Effects of Losartan and Amlodipine on Left Ventricular Remodeling and Function in Young Stroke-Prone Spontaneously Hypertensive Rats

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Background: The purpose of this study was to evaluate the effects of prehypertensive losartan and amlodipine administration on left ventricular (LV) remodeling and function in spontaneously hypertensive rats-stroke prone (SHRSP).

Methods: Spontaneously hypertensive rats-stroke prone were prehypertensively administered losartan, amlodipine, or vehicle. Wistar-Kyoto rats were used as a control. Blood pressure of the rats was determined by tail-cuff method, and LV structure and function were measured by echocardiography and LV cannulation. Collagen volume fraction was analyzed by picrosirius red staining. Protein expressions of brain natriuretic peptide (BNP) and angiotensin II type 1 (AT1R) and type 2 (AT2R) receptors were determined by use of the Western blotting method.

Results: Although both drugs downregulated BNP protein expression, the LV remodeling and function were more improved with losartan than with amlodipine treatment. Losartan upregulated AT1R and downregulated AT2R protein expression.

Conclusions: Both drugs inhibited LV remodeling and improved LV function in prehypertensively treated SHRSP. Losartan provided better continued heart protection, potentially due to its persistent inhibition of AT1R and activation of AT2R in the myocardium.

Key Words: Amlodipine • Blood pressure • Heart • Losartan • Prehypertension

INTRODUCTION

Prehypertension, a transitional phase from normal blood pressure (BP) to hypertension, is defined by a systolic BP (SBP) of 120 to 139 mmHg or a diastolic BP of 80 to 89 mmHg.1 The prevalence of prehypertension in human adults is as high as 50%.2 Because left ventricular (LV) structural changes and dysfunction are already present, prehypertension is a risk factor for cardiovascular events.3-9 Early pharmacological intervention may delay BP progression and protect target organs; however, it is unknown whether these benefits show a ‘persistent effect’.10,11 Furthermore, the possibility that different classes of antihypertensive agents might exert different effects on delaying BP progression, inhibiting cardiac remodeling, and improving heart function in prehypertension has not been systematically studied.

Spontaneously hypertensive rats-stroke prone (SHRSP), which are cultivated from Wistar-Kyoto rats (WKY), tend to develop severe hypertension from 4 weeks of age.12 By about 10 to 12 weeks of age, SHRSP suffer from hypertension. Therefore, the stage from 4 to 10 weeks of
age is equivalent to the prehypertension period in SHRSP. The SHRSP model is considered to be a classic experimental approach for studying hypertension and prehypertension.12

The aims of this study were: 1) to assess the effects of losartan and amlodipine on delaying BP progression and conferring heart-protective properties to SHRSP, and 2) to elucidate whether the heart-protective effects of losartan and amlodipine show a ‘persistent effect’. To address these aims, we studied the effects of prehypertensive treatment with losartan and amlodipine on SHRSP (as a model of hypertension and prehypertension) and WKY (as control group). We examined the BP, LV mass index (LVMI), end-diastolic interventricular septum thickness (IVSTd), LV end-diastolic internal dimension (LVIDd), LV ejection fraction (LVEF), LV end-systolic pressure (LVESP), maximum rate of change of the LV pressure (dP/dtmax), collagen volume fraction (CVF), and protein expressions of LV brain natriuretic peptide (BNP), angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R) in untreated WKY and SHRSP that were prehypertensively treated with losartan, amlodipine, or vehicle.

MATERIALS AND METHODS

Animals and experimental groups

A total of 64 male, 4-week-old SHRSP and WKY (Shanghai SLAC Laboratory Animal Co. Ltd., Shanghai, China) were used in this study. Rats were randomly divided into the following 4 groups of 16 rats each: WKY, untreated WKY as a control group; SHRSP-Veh, vehicle-treated SHRSP; SHRSP-Los, SHRSP treated with 20 mg·kg⁻¹·d⁻¹ losartan (Merck, Sharp & Dohme, Whitehouse Station, NJ, USA); and SHRSP-Aml, SHRSP treated with 10 mg·kg⁻¹·d⁻¹ amlodipine (Pfizer, New York, NY, USA).13

The rats were housed 4 animals per cage in a room with controlled temperature (23 ± 2 °C) under a 12-hour light/12-hour dark cycle. Animals were allowed access to standard food and tap water ad libitum. All procedures were approved by the Animal Ethics Committee of Fujian Medical University and performed in accordance with institutional guidelines.

Experimental protocol

Rats were prehypertensively treated from 4 to 10 weeks of age and followed up until 20 weeks of age. Losartan and amlodipine were dissolved in drinking water and immediately offered to the experimental SHRSP by gavage. The concentrations of losartan and amlodipine, which were stable in water, were adjusted to maintain a constant dosing based on rat body weight, which was measured weekly.

Blood pressure measurement

Systolic BP (SBP) was measured noninvasively in unanesthetized rats every 2 weeks by the tail-cuff method with a specialized pressure transducer (PowerLab ML125/R NIBP System, AD Instruments), as described previously.14 Briefly, SBP was measured when the cuff pressure corresponded to the restoration of the first caudal artery pulse. The average of 3 consecutive readings was used for further analysis. The mean arterial pressure was determined from the SBP.

Remodeling and functional analyses of the heart

At 10 and 20 weeks of age, 8 rats were randomly selected in each group and anesthetized with intraperitoneal chloral hydrate (300 mg/100 g). Transthoracic echocardiography (GE VIVID 7 Dimension, USA) was performed with the animals in the left lateral decubitus position. A vivid echocardiographic system (GE Healthcare, USA) with a 10-MHz transducer was used to obtain M-mode echocardiograms from the long-axis view of the LV. The IVSTd, LVIDd, and LVEF were measured according to the guidelines of the American Society of Echocardiography.15 After echocardiography, rats were fixed supine on the operating table, and LV cannulation was made through the left carotid artery. The pressure signal was inputted into a data acquisition system (ML870 PowerLab 8/30, Australia), which automatically analyzed the LVESP and the dP/dtmax.

LVMI and determination of cardiac fibrosis

After all of the above analyses had been performed, the rat hearts were excised, the LVs (including interventricular septa) were weighed, and the LVMI was assessed as the ratio of the LV weight to body weight. One randomly selected LV section per animal was evaluated.
for cardiac fibrosis. Tissues from LVs were fixed in 10% formaldehyde and embedded in paraffin. Sections (6-μm-thick) were processed for picrosirius red staining. For histomorphometric analysis, 4 randomly selected fields of view per sample were carefully scanned with a light microscope (OLYMPUS CX41-32RFL, Japan; × 100 magnification) that was connected to a computer running the Image-Pro Plus 6.0 software package. As an index of cardiac fibrosis, CVF was determined as the percentage of the total myocardial tissue area that was stained by picrosirius red.

**Western blot analysis of LV BNP, AT1R, and AT2R protein expressions**

Protein expressions of BNP, AT1R, and AT2R in LV sections were determined by Western blot analysis. Briefly, 50 mg of LV muscle were homogenized in lysis buffer. An equal amount of protein was applied to SDS-polyacrylamide gels and electrophoresed under 10% reducing gel conditions. Proteins were transferred to a nitrocellulose membrane, blocked with 5% nonfat milk in TBS containing 0.05% Tween-20 (TBST), and incubated with anti-BNP, anti-AT1R, anti-AT2R (1:500, Abcam), or anti-β-actin antibody (1:1000, sc-1616, Santa Cruz Biotechnology, Dallas, Texas, USA) overnight at 4°C. After 3 washes with TBST, the membranes were incubated for 1 hour at room temperature with horseradish peroxidase-conjugated secondary antibody. The samples were washed 3 times with TBST, and labeled proteins were visualized with electrochemiluminescence (ECL; sc-2048, Santa Cruz Biotechnology) on a high-performance chemiluminescence film. Band intensity was quantified by densitometry with image analysis software. The results were expressed as the ratio of BNP, AT1R, or AT2R over β-actin.

**Statistical analysis**

Data are reported as the mean ± standard deviation (SD) or standard error of the mean (SEM). Statistical analysis was performed with the SPSS 13.0 software program. Differences between groups were compared by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD)-t-test for multiple comparisons when the p-value for the overall ANOVA was < 0.05. A value of p < 0.05 was considered to indicate statistical significance.

**RESULTS**

**Effects of losartan and amlodipine on SBP**

In all groups, the SBP results were equivalent before treatment began. In the prehypertensive phase of treatment, the SBP in the SHRSP-Veh group was increased compared to the pretreatment value (p < 0.05), whereas the SBP in WKY stayed at a nearly constant level. Both losartan and amlodipine effectively delayed BP progression in SHRSP, and no significant difference was observed between the drugs with regard to SBP. After drug intervention was stopped, the SBP showed an accelerated rise in the SHRSP-Aml group. At 14 weeks of age, the difference in SBP between the 2 groups was statistically significant, but this difference gradually weakened with time. At 20 weeks of age, the SBP values in the SHRSP-Los and SHRSP-Aml groups were statistically similar to each other, but they were lower than the SBP in the SHRSP-Veh group (p < 0.05) (Figure 1).

**Effects of losartan and amlodipine on LV remodeling and function**

At 10 and 20 weeks of age, LVMI and IVSd were much higher and LVIDd was much lower in the SHRSP-Veh group compared to the WKY group (p < 0.05). Losartan and amlodipine decreased LVMI and IVSd but increased LVIDd in SHRSP (p < 0.05). No difference in inhibitory effects was observed between these 2 drugs at 10 weeks of age. At 20 weeks of age, LVMI and IVSd were much lower and LVIDd was much higher in the SHRSP-Los group compared to the SHRSP-Aml group (p < 0.05). No difference in LVEF was found among all groups.

![Figure 1. Effects of losartan and amlodipine on systolic blood pressure. Systolic blood pressure in untreated Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats-stroke prone (SHRSP) prehypertensively treated with losartan, amlodipine, or vehicle. Values are the mean ± SEM of n = 8 animals per group. * p < 0.05 vs. WKY and drug-treated SHRSP; # p < 0.05 vs. SHRSP-Los.](image-url)
at 10 or 20 weeks of age (Figure 2).

At 10 and 20 weeks of age, losartan and amlodipine decreased LVESP but increased dP/dtmax in SHRSP (p < 0.05). No differences in LVESP and dP/dtmax were observed between the drugs at 10 weeks of age. At 20 weeks of age, LVESP was much lower and dP/dtmax was much higher in the SHRSP-Los group compared to the SHRSP-Aml group (p < 0.05) (Figure 3).

Effects of losartan and amlodipine on cardiac fibrosis

In the LVs of SHRSP-Veh animals, cardiac fibrosis was evident from the CVF in the myocardial interstitium. At 10 and 20 weeks of age, the amount of CVF in SHRSP-Veh animals was much higher than that of WKY animals (p < 0.05). Both losartan and amlodipine prevented cardiac fibrosis in SHRSP animals (p < 0.05). No difference in inhibitory effects was found between these 2 drugs at 10 weeks of age (p > 0.05), but losartan was superior at 20 weeks of age (p < 0.05) (Figure 4).

Effects of losartan and amlodipine on LV BNP, AT1R, and AT2R protein expressions

No difference in BNP protein expression was found among all groups at 10 weeks of age (p > 0.05). At 20 weeks of age, the BNP protein expression in SHRSP-Veh was significantly higher than that in WKY. Compared with SHRSP-Veh, the BNP protein expression was down-regulated in drug-treated SHRSP and was much lower in SHRSP-Los than in SHRSP-Aml (p < 0.05) (Figure 5). No differences in AT1R and AT2R protein expressions were found between SHRSP-Veh and SHRSP-Aml at 10 and 20 weeks of age (p > 0.05). However, the protein expressions of AT1R and AT2R were downregulated and upregulated, respectively, in SHRSP-Los compared to SHRSP-Veh at 10 and 20 weeks of age (p < 0.05) (Figure 6, 7).

DISCUSSION

Hypertension causes cardiac remodeling and heart failure. Results of clinical trials with amlodipine and angiotensin receptor blockers have indicated that drug-induced changes in BP significantly impact cardiovascular outcome. Different antihypertensive drugs may protect divergent target organs through the same BP re-

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Figure 2. Effects of losartan and amlodipine on left ventricular (LV) remodeling and function. LV mass index (LVMI), interventricular septum thickness (IVSTd), LV end-diastolic internal dimension (LVIDd), and LV ejection fraction (LVEF) in untreated Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats-stroke prone (SHRSP) prehypertensively treated with losartan, amlodipine, or vehicle at 10 and 20 weeks of age. Values are the mean SEM of n = 8 animals per group. *p < 0.05 vs. other groups; †p < 0.05 vs. SHRSP-Aml.
The angiotensin receptor blocker losartan and the calcium channel blocker amlodipine are widely used clinically as antihypertensive drugs to lower BP. However, which drug is better at protecting the heart remains unclear. To date, few studies have addressed the use of prehypertensive treatment with losartan or amlodipine to prevent or slow the progression of cardiac fibrosis.

Figure 3. Effects of losartan and amlodipine on left ventricular (LV) end-systolic pressure (LVESP) and maximum rate of change of the LV pressure (dP/dtmax). (A) Representative pictures of LV cannulation. (B-D) LVESP and dP/dtmax in the LVs of untreated Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats-stroke prone (SHRSP) prehypertensively treated with losartan, amlodipine, or vehicle at 10 and 20 weeks of age. Values are the mean ± SEM of n = 8 animals per group. * p < 0.05 vs. other groups; # p < 0.05 vs. SHRSP-Aml.

Figure 4. Effects of losartan and amlodipine on cardiac fibrosis. (A-B) Representative pictures of cardiac fibrosis at 10 or 20 weeks of age. (C) Collagen volume fraction in the left ventricles of untreated Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats-stroke prone (SHRSP) prehypertensively treated with losartan, amlodipine, or vehicle at 10 and 20 weeks of age. Values are the mean ± SEM of n = 8 animals per group. * p < 0.05 vs. other groups; # p < 0.05 vs. SHRSP-Aml.

Figure 5. Effects of losartan and amlodipine on left ventricular (LV) brain natriuretic peptide (BNP) protein expression. (A-B) Western blot autoradiogram of BNP protein expression in LVs of untreated Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats-stroke prone (SHRSP) prehypertensively treated with losartan, amlodipine, or vehicle at 10 weeks (A) and 20 weeks (B) of age. (C) Ratio of BNP to β-actin at 10 and 20 weeks of age. Data are the mean ± SD of n = 8 animals per group. * p < 0.05 vs. other groups; # p < 0.05 vs. SHRSP-Aml.
losartan and amlodipine to prevent cardiac remodeling and to improve heart function.

In the present study, we investigated the prehypertensive therapeutic aspects of two of the most commonly used antihypertensive drugs, losartan and amlodipine, on SHRSP. With the given dosages, the drugs were similarly effective in delaying BP progression in prehypertension. This result suggested that the use of either drug during the prehypertensive period delayed the development of hypertension. After the drug intervention was stopped, a mildly accelerated rise in SBP was observed in the SHRS-Aml group, and the SBP values in the 2 drug-treated groups were significantly different at 14 weeks of age. By 20 weeks of age, the drug-treated groups showed similar SBP values, which were still lower than those of the SHRSP-Veh animals (Figure 1). These findings suggested that although BP progression was ultimately similar in SHRSP treated with either drug, treatment with losartan led to a weaker BP progression in the short term, and BP development was somewhat accelerated initially after the discontinuation of amlodipine. The SBP values of SHRSP treated with either drug were lower than those of SHRSP-Veh. This result implied that both drugs, especially losartan, demonstrated a ‘persistent effect’ within a certain time period.

Many studies have implied that antihypertensive treatment is beneficial to prohibit LV hypertrophy and improve heart function.19,20 In this study, at 10 and 20 weeks of age, LVMI and IVSTd in the SHRSP-Veh group were much higher than those of the WKY group, whereas LVIdd was much lower. This result showed that BP progression led to LV hypertrophy. Both losartan and amlodipine treatment could inhibit LV hypertrophy, in spite of their different antihypertensive mechanisms, in terms of decreasing LVMI and IVSTd and increasing LVIdd in SHRSP. However, no difference in inhibitory effects between these 2 drugs was found at 10 weeks of age. This finding may be related to the short time of intervention.

Mechanical stress induced by cardiac overload itself could result in LV hypertrophy. As a result, BP reduction alone could partially inhibit adverse cardiac remodeling. At 20 weeks of age, in the case of similar BP, the SHRSP-Los group showed much lower LVMI and IVSTd values and much higher LVIdd values than the SHRSP-Aml group. This finding indicated that prehypertensive losartan was better than amlodipine treatment for the prevention of future myocardial hypertrophy.

No difference was found among the groups for the LVEF or BNP protein expression in 10-week-old animals; however, the LVESP was higher and dP/dtmax was lower.
in SHRSP-Veh as compared to WKY. Treatment with losartan and amlodipine decreased LVESP and increased dP/dtmax in SHRSP. Thus, although the progressively increasing BP had yet to culminate in clinical heart failure by the end of the prehypertensive period, the heart failure process was underway. The drugs showed no differences in their effects on LVESP and dP/dtmax in 10-week-old animals, suggesting that losartan and amlodipine were similarly effective in delaying the progression of potential heart failure at the end of the prehypertensive period. The differences of LVEF in each group remained insignificant. However, significant differences were observed between 20-week-old SHRSP-Veh and WKY in terms of BNP protein expression, LVESP, and dP/dtmax. Thus, the predictive value of BNP, LVESP, and dP/dtmax, especially the latter two parameters, for heart failure was better than that of LVEF, consistent with the results of a clinical study. Compared with SHRSP-Veh, the BNP protein expressions were downregulated in drug-treated SHRSP and were much lower in SHRSP-Los than in SHRSP-Aml. LVESP was much lower and dP/dtmax was much higher in the SHRSP-Los group compared to the SHRSP-Aml group. Therefore, at 20 weeks of age, the heart failure process was underway in SHRSP, and treatment with prehypertensive agents (especially losartan) could delay its progression.

In most hypertensive animal models, LV hypertrophy is reduced when BP is decreased. However, the high risk of cardiac events in hypertensive patients is determined not only by LV hypertrophy, but also by cardiac fibrosis. The differential effects of antihypertensive drugs on cardiac fibrosis have attracted substantial interest from clinical practice. Previous studies have suggested that losartan plays a role in anti-myocardial fibrosis; however, antifibrosis effects have not been reported for amlodipine. The data from this study showed that losartan and amlodipine effectively inhibited cardiac fibrosis in SHRSP. Although both drugs were useful in reducing the increased risk resulting from cardiovascular remodeling, losartan showed an additional advantage in reducing the CVF at 20 weeks of age. Therefore, compared with amlodipine, prehypertensive losartan intervention had a greater benefit in terms of preventing late cardiac fibrosis.

Angiotensin II is the most well-known peptide from the renin-angiotensin system (RAS). Its action on AT1R has been implicated in the pathophysiology of cardiovascular disease, including myocardial remodeling and heart failure, and we previously reported that AT2R can antagonize various AT1R-mediated effects. In this study, the protein levels of AT1R and AT2R in the SHRSP-Veh myocardium were significantly higher than those in WKY. These results implied that RAS activity was increased in the SHRSP myocardium, which might have led to susceptibility to cardiac hypertrophy and heart failure. No differences in AT1R and AT2R protein expressions were found between SHRSP-Veh and SHRSP-Aml at 10 and 20 weeks of age. Therefore, at the given dosage, amlodipine appeared to have had no effect on myocardial RAS, and its inhibition of LV hypertrophy and heart failure was mainly related to its ability to delay BP progression.

In contrast, the protein expressions of AT1R and AT2R were upregulated and downregulated, respectively, in 10- and 20-week-old SHRSP-Los compared to SHRSP-Veh. Therefore, beyond simply delaying BP progression, losartan appeared to inhibit RAS in the rat myocardium, an effect that was sustainable within a certain time period. This observation may serve as the main explanation for why, in the case of rats with the same BP levels, losartan and amlodipine were equally capable of inhibiting myocardial remodeling and improving heart function at the end of the prehypertensive period, whereas losartan showed superiority after drug administration was stopped.

To the best of our knowledge, this is the first study to show that prehypertensive therapy with losartan or amlodipine offers beneficial effects to the heart. With the given dosages, prehypertensive treatments with losartan and amlodipine were effective in delaying BP progression, decreasing LV hypertrophy, inhibiting cardiac fibrosis, and improving heart function at 20 weeks age. These beneficial effects persisted after drug administration was stopped, and losartan was superior to amlodipine. The mechanisms for these beneficial effects may include the ability of both drugs to delay BP progression, and the ability of losartan to inhibit AT1R and activate AR2R in the myocardium within a certain time period after drug discontinuation. These findings provide clinical implications for choosing a drug to protect against target organ damage in prehypertensive patients.
AUTHORS’ CONTRIBUTION

Jin-Xiu Lin and Li-Ming Lin designed the research; De-Hua He and Liang-Min Zhang performed the research, analyzed the data, and drafted the manuscript; and Ruo-Bing Ning, Hua-Jun Wang, and Chang-Sheng Xu participated in the design and coordination of the study and helped to revise the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No. 81070207).

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