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Early treatment with losartan effectively ameliorates hypertension and improves vascular remodeling and function in a prehypertensive rat model

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ABSTRACT

Aims: Pharmacological treatment of prehypertension may ameliorate hypertension and improve vascular structure and function. This study investigated 1) whether early treatment with either losartan or amlodipine at the onset of prehypertension can prevent hypertension and 2) whether losartan and amlodipine equally improve vascular remodeling and function in a rat model of hypertension.
Materials and methods: Stroke-prone spontaneously hypertensive (SHRSP) rats were administered losartan, amlodipine or saline for 6 or 16 weeks at the onset of prehypertension. Wistar-Kyoto rats were used as a control. All groups were observed for 40 weeks. Systolic blood pressure was measured using the tail-cuff method. Vascular structure and function were determined by microscopy and vascular ring contractility assays, respectively. Angiotensin II (Ang II) and aldosterone (Aldo) were measured by radioimmunoassays. Angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) expression was measured by western blot.
Key findings: Losartan effectively reduced progression from prehypertension to hypertension as well as vascular remodeling and improved vascular contractility in SHRSP rats. Long-term losartan (16 weeks) had greater benefits than short-term (6 weeks) treatment. Losartan increased Ang II and decreased Aldo levels in the serum and vessel walls of resistance vessels in a time-dependent manner. Losartan significantly decreased AT1R and increased AT2R vascular expression. Amlodipine had no effect on vascular AT1R and AT2R expression.
Significance: Losartan administered at the onset of prehypertension is more effective than amlodipine in ameliorating hypertension and improving vascular remodeling and function, which is likely mediated by the renin–angiotensin–aldosterone system.

1. Introduction

Vascular disease is in large part affected by elevated blood pressure (BP). Prehypertension, which is defined as the transition from homeostatic BP to hypertension, is determined by a systolic blood pressure (SBP) of 120–139 mm Hg and/or a diastolic blood pressure (DBP) of 80–89 mm Hg [1]. Evidence shows that individuals who have prehypertension are more prone to developing full hypertension and to suffer from cardiovascular events [2]. Vascular lesions are a critical pathology associated with hypertension. Therefore, reducing vascular lesion formation and delaying the onset of hypertension with antihypertensive drugs may reduce cardiovascular and cerebrovascular diseases. Vascular complications, such as decreased arterial compliance, hardening of small arteries, and vascular remodeling, are accompanied by prehypertension. Specifically, prehypertension is associated with an accelerated pulse wave velocity [3,4], an increased intima-media thickness [5], and formation of atherosclerotic plaques [6]. The SPRINT study found that among nondiabetic patients at high risk for cardiovascular events, reducing SBP to <120 mm Hg resulted in a decrease of major cardiovascular events [7]. Thus, antihypertensive therapy at the onset of prehypertension could be a reasonable approach to reduce the risk of adverse cardiovascular events. Some researchers have suggested that treating prehypertension with antihypertensive agents will provide clinical and cost-beneficial effects [8]. Our previous study found that pharmacologic treatment of prehypertension achieved significant benefits in heart and brain tissues [9–11]. However, more research is needed to further understand the effects of early treatment

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with antihypertensive drugs on preventing hypertension as well as maintaining vascular structure and function.

Stroke-prone spontaneously hypertensive (SHRSP) rats, which are derived from Wistar-Kyoto (WKY) rats, serve as a model of human prehypertension, given that the rats naturally progress to hypertension beginning at 4 weeks and continuing to 10–12 weeks of age [12]. Thus, SHRSP rats can be used an acceptable experimental model for investigating prehypertension [12].

The aims of this study were 1) to investigate if early treatment with the antihypertensive agents losartan and amlodipine during prehypertension can prevent hypertension, and 2) to elucidate whether losartan and amlodipine are equally effective at improving vascular remodeling and function in the SHRSP rat model. To address these aims, we studied the effects of losartan and amlodipine (intervention times: 6 and 16 weeks) in SHRSP and WKY rats from the onset of prehypertension. We measured SBP, vascular structure and function, as well as angiotensin II (Ang II) and aldosterone (Aldo) levels in the third branch of the mesenteric arterioles and serum in addition to the protein expression of Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R) in WKY and SHRSP rats treated with the antihypertensive agents.

2. Materials and methods

2.1. Animal treatments

SHRSP and WKY rats were purchased from the SLAK Laboratory Animal Co. Ltd. (Shanghai, China). The study included 144 male, 4-week-old SHRSP and WKY rats that were randomly assigned to one of the following six groups (n = 24 in each group): 1) WKY, untreated WKY rats as a control group; 2) SHRSP-Veh, saline group (control) (2 mL/kg); 3) SHRSP-Los6, 6-week losartan group (20 mg/kg/day); 4) SHRSP-Los16, 16-week losartan group (20 mg/kg/day); 5) SHRSP-Aml6, 6-week amlodipine group (10 mg/kg/day); 6) SHRSP-Aml16, 16-week amlodipine group (10 mg/kg/day). The rats were housed four animals per cage at 23 ± 2 °C under a 12-h light/dark cycle. Animals were allowed standard food and tap water *ad libitum*. Rats were administered the antihypertensive therapeutics or saline at 4 to 10 or 20 weeks after birth and were observed until 40 weeks of age. Losartan and amlodipine were dissolved in drinking water and administered *via* gavage one time a day to the experimental SHRSP rats. The doses of losartan and amlodipine were adjusted based on body weight, which was measured weekly. All procedures were approved by the Animal Ethics Committee of Xiamen University and performed according to institutional guidelines.

2.2. SBP measurements

As described previously, a pressure transducer (PowerLab ML125/R NIBP System, AD Instruments, Sydney, Australia) in combination with a tail cuff was used to measure and record SBP in rats at 4, 10, 16, 20, 24, 28, 32, 36, and 40 weeks of age noninvasively [13]. Briefly, the tail root was fixed to the sleeve of a pressure transducer, leaving the tail artery in close contact with the pulse sensor within the tail cuff. SBP was approximated when the tail cuff pressure corresponded to the pulse pressure in the first caudal artery. Final measurements consisted of three consecutive readings.

2.3. Structural analysis of the third branch of the mesenteric arterioles

The abdominal aorta was cannulated, and blood vessels were perfused with formalin (ZSGB-BIO, Beijing, China). The arteries were harvested in formalin and then fixed with paraffin (ZSGB-BIO). Tissues were sectioned (8 μm; 1512 microtome; Leica Microsystems, Wetzlar, Germany) prior to staining with hematoxylin and eosin (H&E; Sigma-Aldrich, St. Louis, MO, USA). Images of the third branch of the mesenteric arterioles were observed and photographed under an optical microscope (OLYMPUS CX41-32RFL, Tokyo, Japan; 100× magnification). The ratio of the blood vessel wall thickness to that of the lumen (W/L) of these arterioles was analyzed using Image Pro Plus Version 4.5 analysis software (B-Colored Multifunction Imaging Analyzing system; Media Cybernetics, Inc.).

2.4. Vascular contractility of the third branch of the mesenteric arterioles

A 3-mm section of the third branch of the mesenteric arterioles was immersed in a bath solution containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 20 mM $NaHCO_3$, and 11.1 mM glucose (Beyotime Institute of Biotechnology, Shanghai, China; 95% O_2 and 5% CO_2, saturated; 37 °C), and vasodilation and vasoconstriction were measured following treatment with gradually increasing concentrations of norepinephrine (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M; 100 μL; Tianjin Jinyao Amino Acid Co., Ltd., Tianjin, China), acetylcholine (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M; 100 μL; Sigma-Aldrich), and sodium nitroprusside (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M; 100 μL; China Resources Double-Crane Pharmaceuticals Co., Ltd., Beijing, China). A ML870 PowerLab 30 eight-channel recorder and LabChart software 6.0 (AD Instruments, Bella Vista, Australia) were used for data analysis, as described previously [11].

2.5. Serum Ang II and Aldo levels

For serum measurements, blood samples (1 mL) were extracted from rats and stored in tubes containing heparin. The samples were then centrifuged at 1000 ×*g* and 4 °C for 10 min, and the resultant serum samples were analyzed for Ang II and Aldo levels, according to the manufacturer's instructions (No. D02PJB; Beijing North Institute of Biological Technology, Beijing, People's Republic of China).

2.6. Ang II and Aldo levels in the third branch of the mesenteric arterioles

Briefly, rats were anesthetized and the third branch of the mesenteric arterioles were clipped (100 mg) and homogenized using a Polytron in 1 mL of phosphate-buffered saline (PBS) on ice. Samples of lysed tissue were boiled and then centrifuged at 1000 ×*g* at 4 °C for 10 min. Ang II and Aldo levels in the supernatant fractions were analyzed using radioimmunoassay kits, according to the manufacturer's instructions (No. D02PJB; Beijing North Institute of Biological Technology, Beijing, People's Republic of China).

2.7. Western blot for AT1R and AT2R protein expression in the third branch of the mesenteric arterioles

Briefly, 50 mg of the mesenteric arterioles was homogenized in lysis buffer. Equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a 10% reducing gel. Proteins were transferred onto a nitrocellulose membrane, blocked in 5% nonfat milk in Tris-buffered saline containing 0.05% Tween-20 (TBST; Genetech and Geneway Biotech Co., Ltd., Fujian, People's Republic of China). Membranes were incubated with the following antibodies: rabbit polyclonal anti-AT1R (1:500; ab18801; Abcam, Cambridge, UK), rabbit polyclonal anti-AT2R (1:500; ab19134; Abcam), or rabbit polyclonal anti-β-actin (1:1000; sc-1616; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 41 °C. The membrane was washed three times with TBST followed by incubation at room temperature for 1 h with a horseradish peroxidase-conjugated secondary antibody (1:1000, goat anti-rabbit IgG-CFL 488; Santa Cruz Biotechnology). The samples were washed three times with TBST, and the protein was visualized with an electrochemiluminescence reagent (sc-2048; Santa Cruz Biotechnology) using high-performance chemiluminescence film. The band intensity was measured using Image-Pro Plus 5.1 (Media Cybernetics, Rockville,