Original Article

Effects of allisartan, a new AT_1 receptor blocker, on blood pressure and end-organ damage in hypertensive animals

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Aim: To investigate the effects of allisartan, a new angiotensin II type 1 (AT_1) receptor antagonist, on blood pressure (BP) and end-organ damage (EOD) in hypertensive rats and dogs.
Methods: First, a single dose of allisartan was given intragastrically to evaluate the BP reduction in spontaneously hypertensive rats (SHRs), two kidney-one clip (2K1C) renovascular hypertensive rats and dogs, and Beagle dogs with angiotensin II-induced hypertension. Second, allisartan was mixed in rat chow for long-term treatment. After 4 months of drug administration, rats were instrumented to determine BP and baroreflex sensitivity (BRS). Observation of morphologic changes was used to estimate EOD. Third, the acute toxicity of allisartan was compared with that of losartan in mice.
Results: BP was significantly decreased after intragastric administration of allisartan in SHRs, 2K1C rats, 2K1C dogs and Beagle dogs with angiotensin II-induced hypertension. Compared with the control, SHRs that received long-term treatment with allisartan exhibited an improved BRS and organ protective effects. Mice who were administered allisartan experienced less acute toxicity than those treated with losartan.
Conclusion: Allisartan is highly effective for BP reduction and organ protection with low toxicity.

Keywords: hypertension; blood pressure; allisartan; arterial baroreflex; end-organ damage; angiotensin II type 1
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Introduction

Angiotensin II (Ang II) contributes to the development of hypertension and the pathophysiologic alterations of heart and peripheral vasculature. Blockade of the renin-angiotensin system was confirmed to be beneficial in terms of reducing cardiovascular events[1]. As a result, angiotensin II type 1 (AT_1) receptor antagonists (ARBs) are now widely used in clinical settings for the treatment of hypertension and hypertension-related cardiovascular end-organ damage (EOD)[2–4].

Losartan is the representative of ARBs, with a well-established efficacy and safety profile in hypertensive patients[5, 6]. In humans, losartan is mainly metabolized to an active carboxylic acid, EXP3174, which is a selective and noncompetitive AT_1 receptor antagonist. EXP3174 has a longer half-life and 15 times more potency than losartan *in vivo*. *In vitro*, EXP3174 is 30 times more potent than losartan for blocking AT_1 receptor. It was therefore considered that EXP3174 played a major role in the effects of losartan[7, 8].

Allisartan is a newly developed sartan-type antihypertensive drug. Losartan is catalyzed by two cytochrome P450 subfamilies, CYP2C9 and CYP3A4, into many metabolites[9], of which only EXP3174 has the antihypertensive effect. In contrast with losartan, allisartan is only converted to EXP3174 by esterase hydrolysis. Thus, it is reasonable to expect very low toxicity with allisartan. The present study was designed to evaluate the effects of allisartan on blood pressure (BP) reduction and organ protection. At the same time, a preliminary observation was made regarding the acute toxicity of allisartan.

Materials and methods

Animals and chemicals Adult female spontaneously hypertensive rats (SHRs, 200–220 g), male SHRs (320–340 g), male Sprague-Dawley rats (200–220 g), male mongrel dogs (11–13 kg), male Beagle dogs (8–9 kg) and Kunming

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(KM) mice of both sexes (19–21 g) were provided by the animal center of the Second Military Medical University. The animals were housed under controlled conditions (temperature 23–25 °C and lighting 8:00–20:00) and received standard animal chow and tap water *ad libitum*. All animals used in this study received humane care in compliance with the institutional guidelines for the health and care of experimental animals.

The allisartan and losartan used in this study were provided by Allist Pharmaceuticals, Inc, Shanghai, China.

Preparation of renovascular hypertensive model in rats Renovascular hypertensive rats (RVHRs) were prepared as previously described[10, 11]. Briefly, Sprague-Dawley rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). The left renal artery of the rat was isolated through a flank incision and a silver clip (0.2 mm internal gap) was placed on the renal artery. Four weeks after placement of the clip, BP was measured in conscious rats. Rats with systolic BP (SBP) greater than 140 mmHg were used in this study.

Preparation of renovascular hypertensive model in dogs Renovascular hypertensive dogs (RVHDs) were prepared using mongrel dogs. The dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv). The left renal artery was exposed through a retroperitoneal flank incision. The renal artery was constricted to reduce the blood flow to approximately 30%–40%[12] of the baseline value with nylon threads. Six weeks after the operation, the dogs were anesthetized for BP determination. The experiments were conducted in RVHDs with a steady SBP ≥160 mmHg.

BP measurement in conscious rats SBP, diastolic BP (DBP) and heart period (HP) of conscious rats were continuously recorded using a previously described technique[13, 14]. Briefly, rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A polyethylene catheter was inserted into the lower abdominal aorta *via* the left femoral artery for BP measurement, and another catheter was placed into the stomach *via* a mid-abdominal incision for drug administration. The catheters were exteriorized through the interscapular skin. After a 2-d recovery period, the animals were placed in individual cylindrical cages containing food and water for BP recording. The aortic catheter was connected to a BP transducer *via* a rotating swivel that allowed the animals to move freely in the cage.

Morphological examination Morphological examinations were carried out after BP recording and BRS measurement. The animals were weighed and killed by decapitation. The thoracic and peritoneal cavities were opened immediately. The right kidney, abdominal aorta, and heart were excised and rinsed in cold physiological saline. The right kidney was blotted and weighed. The left ventricle was isolated, blotted, and weighed. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 30-mm-long segment of thoracic aorta was harvested, blotted, and weighed. Ratios of ventricular weight to body weight (VW/BW), left ventricular weight to body weight (LVW/BW), right kidney weight to body weight (RKW/BW), and aortic weight to the length of the aorta (AW/length) were calculated[15-17].

Protocols

Experiment 1: Acute effects of allisartan on BP in female SHRs and RVHRs An aortic catheter was connected to the BP monitoring system. After approximately 4 h, BP signal was recorded in conscious rats for 1 h to serve as the basal value. Thereafter, a single dose of allisartan (7.5, 15, and 30 mg/kg) or losartan (30 mg/kg) was given *via* the intragastric catheter. BP was continuously recorded for 6 h. SBP, DBP, and HP were calculated hourly to serve as the data after drug administration.

Experiment 2: Acute effects of allisartan on BP in RVHDs Dogs were anesthetized and a polyethylene catheter connected to a BP transducer was inserted into the left femoral artery for BP measurement. About 30 min after catheterization, BP was recorded for 10 min to serve as the basal value. Then, a single dose of allisartan (4, 8, and 16 mg/kg) or losartan (16 mg/kg) was given intragastrically. BP was recorded for 10 min at 30, 60, 90, 120, 150, and 180 min after drug administration.

Experiment 3: Acute effects of allisartan on BP in Ang II-induced hypertension in Beagle dogs Adult male Beagle dogs were anesthetized with urethane (2 g/kg, iv). A continuous intravenous perfusion of Ang II (2 μg/kg) was given to maintain the SBP at approximately 180 mmHg. A basal BP over 10 min was recorded and a single dose of allisartan (5, 10, and 20 mg/kg) or losartan (20 mg/kg) was administered intragastrically. Thereafter, BP was recorded for 10 min at 5, 15, 30, 60, 120, 180, and 240 min after drug administration.

Experiment 4: Effects of long-term treatment with allisartan on BP, BRS, and EOD in SHRs In long-term treatment experiments, allisartan (7.5, 15, and 30 mg/kg) or losartan (30 mg/kg) was mixed into the rat chow. The rat chow containing the drug was prepared according to the previously determined food consumption and the estimated drug dose. Rats were given the rat chow containing the drug for 4 months and the control group received normal rat chow without the drug. Thereafter, BP was recorded for 4 h and baroreflex sensitivity (BRS) was measured using our previously described method[18, 19]. After the determination of BRS, rats were

killed and the morphological examination was performed.

Experiment 5: Acute toxicity of intragastric allisartan in mice Acute toxicity of allisartan and losartan was preliminarily observed. Mice were orally administered 5 mg/kg of allisartan or losartan (n=6 in each group) with a seven-day observation period. Because no deaths occurred in the allisartan group, 10 mg/kg of allisartan and 6 mg/kg of losartan were given to a fresh batch of mice (n=6 in each group). Mice were observed for seven days to determine the number of resultant deaths.

Statistical analysis Data were expressed as mean±SEM. Comparisons between values obtained in the same group before and after drug administration were made using the paired t-test. Comparisons between groups were made using unpaired Student's t-test. P<0.05 was considered statistically significant.

Results

Acute effects of allisartan on BP in SHRs Compared with the basal values, levels of SBP and DBP were significantly decreased dose-dependently by allisartan. At a concentration of 30 mg/kg, allisartan produced a similar BP reduction as losartan at 30 mg/kg. The maximal hypotensive effect was seen 3 h after the administration of allisartan or losartan. HP was not affected in any groups (Figure 1).

Acute effects of allisartan on BP in RVHRs Allisartan and losartan produced long lasting hypotensive effects in 2K1C rats. Both SBP and DBP were significantly decreased (>20 mmHg) and HP was not altered (Figure 2). The maximal hypotensive effect was seen 6 h (the last hour of our calculated time period) after the administration of allisartan or losartan.

Acute effects of allisartan on BP in RVHDs Levels of SBP and DBP were significantly decreased by allisartan (8 and 16 mg/kg) and losartan (16 mg/kg) in 2K1C dogs. The maximal BP reduction was seen 30 min after drug administration in anesthetized dogs. HP was significantly increased by allisartan and losartan at the dose of 16 mg/kg (Figure 3).

Acute effects of allisartan on BP in Ang II-induced hypertension in Beagle dogs In these hypertensive Beagle dogs, a low dose (5 mg/kg) of allisartan exhibited only a tendency in BP reduction (Figure 4). Allisartan (10 and 20 mg/kg) and losartan (20 mg/kg) significantly decreased SBP and DBP with no impacts on HP.

Effects of long-term treatment with allisartan on BP, BRS and EOD in SHRs Compared with the control group, SBP and DBP were significantly decreased in a dose-dependent manner after the administration of allisartan for 4 months in SHRs. At the same dose (30 mg/kg), the antihypertensive effect of allisartan was similar to that of losartan. In all drug-treated groups, HP was not affected. In terms of arterial baroreflex (ABR) function, BRS was markedly enhanced in all drug-treated groups. End-organ protective effects were also seen in allisartan-treated SHRs. The ventricle weight/body weight (VW/BW), left ventricle weight/body weight (LVW/BW), and aorta weight/length (AW/length) ratios were significantly decreased and the right kidney weights/body weight (RKW/BW) ratio was significantly increased when compared with untreated rats. Losartan presented similar effects on EOD as allisartan but had no effect on RKW/BW (Figure 5).

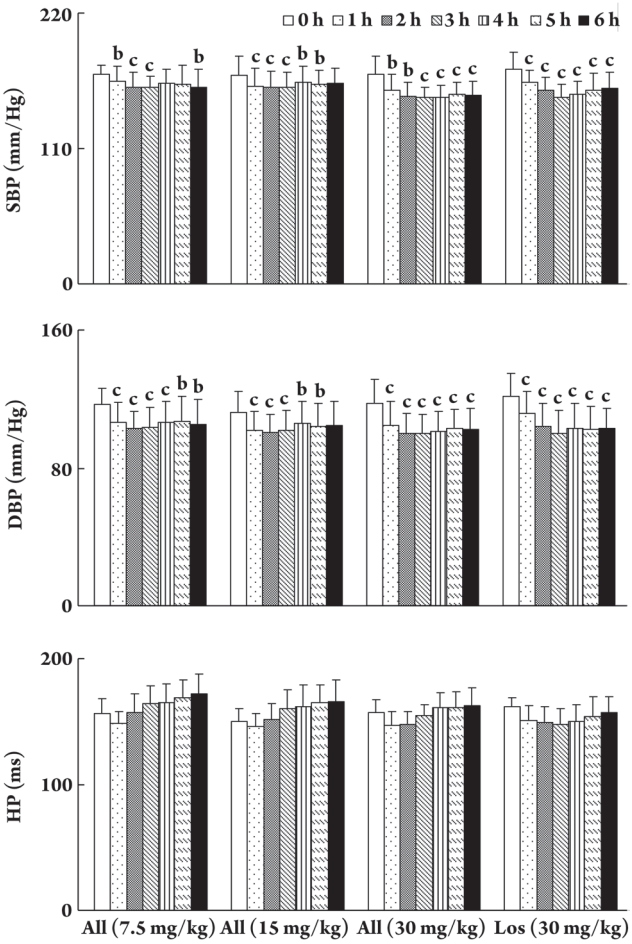

Figure 1. Effects of a single dose of allisartan or losartan on SBP, DBP, and HP in SHRs. All (7.5 mg/kg), n=9; All (15 mg/kg), n=10; All (30 mg/kg), n=11; Los (30 mg/kg), n=10. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period; All, allisartan; Los, losartan. Mean±SEM. ^bP<0.05, ^cP<0.01 *vs* basal values (0 h).

Acute toxicity of allisartan in KM mice After intragastric administration of 5 g/kg of losartan or allisartan,

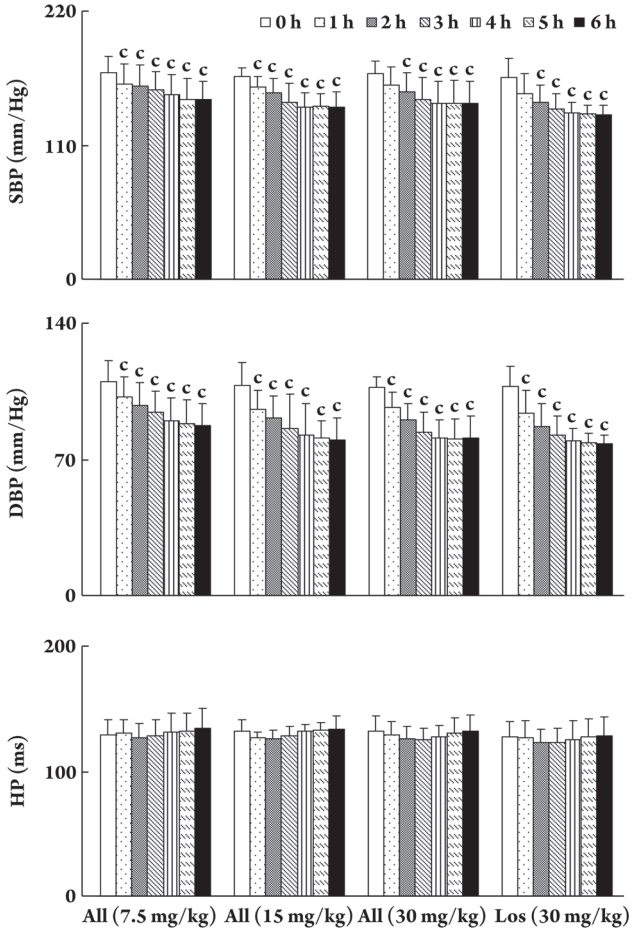

Figure 2. Effects of a single dose of allisartan or losartan on SBP, DBP, and HP in 2K1C renovascular hypertensive rats. All (7.5 mg/kg), *n*=11; All (15 mg/kg), *n*=8; All (30 mg/kg), *n*=9; Los (30 mg/kg), *n*=10. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period; All, allisartan; Los, losartan. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* basal values (0 h).

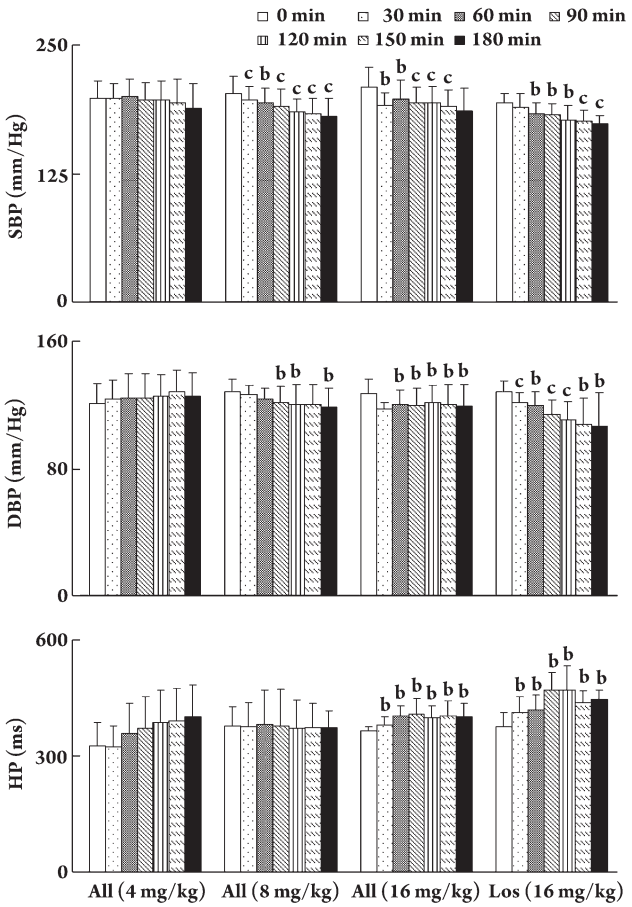

Figure 3. Effects of a single dose of allisartan or losartan on SBP, DBP, and HR in 2K1C renovascular hypertensive dogs. *n*=6 in each group. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; All, allisartan; Los, losartan. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* basal values (0 min).

the death rate was 5/6 in the losartan group and 0/6 in the allisartan group over a seven-day observation period. When the dose of losartan was increased to 6 g/kg, all 6 mice died within 30 min. However, when the dose of allisartan was increased to 10 g/kg, no mice died. These results indicated that the acute toxicity of allisartan was much less than that of losartan in mice.

Discussion

Hypertension is one of the major risk factors for the main cause of death in adult populations worldwide. If not properly treated, hypertension can lead to stroke, heart attack, heart failure and kidney disease[20–23]. Hypertension often requires lifelong treatment with one or more antihypertensive medications. Treatment with medication for hypertension can reduce the incidence of stroke (by about 35%–40%), heart attack (by 20%–25%), and heart failure (by more than 50%)[24].

Clinically, BP reduction is important but is not the final purpose of hypertension therapy. Preventing and reversing EOD is the ultimate objective in the treatment of hypertension. A high BP level induces organ damage and decreased BP can help to prevent EOD[25]. However, a high BP level is not the only determining factor of hypertensive EOD. Previous studies have demonstrated that the severity of EOD was positively related to BP variability and BRS in SHRs[26–28].

AT_1 receptor antagonists are highly effective at controlling BP and therefore are widely used in the clinic for the treatment of hypertension. In the present study, the antihypertensive effect of allisartan, a new sartan-type antihyper-

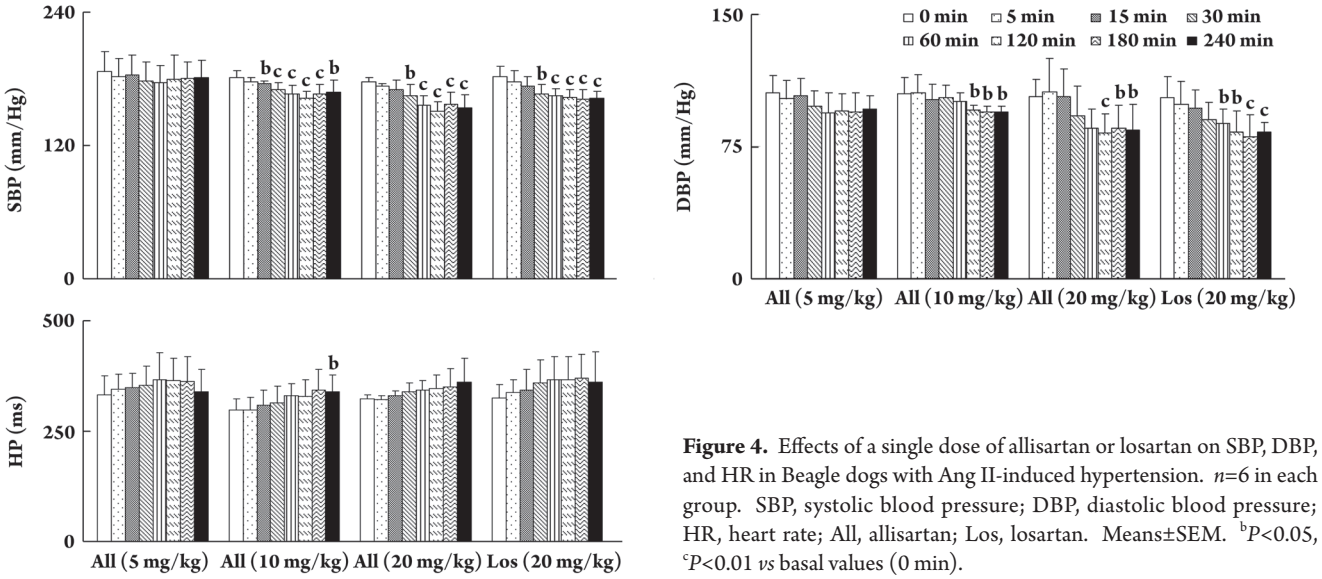

Figure 4. Effects of a single dose of allisartan or losartan on SBP, DBP, and HR in Beagle dogs with Ang II-induced hypertension. *n*=6 in each group. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; All, allisartan; Los, losartan. Means±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* basal values (0 min).

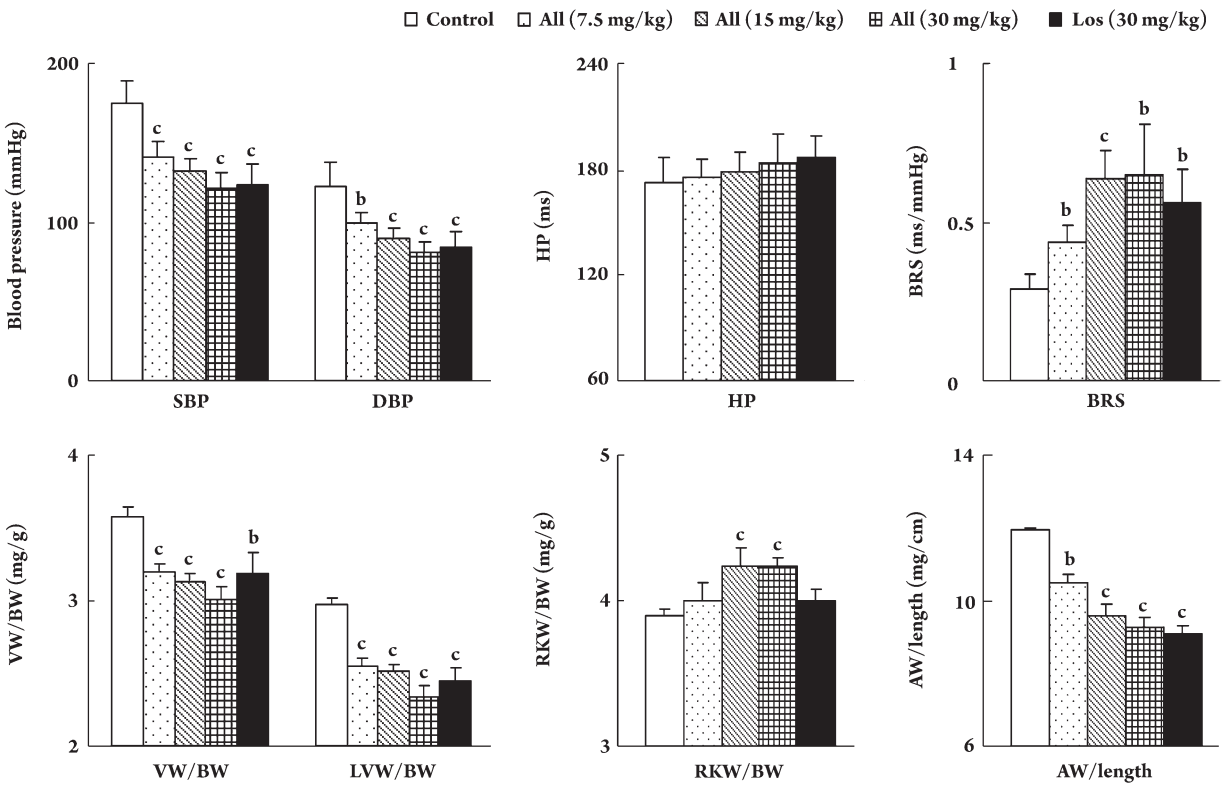

Figure 5. Effects of long-term treatment with allisartan or losartan on blood pressure, baroreflex sensitivity, and end-organ damage in SHRs. Control, *n*=12; All (7.5 mg/kg), *n*=11; All (15 mg/kg), *n*=11; All (30 mg/kg), *n*=9; Los (30 mg/kg), *n*=11. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period; BRS, baroreflex sensitivity; VW, ventricle weight; LVW, left ventricle weight; AW, aorta weight; RKW, right kidney weight; BW, body weight. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

tensive drug, was examined in different hypertensive models. The results of short-term treatment showed that allisartan reduced BP dose-dependently and presented similar anti-hypertensive effects as losartan at the same dose. With

long-term treatment, allisartan not only reduced BP but also enhanced BRS and had great organ protection effects in SHRs. Allisartan greatly reversed left ventricle hypertrophy, prevented renal cortex atrophy, and lessened thickening of the aorta. These effects were similar to or slightly greater than those of losartan at the same dose. The doses of losartan and allisartan used in this study were selected mainly according to the results of the preliminary tests (results were not shown).

In the present study, allisartan presented a different capability and tendency to reduce BP in SHRs and 2K1C hypertensive rats. Allisartan produced a long-lasting BP reductive effect in 2K1C hypertensive rats. SBP was reduced time-dependently and the maximum hypotensive effect was seen during the last hour of our calculated time period (6 h) after drug administration (Figure 2). In SHRs the maximum hypotensive effect occurred 3 h after drug administration; subsequently, the BP had a tendency to increase (Figure 1). In addition, allisartan produced a greater hypotensive effect in 2K1C hypertensive rats than in SHRs. The differences in hypotensive effects between these two animal models may be caused by different mechanism with regard to the formation of hypertension. The SHR is a genetic model of naturally developing hypertension, but the etiopathogenesis and pathogenesis remain unclear. SHR is used as a model for essential hypertension in humans[29, 30]. The activity of the renin-angiotensin system (RAS) is normal or low in SHR[31]. However, the BP can be lowered by the commonly used ACE inhibitor and AT_1 receptor blockers[32, 33]. This is consistent with our present results that allisartan reduced BP in SHR. The 2K1C hypertensive rat is a renovascular hypertensive model, which is characterized by elevated Ang II expression caused by ischemia in clipped kidney and shear stress in nonclipped kidney. The activity of RAS plays a key role in the development and maintenance of high BP through the production of Ang II[34, 35]. Ang II has a high affinity for AT_1 receptors, which are responsible for Ang II-induced hypertension. Thus, the AT_1 receptor blocker, allisartan, exhibited a more powerful effect on BP reduction in 2K1C hypertensive rats than in SHRs in our current study, suggesting that allisartan is more suitable for the treatment of renovascular hypertension.

Toxicity is another important index in evaluating a drug. Because antihypertensive treatment may be lifelong, the safety of an antihypertensive drug is important. Allisartan is a new sartan-type antihypertensive drug, which has many similarities to losartan. The acute toxicity of allisartan was compared with that of losartan after pharmacodynamic studies and found to be much lower.

In conclusion, allisartan is highly effective in BP reduction and organ protection with low toxicity. It may become a potent antihypertensive drug in the future.

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Author contribution

Ding-feng SU and Jian-guo LIU designed research; Ming-yue WU, Ai-jun LIU and Xiu-juan MA performed research; Xia TAO contributed new analytical tools and reagents; Ming-yue WU and Chu YANG analyzed data; Ming-yue WU and Xiu-juan MA wrote the paper.

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