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Beneficial Effects of the Combination of Amlodipine and Losartan for Lowering Blood Pressure in Spontaneously Hypertensive Rats

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A combination of antihypertensive agents can better control blood pressure and reduce the number and severity of side effects than a monotherapy. Since both CCBs (calcium channel blockers) and ARBs (angiotensin II receptor type-1 blockers) are current and effective antihypertensive drugs, this study assessed the synergistic antihypertensive effects as well as the optimal combination ratio of these two drugs. Amlodipine (3 mg/kg) or losartan (30 mg/kg) alone or a combination of each drug at a ratio 1:10 and 1:20 was administered orally to spontaneously hypertensive rats (SHR). A four-week treatment of either 3 mg/kg amlodipine or 30 mg/kg losartan alone decreased the systolic blood pressure (SBP). However, their combination significantly lowered the SBP from the 3rd week, and there was a positive correlation between this reduction in blood pressure and the improvement in arterial endothelium-dependent relaxation. In addition, the combination therapy (1:20) decreased both the cardiac mass and left ventricular weight to a greater extent than with either amlodipine or losartan alone. The collagen content in the cardiac tissue was also significantly lower after the 4-week combination therapy (1:10). These results suggest that the combined use of amlodipine and losartan might be more effective in treating hypertension than a monotherapy.

Key words: Amlodipine, Losartan, Combination, Spontaneously hypertensive rat, Systolic blood pressure

INTRODUCTION

Hypertension is one of the major risk factors for cardiovascular complications and can cause cerebrovascular and renal damage if not properly controlled. The optimal blood pressure is < 120/80 mmHg and the recommended blood pressure goal is <130/85mmHg, which is lower than the previous limit of 140/90mmHg (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003; Chobanian et al., 2003). However, in achieving these more strict blood pressure limits, there are some limitations in monotherapy within the framework of efficacy. In practice, < 50% of hypertensive patients reach their optimal blood pressure with monotherapy using diuretics, β-blockers, ACEI (angiotensin-converting enzyme inhibitor), and CCB (calcium channel blocker) (Materson et al., 1993). Moreover, the response rate can fall to almost 10% when the half-maximal doses are used (Lindholm et al., 2002).

Therefore, various combinations of antihypertensive agents have been marketed, and recent clinical guidelines for the treatment of hypertension recommend a combination therapy for appropriate blood pressure control (Chobanian et al., 2003; Whitworth, 2003; Gavras and Rosenthal, 2004; Rosenthal and Gavras, 2006). The advantage of a first-line treatment for hypertension with combination therapy compared with monotherapy are as follows: 1) there is some synergistic action when using two or more drugs that have different mechanisms of action – i.e. the blood pressure is controlled more effectively and the hypertension related complications are prevented, and 2) the number and severity of side effects can be reduced using low-dose range, thereby optimizing the patient's compliance (European Society

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of Hypertension-European Society of Cardiology Guidelines Committee, 2003).

Among the many combinations of antihypertensive drugs, it has been demonstrated that the calcium channel blocker (CCB) and angiotensin II receptor type-1 blocker (ARB) combination is useful for lowering blood pressure and preventing an impairment of various organs such as the heart and kidney (Yao et al., 2003a; Okuda et al., 2005; Yao et al., 2003b; Kohlmann et al., 2006). Therefore, a combination of dihydropyridine CCB (Amlodipine) and ARB (Losartan) is expected to be a promising option for the treatment of hypertension. This study assessed the therapeutic value of an amlodipine and losartan combination on both blood pressure reduction and organ protection in a SHR model.

MATERIALS AND METHODS

Animals

Male spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats, 12 weeks of age (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu, Japan), were used in this study. The rats were kept at 23±2°C under a 12-h light–dark cycle, and allowed access to a commercial chow (5L79 diet, Labdiet, Indiana, USA) and tap water *ad libitum*. This study was performed in accordance with the institutional Standard Operation Procedure for Animal Care and Experiments (SOP-ANC) of the Dong-A Pharmaceutical Company and with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Drugs

Amlodipine orotate (Lot No. 6301) and losartan (Lot No. 6501) were synthesized at Dong-A Pharmaceutical Company Research Laboratories. The other chemicals were of reagent grade and purchased from commercial sources.

Experimental protocols

The rats were divided into 6 treatment groups (5 rats in each group) as follows: (i) untreated age-matched WKY rats; (ii) vehicle-treated SHR-SP used as the control group; (iii) amlodipine orotate 3 mg/kg/day; (iv) losartan 30 mg/kg/day; (v) amlodipine 3 mg/kg/day combined with losartan 30 mg/kg/day (1:10 ratio); (vi) amlodipine 3 mg/kg/day combined with losartan 60 mg/kg/day (1:20 ratio). The drugs were suspended in 1% hydroxypropylmethylcellulose (HPMC) in a volume of 5 mg/kg body weight and administered orally over a four-week period.

Body weight and blood pressure measurement

The body weight and systolic blood pressure (SBP) were measured at weekly intervals from the baseline period until sacrifice. The SBP was monitored indirectly using tail-cuff plethysmography (L120: IITC Life Science, San Diego, USA). Briefly, the rats were kept in a box warmed to 38×C for 10 min and then placed in a holder. The SBP was measured as the internal pressure of a latex cuff fixed around the tail of the animals, and the average of three readings was used for further analysis.

Endothelial function analysis

The rats were sacrificed at the end of the treatment period, and the thoracic aorta was excised immediately and placed in a modified Krebs-Henseleit solution, containing (mmol/L) : NaCl 118.0, KCl 45, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.0. The aortic tissue was dissected from the adherent fat and connective tissues, and the arteries were cut into 2-3 mm rings. Extreme care was taken to avoid stretching the arteries during the dissection. The contractile force was measured by mounting the aortic rings in 15 ml organ baths containing Krebs-Henseleit solution under a basal tension of 2 g and attaching then to a force displacement transducer (GRASS TELEFACTOR, Astro-Med, Inc., USA). The data was recorded on a polygraph (ML785 PowerLab, ADInstruments). The tissue bath was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Each preparation was allowed at least 60 min to stabilize before performing the experiment. During this period the incubation medium was changed every 15 min. The presence of an endothelium was confirmed by the occurrence of relaxation in the rings contracted with norepinephrine (10^{-5} mol/L) in response to acetylcholine (10^{-6} mol/L). After washing and re-equilibration, the aortic rings were precontracted with norepinephrine (10^{-5} mol/L). When the contractile response was stable, the endothelium-dependent relaxation was measured by adding acetylcholine at increasing cumulative concentrations (10^{-9} to 10^{-5} mol/L). The relaxation is expressed as the percentage decrease in the maximum tension obtained using the norepinephrine-induced contraction.

Evaluation of cardiac hypertrophy

At the end of experiment, all the animals were sacrificed by a cervical dislocation. The isolated heart was washed out with warmed saline to eliminate the excess blood, and then fixed in 10% neutralized formalin solution. After fixation, the heart

was weighed, and dissected into the left ventricle, inter ventricular septum and the right ventricle, which were then weighed individually. The portion of the middle one-third near the apex was embedded in paraffin and stained using Masson's trichrome technique (Sigma Diagnostics, St. Louis, Mo. USA) to determine the collagen level. The areas of collagen were determined and added to obtain the total square using an Axioskop 2 microscope (Zeiss Co. Ltd., Germany) and an image-analyzer (Image-pro plus, media cybernetics USA).

Statistical analysis

The data is expressed as the mean ± SD. One-way analysis of variance (ANOVA) followed by a Dunnett' test was used to determine the significance of the difference between the different groups. A p value <0.05 was considered significant.

RESULTS

Effects of amlodipine and losartan alone, and their combination on the systolic blood pressure

Fig. 1 shows the hypotensive effects in SHR of each drug and their combination. At the beginning of the experiment, the SBP in the SHR was almost double that in the normotensive WKY rats. Before administering the drugs, there were no significant differences between the SHR groups. Although 3 mg/kg amlodipine and 30 mg/kg losartan alone lowered the SBP after 3 and 4 weeks treatment respectively, their combination significantly lowered the SBP from the 3rd week. Therefore, the combination of almodipine and losartan decreased the blood pressure of the SHR more potently than either amlodipine or losartan alone.

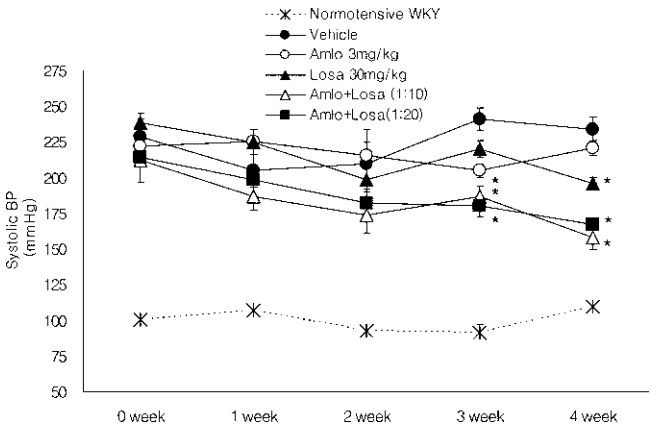

Fig. 1. The changes in blood pressure by the amlodipine and/or losartan treatment. The systolic pressure was measured every week for 4 weeks. *significantly different from the vehicle-treated group, P<0.05. The data is expressed as the mean±SD.

Effects on Ach-induced aorta relaxation

Fig. 2 shows the vasodilatation response of the thoracic aorta to cumulative doses of acetylcholine. The EC_{50} of the SHR rats was almost 45 times higher than that of the normotensive WKY rats (1.483 μmol/L vs 0.033 μmol/L, respectively). Treatment with losartan alone or combined with amlodipine significantly ($p<0.05$) improved the endothelium-dependent relaxations compared with the vehicle treated animals. However, there was a similar level of endothelium-dependent relaxation between the combination ratios of these two drugs (EC50 of Ach in 1:10 and 1:20 combination ratio was 0.226 and 0.209 μmol/L, respectively).

Effects on body weight and cardiac hypertrophy

Table I shows the body weight, heart and left ventricular weight of all the experimental groups. There were no significant differences in body weight observed during the entire experimental period. The relative heart and left ventricular weight were significantly ($p<0.05$) higher in the SHR rats (32 and 42%, respectively) than in the normotensive rats. This increase in heart weight was attenuated significantly by losartan alone and when combined with amlodipine (1:20 ratio). The degree of decrease in the cardiac weight was 14.4% in losartan alone

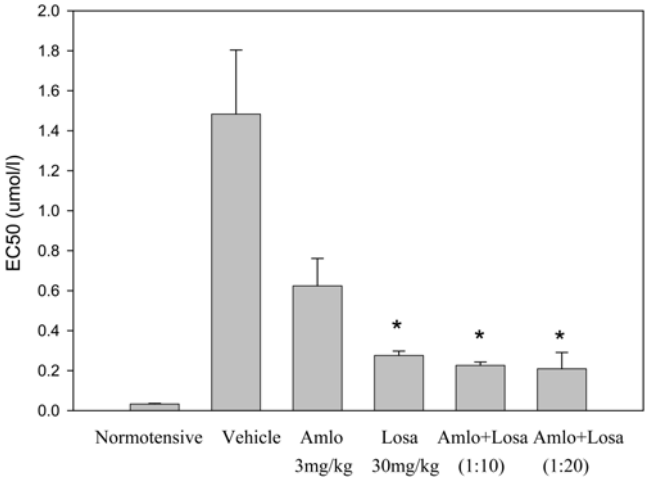

Fig. 2. Effect of amlodipine and/or losartan on the acetylcholine-induced thoracic aorta relaxation. *significantly different from the vehicle-treated group, P<0.05. EC50, effective concentration of acetylcholine that induced a 50% relaxation of the pre-contracted thoracic aorta. The data is expressed as the mean±SEM.

Table I. Body weight, cardiac weight, and left ventricular weight in each group of SHR

	BW (g)	Heart (mg/g BW)	Left ventricle (mg/g BW)
Normotensive	370.8±19.7	3.49±0.05	1.54±0.16
Vehicle	337.8±15.6	4.59±0.39[a]	2.19±0.14[a]
Amlo 3 mg/kg	339.8±17.2	4.38±0.28[a]	2.28±0.20[a]
Losa 30 mg/kg	344.6±12.0	3.93±0.06[a,b]	1.95±0.11[a]
Amlo 3 mg/kg + Losa 30 mg/kg (1:10)	326.0±18.6	4.17±0.12[a]	2.08±0.14[a]
Amlo 3 mg/kg + Losa 60 mg/kg (1:20)	327.0±15.8	3.75±0.16[a,b]	1.85±0.07[b]

Amlo, amlodipine; Losa, losartan; BW, body weight.
[a] significantly different from normotensive rats, $p<0.05$.
[b] significantly different from vehicle-treated group, $p<0.05$.

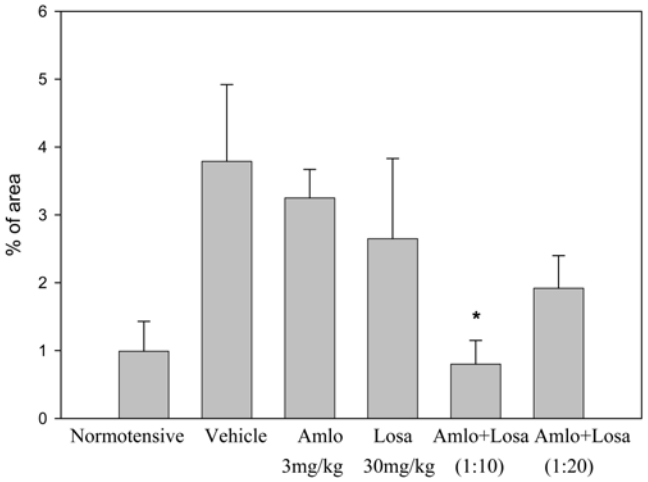

Fig. 3. Effect of amlodipine and/or losartan on the cardiac collagen content. The results are presented as a percentage of the collagen positive area per total area. *significantly different from the vehicle-treated group, $P<0.05$.

group and 18.3% in amlodipine plus losartan (1:20) group. In addition, only the combination of amlodipine with losartan (1:20) treatment significantly (15.5%, $p<0.05$) lowered the relative left ventricular weight compared with the vehicle.

Effects on cardiac morphometry

Fig. 3 shows the changes in the collagen content in the cardiac tissue. The collagen content of the vehicle treated group was 4 times higher than in the normotensive WKY rat. Amlodipine combined with losartan (1:10 ratio) significantly ($p<0.05$) decreased this increase in collagen content to almost the level of the vehicle group. Fig. 4 shows the representative photographs of the left ventricle from the normotensive WKY rats, vehicle treated SHR, and SHR treated with amlodipine combined with losartan (1:10).

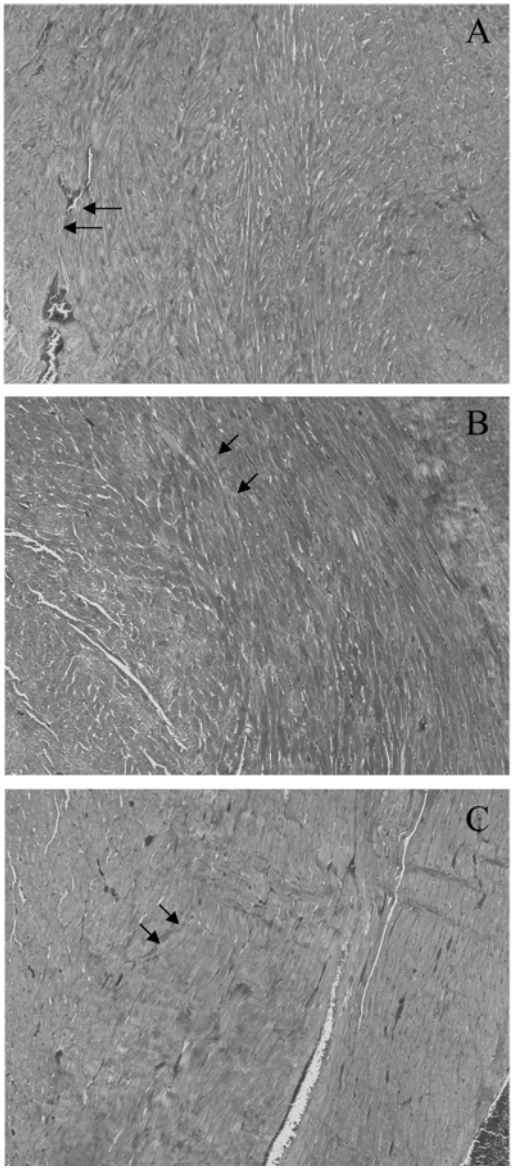

Fig. 4. Representative cardiac collagen content in each group. A, normotensive WKY rat; B, vehicle-treated SHR; C, Amlo 3 mg/kg combination with Losa 30 mg/kg (1:10). The interstitial collagen fibers staining blue (arrows) were combined using a computer-assisted analysis system.

DISCUSSION

In a previous study, the efficacy and tolerability of a fixed-combination of amlodipine and losartan (1:20) was evaluated in 204 Brazilian patients (Kohlmann et al., 2006). Their study demonstrated that the combined use of amlodipine and losartan has a high antihypertensive efficacy, which can be sustained for a long-time. In Indian post-marketing surveillance recorded from 728 patients, most of the patients prescribed the losartan-amlodipine combination (93.8%) showed a good to excellent response (Gokhale et al., 2002).

Based on this clinically proven combination ratio, the present study further evaluated the combination ratio to determine the rationale for the most appropriate combination ratio. These observations provide evidence that the lower losartan combination had as much effect on lowering the blood pressure and alleviating the related symptoms as the established combination ratio. These results also reconfirmed that the amlodipine-losartan combination has a larger hypotensive effect in the hypertension animal model compared with either of the antihypertensives used alone. In addition, the combined treatment with amlodipine and losartan also alleviated the cardiac hypertrophy and endothelial dysfunction more effectively than the monotherapy.

Recent studies have shown that amlodipine reduces the extent of left ventricular hypertrophy and regressed collagen deposition (Tomassoni et al., 2003), which persisted after treatment withdrawal (Sevilla et al., 2004). This beneficial effect of amlodipine can be explained by the inhibition of calcium entry in the myocytes (Sevilla et al., 2004). Losartan also has a similar beneficial effect on the heart, which affects not only the myocytes but also the production of collagen and other matrix tissues (Makino et al., 1997).

Evidence from experimental and clinical studies suggests that a multitherapy-based antihypertensive regimen, which includes inhibitors of the renninangiotensin system and calcium channel antagonists, can improve the endothelial function (Schulman et al., 2005). It was reported that in addition to its well-known antihypertensive effect, amlodipine improves the endothelial function in patients with arterial hypertension (On et al., 2002). Basically, the molecular mechanism of this effect can be explained by its antioxidative effect (Franzoni et al., 2004). Amlodipine prolongs the half-life of NO through its reactive oxygen species (ROS) scavenging capacity, accompanied by the NO formation enhancing activity (Berkels et al., 2004), which can improve the NO bioavailability. The improvement in the endothelial function by losartan appears to be dependent on the increased relaxant factor such as NO availability (Maeso et al., 1998) similar to that of amlodipine, and the normalization of the constricting factor such as endothelin-1 content (d'Uscio et al., 1998).

Based on these results, it is concluded that the combined treatment of amlodipine and losartan is more effective in treating hypertension and cardiovascular disease than a monotherapy. Furthermore, it was confirmed that a lower losartan ratio (1:10) than the clinically proven combination ratio has a similar effect in the treatment of cardiovascular hypertrophy and remodeling.

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