

Recombinant adeno-associated virus-mediated delivery of antisense angiotensin II receptor 1 gene attenuates hypertension development[1]

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Key words

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Abstract

Aim: The renin-angiotensin system plays a crucial role in the development and establishment of hypertension, and the pharmacological blockade of the system results in a reduction in blood pressure. In the present study, we investigated whether the effects of a novel, double-stranded, recombinant adeno-associated virus vector (rAAV)-mediated antisense angiotensin II receptor 1 (AT$_1$R) gene efficiently prevents the development of hypertension induced by a high-salt diet in adult, male Sprague-Dawley (SD) rats. **Methods:** A rAAV was prepared with a cassette containing a cytomegalovirus promoter and partial cDNA (660 base pairs) for the AT$_1$R inserted in the antisense direction (rAAV-AT$_1$-AS). A single tail vein injection of the rAAV-AT$_1$-AS or rAAV-GFP (green fluorescent protein, a reporter gene) was performed in adult, male SD rats. Two weeks after injection, the animals were fed a diet containing 8% NaCl, and the systolic blood pressure was measured weekly using the tail-cuff method for 12 weeks. **Results:** The high-salt diet induced a significant rise in systolic blood pressure in the rAAV-GFP-treated animals; however, the rAAV-AT$_1$-AS treatment attenuated the rise in blood pressure (142.7±4.5 mmHg *vs* 117±3.8 mmHg, *P*<0.01), and the hypotensive effect was maintained until the experiments ended at 12 weeks. In the rAAV-GFP-treated animals AT$_1$ was overexpressed in various tissues, especially in the aorta and kidney at mRNA levels; in contrast, rAAV-AT$_1$-AS treatment markedly attenuated AT$_1$ expression. Furthermore, rAAV-AT$_1$-AS treatment prevented target organ damages from hypertension, including cardiac dysfunction and renal injury compared to the rAAV-GFP group. **Conclusion:** These results suggest that rAAV-mediated anti-AT$_1$ delivery attenuates the development of hypertension and protects against renal injury and cardiac remodeling.

Introduction

Epidemiological data have suggested a correlation between dietary salt and blood pressure regulation in the prevalence and progression of essential hypertension, although the salt-blood pressure theory has remained the subject of ongoing controversy in a number of studies[1–4]. It has been demonstrated that the renin-angiotensin (RAS) is important in the development and maintenance of hypertension in both essential hypertensive patients and animal models of hypertension[5,6]. Moreover, many studies have revealed that high salt could stimulate the RAS and induce angiotensin II

receptor 1 (AT$_1$R) overexpression, which may be responsible for the elevation of blood pressure[7,8]. The blockade or interruption of RAS by pharmacological agents, such as the angiotensin-converting enzyme inhibitor and AT$_1$R antagonists, have been extremely successful in the treatment of hypertension and the prevention of it complications. The findings indicate that the RAS components are crucial for the development of hypertension and cardiovascular function.

Gene therapy is a promising strategy for the treatment of hypertension[9]. Applications of antisense RAS genes to block the expression of either angiotensinogen or AT$_1$R have

demonstrated efficiency of this therapeutic strategy. Iyer *et al* reported that retroviral vector-mediated antisense AT_1R cDNA gene delivery resulted in a chronic reduction up to 30–60 mmHg in hypertension in spontaneously hypertensive rats (SHR) rather than Wistar Kyoto normotensive rats[10]. The intracerebroventricular application of antisense oligonucleotides, directed to either AT_1R or angiotensinogen mRNA also significantly reduced blood pressure in hypertensive animals with a single injection[11–13]. Moreover, an injection of antisense cDNA or oligonucleotides of the angiotensin-converting enzyme, renin, or angiotensinogen genes also induced a reduction of blood pressure in hypertensive animals[14–17]. All these findings suggest that blocking RAS genes may serve as target or candidate genes for hypertension gene therapy.

Another concern is which vector could be used to carry genes for chronic or permanent reduction in blood pressure. Several viral vectors, including retroviruses, lentivirus virus, and recombinant adeno-associated virus vector (rAAV) can be adaptive for gene therapy of genetic or chronic diseases. However, the retrovirus vector should not be considered due to the carcinogenic effects. rAAV, which either integrates into the genome or remains in the nucleus as a stable episome, offers the most attractive advantages and may be the most promising gene vector for long-term therapy[18,19]. The adeno-associated virus (AAV) is safe to use. It does not induce any pathogenic responses and does not replicate inside cells. The AAV is a defective parvovirus which cannot replicate in cells without the presence of a wild-type adenovirus[20,21]. The AAV is effective as a vector as it does not produce viral proteins that stimulate inflammatory reactions and contains sufficient carrying capacity for the insertion of an antisense cDNA for AT_1R. More recently, a new double-stranded rAAV was developed, and this double strand can express more rapidly and efficiently than a single-strand rAAV, which makes it more attractive[22]. In the present study, the rAAV was used to carry the rat antisense AT_1R gene (rAAV-AT_1-AS) for hypertension therapy. It was demonstrated that the rat rAAV-AT_1-AS delivery via intravenous injection prevented the long-term, salt-induced development of blood pressure and attenuated cardiovascular complications of hypertension.

Materials and methods

Preparation of rAAV-AT_1-AS and rAAV-GFP (green fluorescent protein, as control) The rAAV-D(+) vector (double-stranded rAAV Vector plasma), the adenovirus helper plasmid pXX_6, and the packaging plasmid pXX_2 have been described previously[21]. A 660 bp fragment of rat vascular AT_1R cDNA (127–786) was amplified with RT-PCR from rat kidney total RNA and ligated to the rAAV-D(+) vector in the antisense orientation after being identified by sequencing. The rAAV-AT_1-AS and rAAV-GFP vector plasmids were constructed by inserting the rat AT_1 antisense cDNA and GFP cDNA into the rAAV-D(+) vector driven by the cytomegalovirus promoter at BamHI and NotI sites, respectively. The vectors for rAAV-AT_1-AS and rAAV-GFP were prepared using a triple plasmid cotransfection method in 293 cell lines, as described previously[21,23]. A total of 50 µg plasmid DNA per 15 cm plate (rAAV-AT_1-AS or rAAV-GFP vector plasmids /pXX_2/pXX_6 were 1:1:1 molar ratios) were used for transfection. For large-scale rAAV preparations, 40×15 cm plates, each containing $5×10^6$ 293 cells were used, and a single-step gravity-flow column purification method was carried out, as previously described[24]. The eluted rAAV was aliquoted and stored at -80 °C. To determine the titer of the AAV vectors, 5 µL of the rAAV vector was digested with 5 U DNase I at 37 °C for 1 h followed by digestion with 100 µg proteinase K in proteinase K buffer for 1 h at 37 °C. The reaction was then extracted with an equal volume of phenol/chloroform/isoamyl alcohol, and the titer of rAAV-AT_1-AS and rAAV-GFP was determined by dot blot hybridization with a ^{32}P-labeled probe[23].

Animals Male Sprague-Dawley (SD) rats weighing 250–270 g were obtained from the Tongji Medical College Animal Center (Wuhan, Hubei Province, China). All of the animals were housed at room temperature with 12 h light/dark cycles and allowed free access to normal rat chow and water ad libitum. The experimental protocols complied with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Chinese Academy of Sciences. The animals were randomly allocated to the groups of rAAV-AT_1-AS, rAAV-GFP and Saline.

Gene delivery The animals were anesthetized intraperitoneally with pentobarbital at a dose of 40 mg/kg body weight. rAAV-AT_1-AS, rAAV-GFP (about $2×10^{10}$ virion particles), or an equal volume of saline was then administered into the SD rats by tail vein injection. After the injection, the rats were kept warm under an infrared lamp until they recovered. After 2 weeks, the animals of rAAV-AT_1-AS and rAAV-GFP groups were exposed to a high-salt diet (8% NaCl) and the animals in saline group received normal diet for 12 weeks, and then cardiac catheterization was performed for the hemodynamic measurements before the animals were killed under pentobarbital anesthesia (40 mg/kg body weight). The hearts, lungs, brains, aorta, and livers were harvested, snap-frozen in liquid nitrogen, and stored at -80 °C.

Blood pressure measurements The systolic blood pressure of the rats was measured with a manometer–tachometer

(Rat tail NIBP system, ADI Instruments, Bella Vista, NSW, Australia) by cuffing the tails of the animals. The rats were restrained in a plastic cage for approximately 15–30 min to acclimatize and were minimally warmed before the blood pressure determination. Five to ten stable readings of systolic blood pressure were taken weekly for each animal. All of the measurements were performed between 21:00 and 24:00. The systolic blood pressure was represented by the mean of these stable recordings.

Serum and urine collection Urine samples were collected for 24 h in metabolic cages with 500 μL toluene to prevent the decay of the urine. The samples were stored at -80 °C before the urinary microalbumin concentration was measured.

Whole heart and kidney weight measurements At 12 weeks after the high-salt diet, the animals were anesthetized with pentobarbital and the hearts were removed. The whole heart, double kidney, and body were then weighed. The ratios of whole heart/body weight and double kidney/body weight were calculated.

RT-PCR analysis of rat AT$_1$ mRNA Total RNA was extracted from fresh rat tissues using TRIZol reagent (Gibco, Carlsbad, CA, USA), according to the manufacturer's instructions. RT-PCR for the rat AT$_1$ transcription level was then performed using the RT-PCR kit (TaKaRa Biotechnology, Dalian, Liaoning, China), according to the manufacturer's instructions. PCR primers specific for rat AT$_1$ were 5'-GGA TGG CGG GAA GTC TAT T-3' for upstream and 5'-TGG GAA TCT TTG AGA ACT GG-3'for downstream (1151–1651 bp, which will not amplify transected antisense cDNA and RNA) and β-actin 5'-TCC TCC CTG GAG AAG AGC TA-3' for upstream and 5'-TCA GGA GGA GCA ATG ATC TTG-3' for downstream.

Urinary microalbumin and sodium measurement Urinary microalbumin was measured by ELISA. The albumin antibody-coated and blocked microtiter plates (96 wells), labeled with albumin antibody, albumin standard, and O-phenylenediamine(OPD) substrates were stored at 4 °C. The albumin standard (0.1–6.4 μg/mL) and rat urine samples were added to individual wells in a total volume of 50 μL dilution buffer. The labeled albumin antibody (50 μL) was added to the dilution buffer and mixed gently. The plates were incubated at 37 °C for 90 min, and after incubation, the plates were washed 4 times with wash solution. The color reaction was performed by adding 100 μL freshly prepared OPD substrate solution at 37 °C for 20 min. Then the plates were read at 490 nm on an Elx 800 ELISA reader (Biotek Instruments,Winooski, Vermont, USA). Urinary sodium was measured at the clinical laboratory of Tongji Hospital using an automatic analyzer.

Hemodynamic variables The hemodynamic variables were monitored using a catheter tip manometer (AD Instruments, Bella Vista, NSW, Australia) advanced from the right carotid artery via the aortic arch into the left ventricle (LV) at the end of the experiment. Global systolic function was measured as LV peak pressure (LVPSP), LV end-diastolic pressure (LVEDP), and the maximum–minimum rate of pressure increase (dp/dt_{max}). Global LV end-systole was defined as the point of minimum dp/dt and LV end-diastole as the beginning of the sharp upslope of the LV dp/dt tracing.

Analysis of morphology and collagen deposit The rat kidneys and hearts were preserved in 4% phosphate-buffered saline-formaldehyde solution and then embedded in paraffin. Four micrometer-thick sections were cut and stained with Sirius Red (collagen stained with Sirius Red), using the method described by Dobrzynski *et al*[25]. All of the sections were evaluated by investigators who were blind to the experiment. The percentage of extracellular matrix (ECM) production was quantified at the Department of Ultrastructural Pathology, Tongji Hospital with the use of the HAIPS pathological imagic analysis system (Wuhan, China). To quantify the ECM, red staining was expressed as a percentage of the total tissue area[26].

Statistical analysis The statistical significance of the difference in the systolic blood pressure between rats receiving rAAV-GFP and rats receiving rAAV-AT$_1$-AS was determined by ANOVA. In addition, we used unpaired Student's t-test to assess the differences in urine microalbumin content, the ratios of total heart/body weight, and the results of the ECM quantification between the rAAV-GFP and rAAV-AT1-AS groups after gene delivery. The results were expressed as mean±SEM.

Results

Hypotensive effects of rAAV-AT$_1$-AS The systolic blood pressure of all animals was monitored weekly before and after exposed to a high-salt diet. The results showed that a high-salt diet induced a significant elevation in the systolic blood pressure up to 132.3±12.9 mmHg ($P<0.01$. $n=8$. ANOVA) in the rAAV-GFP-treated rats compared with the rAAV-AT$_1$-AS and saline solution-treated rats at 1 week. The systolic blood pressure kept elevating and the maximal blood pressure difference was up to 27.8 mmHg. In contrast, in the rAAV-AT$_1$-AS-treated animals and normal controls, the blood pressure was stable for the duration of the experiment (12 weeks; Figure 1). These results indicated that rAAV-AT$_1$-AS delivery prevents the development of high blood pressure in high-salt diet rats long term.

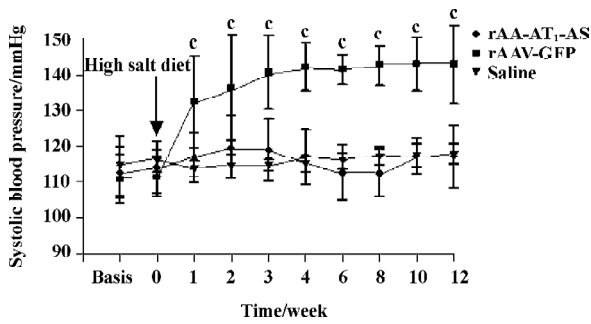**Hemodynamic variables** The hemodynamic variables

Figure 1. Measurements of systolic pressure in rats after high-salt diet using the NIBP system by cuffing the tails. Blood pressure is expressed as mean±SEM. $^cP<0.01$ *vs* rAAV-AT_1-AS and saline groups, $n=8$, ANOVA. There was no significant difference in blood pressure between the rAAV-AT_1-AS-treated rats and the saline-treated, and this hypotensive effect lasted untile the experiments ended at 12 weeks. rAAV-GFP delivery induced a significant elevation in systolic blood pressure in 1 week.

were monitored using a catheter tip manometer advanced from the right carotid artery via the aortic arch into the LV. First, the intra-arterial blood pressure was recorded (126.5 ± 2.95 mmHg in the normal control, 129.2 ± 7.72 mmHg in rAAV-AT_1-AS *vs* 148.0 ± 7.57 mmHg in rAAV-GFP, $P<0.01$, $n=5$) indicating consistency with tail artery blood pressure monitoring. The LVPSP, the left LVEDP, and the maximal/minimum ratio of LVP ($\pm dp/dt_{max}$) were measured and evaluated. The

results showed that significant differences in hemodynamics between different groups were observed (Figure 2). In the rAAV-GFP-treated animals, the LVPSP (190.3 ± 6.02 *vs* 167.5 ± 4.94 mmHg, $P<0.05$, $n=5$), $+dp/dt_{max}$ (9703.8 ± 131.4 *vs* 7837 ± 930.3 mmHg/s, $P<0.05$, $n=5$), and $-dp/dt_{max}$ (5830.4 ± 165.4 *vs* 4366 ± 353.2 mmHg/s, $P<0.05$, $n=5$) were significantly lowered, and LVEDP increased (4.04 ± 0.07 *vs* 5.01 ± 0.13 mmHg, $P<0.05$, $n=5$) compared with the normal control. In contrast, rAAV-AT_1-AS treatment prevented the deterioration of the cardiac function index (190.3 ± 6.02, 9703.8 ± 131.4, 5830.4 ± 165.4, and 4.04 ± 0.07, respectively) without significant differences compared with the normal control, but was significantly different compared with the rAAV-GFP-treated animals ($P<0.05$; Figure 2). These data indicate that rAAV-AT_1-AS treatment significantly attenuates systolic and diastolic dysfunction in salt-induced, hypertensive rats as high blood pressure was reduced.

Attenuation of cardiovascular hypertrophy and fibrosis Myocardial hypertrophy is an important part of heart injury resulting from hypertension, and serves as an independent predictor of cardiovascular events in clinical practice. In this study, we measured the ratio of heart weight/body weight to analyze the effects of rAAV-AT_1-AS treatment on myocardial hypertrophy and remodeling. As shown in Figure 3, this ratio was significantly reduced in the rAAV-AT_1-AS-treated rats compared with the rAAV-GFP-treated animals (2678.1 ± 105.5 *vs* 2904.2 ± 34.4 mg/kg, $n=8$, $P<0.05$). Sirius Red staining
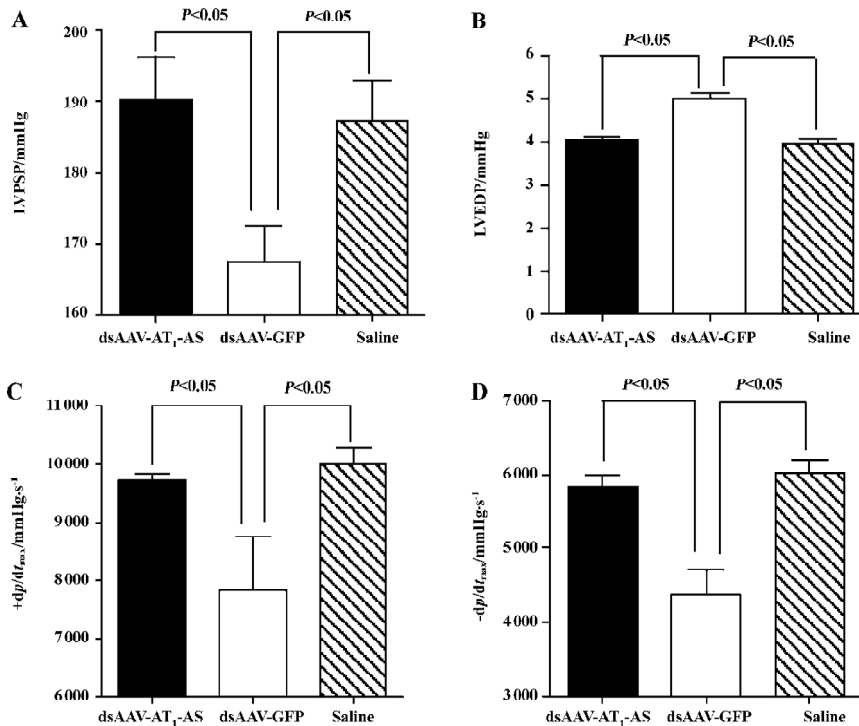

Figure 2. Hemodynamic variables using a cardiac catheter tip manometer advanced from the right carotid artery via the aortic arch into the left ventricle 12 weeks after the high-salt diet. Results show that rAAV-AT_1-AS delivery markedly improves systolic and diastolic functions. Data are expressed as mean±SEM. $n=5$.
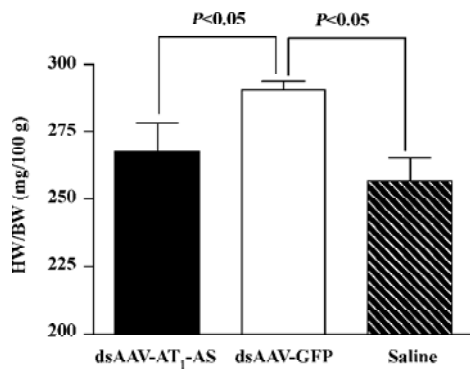
Figure 3. Inhibition of cardiac hypertrophy in rats after rAAV–AT$_1$-AS treatment. Comparison of the heart weight (HW)/body weight (BW) ratio shows that the rAAV-AT$_1$-AS-treated rats have a lower heart weight/body weight ratio than rAAV-GFP-treated rats (*n*=8).

for collagen I-VI, representing myocardial fibrosis and matrix proliferation, showed that the red-stained area significantly increased in the rAAV-GFP-treated animals compared with the rAAV-AT$_1$-AS-treated rats and normal control groups, suggesting an increased collagen deposit in salt-induced, hypertensive rats (Figure 4). Meanwhile, the structures were deranged and the sizes of the cardiac myocytes were not

uniform in the control rats in LV sections. A further quantitative analysis of the Sirius Red-stained sections was performed using HAIPS pathological imagic analysis system and was expressed as the ratio of collagen to the kidney area (percentage of ECM). The results demonstrated that AT$_1$-AS significantly reduced ECM accumulation compared with the rAAVGFP-treated animals (4.26±3.51 *vs* 15.58±6.25% ECM, *n*=8, *P*<0.05; Figure 4D).

AT$_1$–AS treatment decreases urinary microalbumin secretion An increased albumin concentration in the urine is a marker of kidney injury, as well as an independent predictor of cardiocerebral vascular disease. In this study, the microalbumin level in the urine was measured by ELISA. Initially, the urine microalbumin levels had no difference between the 3 groups. However, after exposure to a high-salt diet, the urine microalbumin level in the control animals receiving rAAV-GFP reached 2.95 ±0.82 ng/mL and 3.05±0.76 ng/mL, respectively, at weeks 6 and 12), which was significantly higher than that in rats receiving rAAV-AT$_1$-AS (2.65 ±0.69 ng/mL and 2.72±0.86 ng/mL at weeks 6 and 12, respectively, *P*<0.05) and saline injection (2.56±0.41 ng/mL and 2.52±0.58 ng/mL, at week 6 and 12, respectively, *P*<0.05, Figure 5). The results suggest that rAAV-AT$_1$-AS treatment markedly attenuates the renal damage induced by hypertension from a high-salt diet.
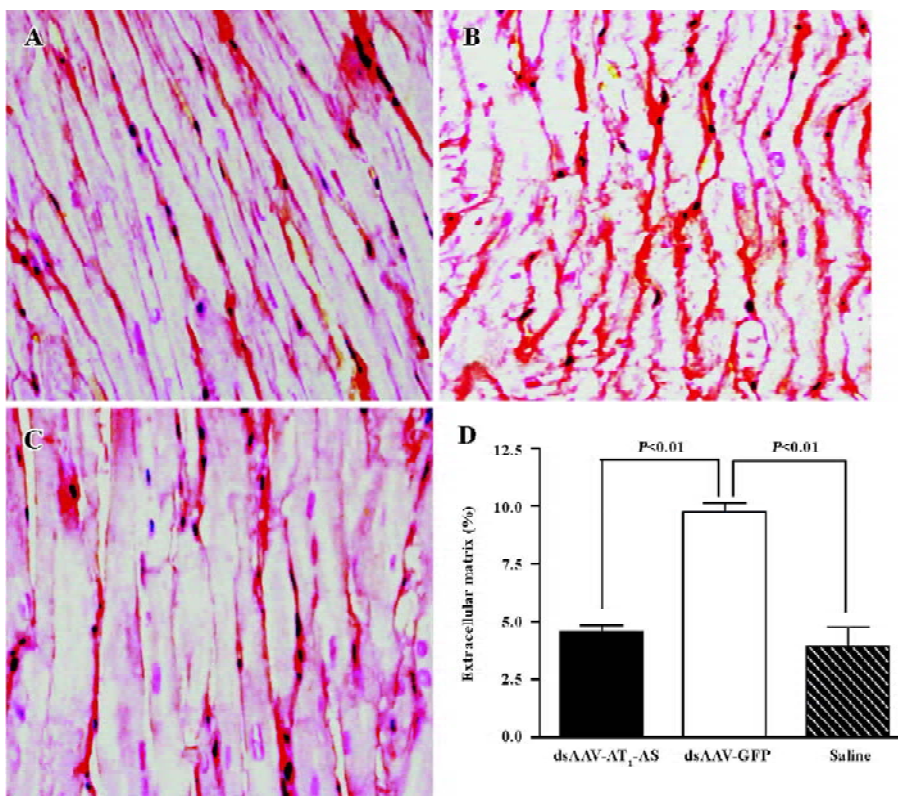

Figure 4. Inhibition of fibrosis and collagen deposit in rat hearts determined by Sirius Red staining under light microscope. (A, B) Sirius Red-stained microscopy of hearts of high-salt diet rats receiving rAAV-AT$_1$-AS and rAAV-GFP, respectively, showed fibrosis and collagen deposit in the heart. (C) rats treated with saline. (D) Sirius Red-stained sections were analyzed by using HAIPS pathological imagic analysis system for the percentage of ECM/collagen area surface. ECM was markedly reduced in rAAV-AT$_1$-AS-treated animals compared with the control groups.
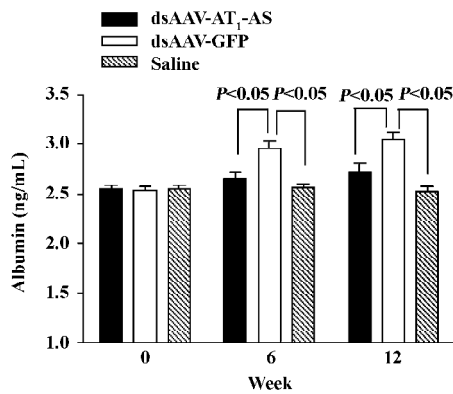
Figure 5. Levels of urine microalbumin were measured by ELISA and the data are expressed as mean±SEM (ng/mL). *n*=8.

Protective effects of AT$_1$-AS on kidneys To evaluate the beneficial effects of AT$_1$-AS gene delivery in high-salt diet rats, collagen deposit in the kidneys was examined by Sirius Red staining. In the rAAV-GFP rats, there was more intense and diverse red staining in glomerules than that found in the AT$_1$-AS-treated and normal control animals (Figure 6A–6C). The ECM accumulation was then quantified and was markedly lower in the group receiving rAAV-AT$_1$-AS (11.80%±1.22%)

than that in the rats receiving GFP vectors (16.22%±0.85%, *P* <0.05; Figure 6D). The results further indicate that AT$_1$-AS treatment significantly attenuates renal damage induced by hypertension.

Expression of rat AT$_1$-AS The efficient expression of antisense AT$_1$R mRNA is crucial for blocking AT$_1$R and therefore in the present study, the expression of the rat AT$_1$-AS was analyzed using semiquantitative RT-PCR. Total RNA was prepared from animal hearts, kidneys, and aorta 12 weeks after exposure to a high-salt diet. RT-PCR was carried out using primers specific to rat native AT$_1$R cDNA (base pair 501) rather than transfected antisense cDNA or its mRNA. As shown in Figure 7, in the salt-induced, hypertensive animals (rAAV-GFP group), the AT$_1$R mRNA level was significantly upregulated in the heart, kidney, and aorta compared with the normal control group. In contrast, rAAV-AT$_1$-AS treatment markedly downregulated the transcription of AT$_1$R in rats compared with the control groups, including both rAAV-GFP-treated and normal control rats. Furthermore, we reviewed the AT$_1$R protein expression and found that AT$_1$R was unregulated in the heart, kidney, and liver of the salt-induced, hypertensive rats; rAAV-AT$_1$-AS treatment dramatically attenuated the effect to a lower level than the normal control (Figure 7). The results suggest that the rAAV-mediated partial AT$_1$R antisense cDNA delivery was not only
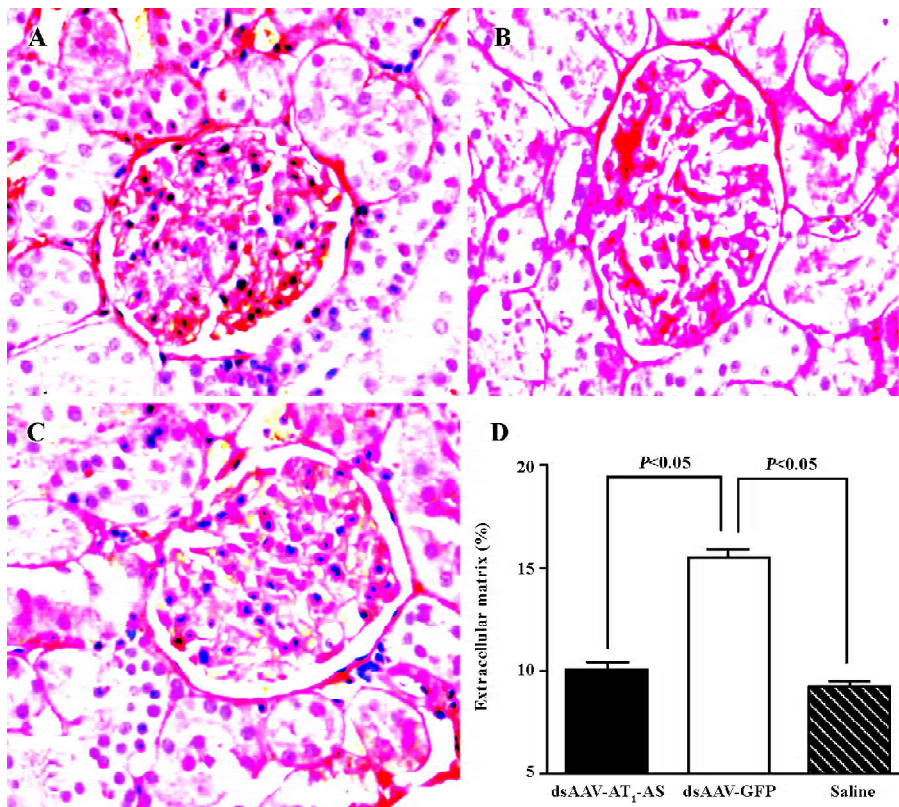

Figure 6. Sirius Red staining of kidney showed that ECM (red) was accumulated in the renal glomerulus of high-salt diet rats (B), but rAAV-AT$_1$-AS treatment attenuated ECM accumulation (A). Data are expressed as mean±SEM. *n*=8. (A, B) high-salt diet rats injected with rAAV-AT$_1$-AS and rAAV-GFP, respectively, and (C) saline-treated rats. (D) Sirius Red-stained sections were analyzed by HAIPS pathological imagic analysis system for the percentage of ECM/collagen area surface. ECM was markedly reduced in the rAAV-AT$_1$-AS-treated animals compared with the control groups.
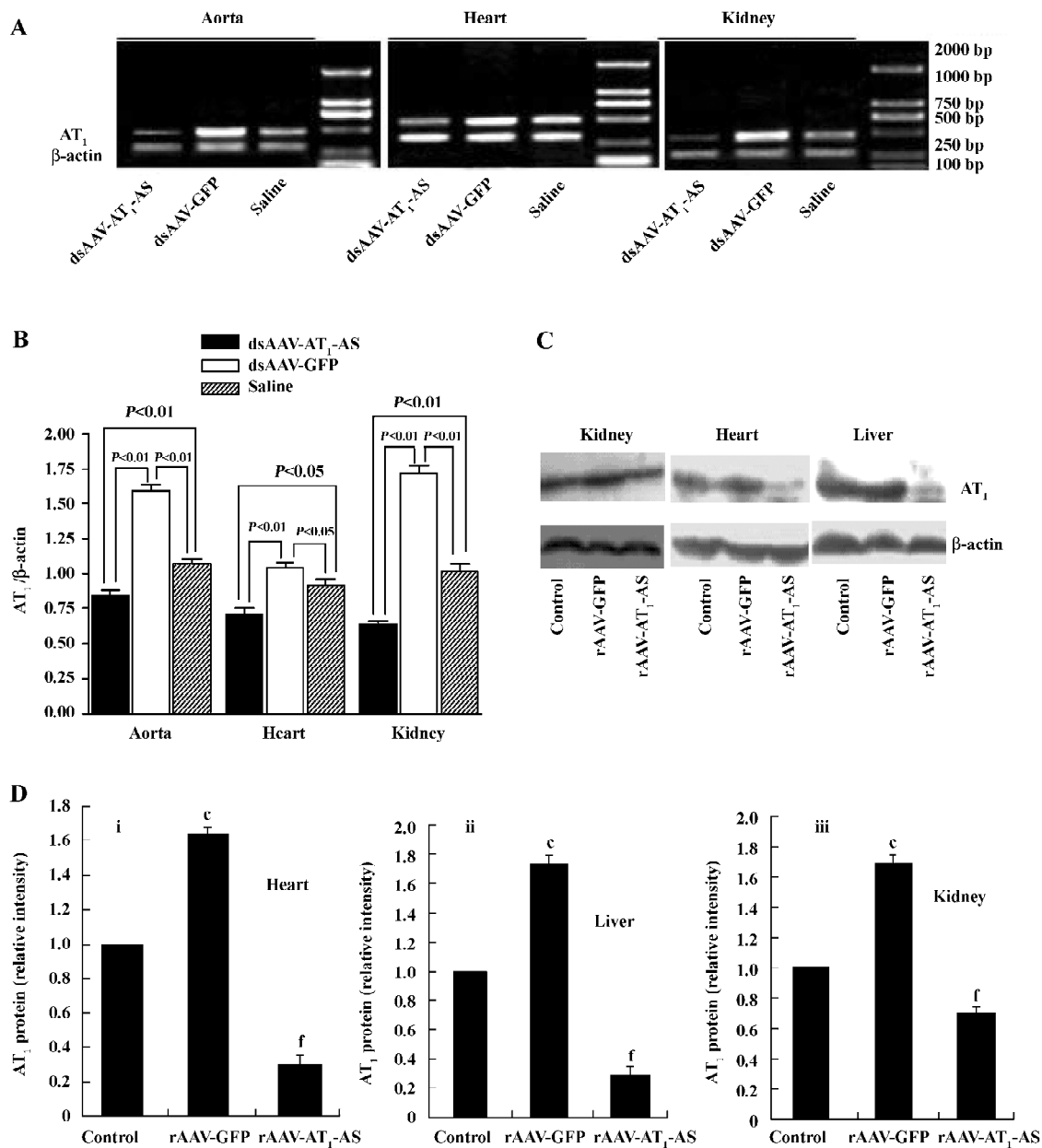
Figure 7. Detection of rat AT$_1$ by RT-PCR and Western blotting. (A) representative RT-PCR results of 3 independent experiments for AT$_1$R using the primers specific for AT$_1$, which amplify AT$_1$ rather than antisense AT$_1$ because transfected antisense AT$_1$ is not full length and the used primers are over the antisense AT$_1$. (B) average level of density scanning of RT-PCR gels from 3 independent experiments showing that AT$_1$ was significantly unregulated in the different tissues of salt-induced, hypertensive rats, but the upregulated effects were dramatically attenuated by rAAV-AS treatments. (C) representative Western blots for AT$_1$R and β-actin in the kidney, heart, and liver; (D) average relative levels of density scanning of western blots for AT$_1$R to β-actin. $^cP<0.01$ *vs* normal control; $^fP<0.01$ *vs* obesity control.

transcripted, but also efficiently blocked the transcription of AT$_1$R mRNA in the main organs, therefore preventing the development of hypertension and protecting against hypertension-induced target organ injury.

Discussion

The AAV has many advantages and properties compared with other vectors, such as retroviruses or adenovirus, including safety, the ability to infect both the dividing and

non-dividing cells, and the lack of pathogenesis or inflammatory response. In the present study, therefore, we constructed the rAAV with antisense to AT_1R. The rAAV vector has been shown to drive an efficient and prolonged transgene expression, either via persistent episomal concatamers[27,28] or by genomic integration[21,22].

It is well known that a high-salt diet is an important inducer for hypertension[29–31]. Scientists have found that high salt can induce the overexpression of AT_1R in vascular smooth muscles both *in vivo* and *in vitro*[7,8], and subsequently enhance the re-absorption of water and salt; that is why a high-salt diet induces hypertension. Moreover, high salt can induce the overexpression of AT_1 in the kidneys and promotes fibrosis and hypertrophy of the kidneys and heart[32-35]. The clinical applications of a new class of antihypertensive drug AT_1 blockades resulted in efficient reduction, organ protection, and improved long-term prognosis[36,37], which provides further support for our hypothesis. Recently, Phillips *et al* exploited the AAV and retroviral vectors to mediate AT_1-AS-induced hypotension in SHR and 2K1C rats[11,38–42], which suggests the possibility of the antisense AT_1 gene to treat hypertension through systemic delivery. However, the hypotensive effect of rAAV-mediated antisense AT_1 on high-salt diet-induced hypertension has not been documented.

In this study, we used rAAV, an efficient and rapid expression vector, to mediate partial AT_1-AS (660 base pairs) delivery. The results showed that 1 bolus injection of rAAV-AT_1-AS prevented a rise in blood pressure in high-salt diet-induced hypertensive rats in the long term, and this effect can come as early as 1 week after injection.

RT-PCR showed that systemic rAAV-AT_1-AS delivery markedly reduced the transcription level of AT_1 in the heart, aorta, and kidney of rats in the long term, which not only led to a sustained reduction in the systolic blood pressure, but also significantly decreased the urine levels of microalbumin and protected the kidneys from injury and fibrosis of glomerulus induced by hypertensive rats. A morphological analysis showed that the damage in the rAAV-GFP-treated rats also included tubular dilatation, loss of brush border in the proximal tubule, and glomerular sclerosis. Large areas of intense focal fibrosis in the cortex and the medulla vascular area, indicated by ECM staining, were observed in the control group, whereas AT_1-AS gene delivery greatly attenuated all these renal damages and reduced the size and number of protein casts present in the tubules.

Previous studies have documented that high salt plays an important role in cardiovascular remodeling. The mechanisms through which high salt induces cardiovascular and renal remodeling may be direct biological effects[33–35]. Pachori *et al* administered the retroviral vector containing

AT_1R antisense in the neonatal rat, which resulted in a significant attenuation of cardiac hypertrophy despite its failure to normalize high blood pressure in renin-transgenic rats[43]. In our study, rAAV-AT_1-AS treatment significantly attenuated cardiomyocyte diameter and fibrosis, as observed by reduced ECM staining, as well as homodynamic variable deterioration and a marked alleviation of diastolic and contractile dysfunction compared with the rAAV-GFP-treated animals. The results show that the long-term, stable expression of the AT_1-AS treatment efficiently protects the heart and improves cardiac function in salt-induced, hypertensive rats.

In summary, this study shows that the delivery of rAAV-mediated partial antisense cDNA to the AT_1R has the potential for chronically preventing hypertension in high-salt diet animals. The hypotensive effect was sustained and persisted for over 3 months in the high-salt diet rats, and protected the heart and kidney from damage. These findings suggest the feasibility of rAAV-AS for the gene therapy of hypertension and its complications. No adverse effects induced by rAAV injection were observed during the course of the study. The high level of expression from rAAV makes it attractive for human gene therapy. More experiments are needed to study the immunological aspects and the influence of the overexpression of the AT_1-AS gene on diverse biological processes before this approach is utilized clinically in the therapy of hypertension and other cardiovascular and renal diseases.

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