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Original

Protective Effects of Olmesartan and Azelnidipine against Cardiovascular Organ Injuries in Spontaneously Hypertensive Rats

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SUMMARY

In the treatment of hypertension, care should be taken for preventing of hypertensive organ injuries as well as lowering blood pressure to the adequate level in order to reduce the risk of cardiovascular diseases. The purpose of this study is to examine the effects of angiotensin II receptor blockers (ARB), calcium channel blockers (CCB) and their combination on the development of cardiovascular organ injuries in spontaneously hypertensive rats (SHR). Four groups of male 8-week-old SHR (n＝9 each) were given vehicle (control), 10 mg/kg azelnidipine (AZL), 10 mg/kg olmesartan (OLM, n＝9), or the combination of AZL and OLM (5 mg/kg each) for 12 weeks, and their effects on cardiovascular organ injuries were evaluated. Tail-cuff blood at 12 weeks was similarly lowered by AML, OLM and the combination therapy (148, 143 and 143 mmHg, respectively) as compared with the control SHR (198 mmHg). Pulse rate was significantly less in the AZL group but not in the OLM group or the combination therapy group than in the untreated control group (－27, －12, ＋6 bpm, respectively). The cardiac ventricular weight (AZL －12%, OLM －15%, combination －18% vs. control) and aortic thickness (AZL －17%, OLM －16%, combination －19% vs. control) were reduced by similar extents in the three groups given antihypertensive treatments. Regarding the myocardial fibrosis, left ventricular hydroxyproline content was reduced in the OLM and the combination groups but the change was not significant in the AZL group (AZL －14%, OLM －30%, combination －27 % vs. control). In the echocardiographic evaluation of cardiac function, the index of left ventricular diastolic function is significantly improved in the OLM and the combination groups but not in the AZL group, while the index of systolic function was not different between the four groups. It is suggested that the antihypertensive therapy including ARB is superior to the monotherapy by CCB in preventing the myocardial fibrosis and preserving the left ventricular diastolic function.

Key Words : hypertension, spontaneously hypertensive rat, angiotensin II receptor antagonist, calcium channel blocker, left ventricular hypertrophy, arteriosclerosis

INTRODUCTION

The ultimate goal of antihypertensive therapy is not only to normalize the blood pressure level but also to prevent end-organ damage, such as cardiac hypertrophy and renal dysfunction, and to prevent the cardiovascular disease, such as stroke and myocardial infarc-

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tion. Therefore, the efficacy of antihypertensive drugs on inhibition of hypertensive tissue injuries and preservation of cardiovascular organ function has to be taken into consideration in order to achieve maximal improvement of long-term prognosis of hypertensive patients. In the real world clinical practice, angiotensin II receptor antagonists (ARB) and long-acting calcium channel blockers (CCB) are widely used for the treatment of hypertensive patients because these classes of antihypertensive drugs have few chances to cause adverse effects. In addition, CCB exerts consistently certain hypotensive effects by dilating arteries directly and ARB is expected to have protective effects against the cardiac and the renal injuries partially by the mechanism independent of blood pressure lowing[1,2]. Thus, it is obviously important to collect the information as to the effects of CCB and ARB on the development and the progression of hypertensive injuries in cardiovascular organs such as the heart, the kidneys and the arteries.

It should be also kept in mind that generally only one third of hypertensive patients achieve the blood pressure level lower than 140/90 mmHg with a single antihypertensive agent irrespective of the class of drug used and two thirds of patients require a combination of two or more drugs in order to obtain adequate blood pressure reduction[3,4]. Therefore, it is also a matter of concern to delineate the therapeutic efficacy of combination therapy especially with ARB and CCB in terms of protecting cardiovascular organs from hypertension. In this experimental study, the efficacies of CCB, ARB and their combination therapy were examined and compared focusing on the inhibition of cardiovascular organ injuries in spontaneously hypertensive rat (SHR) which is assumed to be an appropriate animal model of human essential hypertension.

METHODS

Treatment of rats

Male 8-week-old SHR (n=36) were purchased from Charles River Japan (Atsugi, Kanagawa, Japan). They were fed standard chow and tap water, and were housed in a temperature- and light-controlled room throughout the study period. They were divided into 4 groups of 9 SHR each. As a dihydropyridine derivative CCB, 10 mg/kg azelnidipine (AZL) suspended in 0.5 ml of 0.5% methyldellulose was given once daily by gastric tube in morning hours to 9 rats. As an ARB, 10 mg/kg olmesartan (OLM) was given in the similar way to another 9 rats. In addition, 5 mg/kg AZL and 5 mg/kg OLM were simultaneously given in 9 rats of the combination therapy group, and 9 rats of the control group were given vehicle.

The treatments were continued for 12 weeks and the systolic blood pressure was measured biweekly during the study period using the tail-cuff method. At the end of 12-week period, the rats were evaluated for the physiological, biochemical and histological evaluations described below. The experiments were performed in accordance with the institutional guide for care and use of laboratory animals, and the study protocol was approved by the institutional animal research committee.

Echocardiography

After 12 weeks, transthoracic echocardiographic studies were performed under light anesthesia with intraperitoneal injection of ketamine HCl (10 mg/kg) and xylazine (10 mg/kg). Two-dimensional echocardiography and M-mode tracing were recorded at the level of the papillary muscles using a Toshiba (Tokyo, Japan) SSH-260A unit with a 7.0 MHz transducer placed on the shaved left hemithorax of the rats in the left decubitus position. M-mode measurements included left ventricular end-systolic and end-diastolic diameters (LVDs, LVDd), end-diastolic left ventricular posterior wall thickness (PWT), and interventricular septal thickness (IVST). Midwall fractional shortening (mFS) was calculated as follows[5,6] : $mFS = [(LVDd + IVST/2 + PWT/2) - \{(LVDd + IVST/2 + PWT/2)^3 - LVDd^3 + LVDs^3\}^{1/3}]/(LVDd + IVST/2 + PWT/2)$. The values of mFS were multiplied by 100 and expressed as % values.

Pulsed-wave Doppler spectra of mitral inflow velocities were recorded from the apical 4-chamber view with the sample volume placed near the tips of the mitral leaflets and adjusted to the position where the velocity was maximal and the flow patterns were laminar. The Doppler spectra were recorded on paper at 100 mm/s and analyzed off-line to determine peak early diastolic filling velocity (E) and peak filling velocity at atrial contraction (A). The heart rate was main-

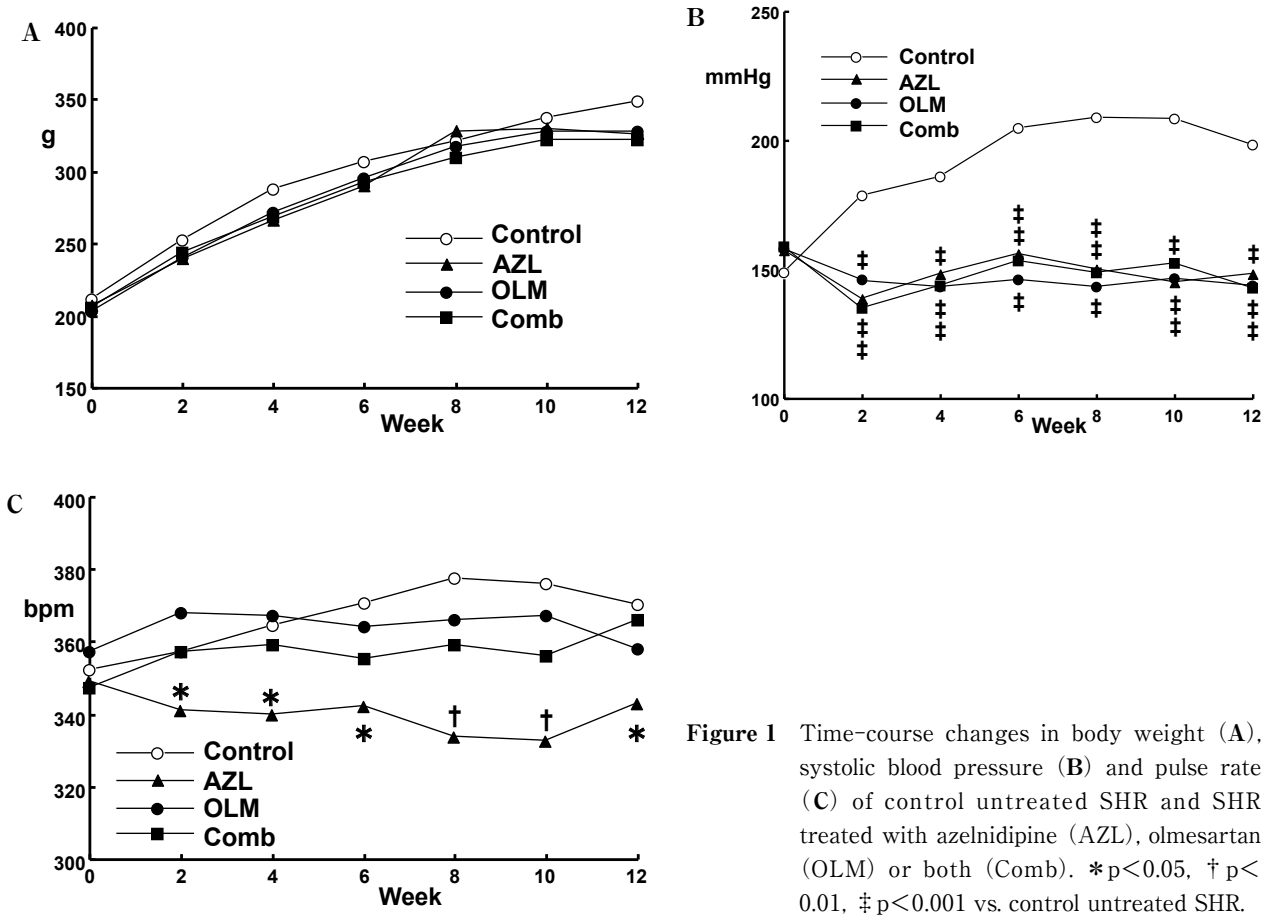

Figure 1 Time-course changes in body weight (**A**), systolic blood pressure (**B**) and pulse rate (**C**) of control untreated SHR and SHR treated with azelnidipine (AZL), olmesartan (OLM) or both (Comb). ＊p<0.05, † p< 0.01, ‡ p<0.001 vs. control untreated SHR.

tained around 300 bpm with the anesthesia during the recording, which enabled the separation of E and A wave peaks in each rat. Measurements represent the mean of at least 3 consecutive cardiac cycles, and the E/A ratio was used for the evaluation of left ventricular diastolic function[7].

Biochemical Assay

After performing the echocardiogram, blood samples were drawn from the inferior vena cava, transferred into ice-cooled tube containing 1 mg/ml EDTA and centrifuged at 4℃ to obtain plasma. Plasma renin activity and concentrations of angiotenisin II and aldosterone were measured by respective radioimmunoassays using the commercial kits (SRL, Inc., Tokyo, Japan).

A portion of the left ventricular free wall tissue was homogenized in ten equivalent volumes of saline. A 0.5 mL aliquot of the homogenate was then mixed with 36% hydrochloride and heated to 100℃ for 20 hours. Next, the mixture was centrifuged at 1,500 g for 30 minutes, and a 0.1 mL aliquot of the supernatant was mixed with 1.5 mL of 0.3 N hydroxylithium. Hydroxyproline content in the reaction product was determined by high-performance liquid chromatography, and the value was expressed relative to tissue weight[8].

Histological Examination

The cardiac ventricles and descending thoracic aorta were excised and weighed. The weight of cardiac ventricles was corrected with the body weight and the weight of aorta was expressed as weight per unit area. The upper half of cardiac ventricles were fixed in neutral-buffered 8% formaldehyde solution and embedded in paraffin. The 2-mm sections were cut for histological examination which was conducted in a blind manner. The sections were stained with Masson trichrome and the fibrosis of the left ventricular wall was evaluated. The area stained in blue with aniline was quantified in ten randomly-selected high-power fields (x200) using a computer system (Image Quest, Hamamatsu Photonics ; Hamamatsu and MacScope, Mitani Co., Fukui, Japan), and the average percent value was

Table 1 Physical measurements at the end of 12-week study period.

Parameter	Control (n=9)	Azelnidipine (n=9)	Olmesartan (n=9)	Combination (n=9)
Body weight, g	337±7	327±7	326±7	322±8
Systolic blood pressure, mmHg	198±5	148±3‡	143±3‡	143±3‡
Pulse rate, bpm	370±10	343±7*	358±9	376±10

Values are means±SE. * $p<0.05$, ‡ $p<0.001$ vs. control untreated SHR.

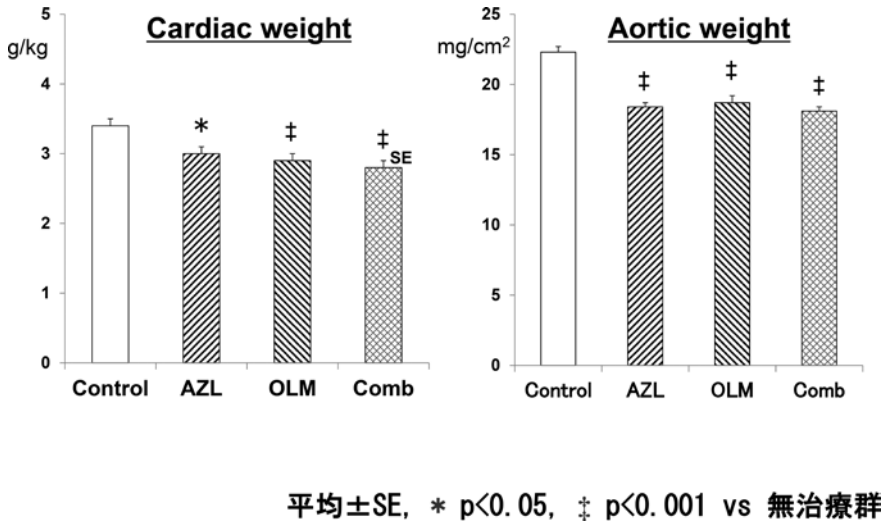

Figure 2 Cardiovascular organ weights in control untreated SHR and SHR treated with azelnidipine (AZL), olmesartan (OLM) or both (Comb) for 12 weeks. ＊$p<0.05$, ‡$p<0.001$ vs. control untreated SHR.

used for comparison.

Statistical Analysis

Values are expressed as means±SE. Comparison of the 4 groups was performed by one-way ANOVA and the post-hoc analysis by Dunnett's multiple-range test. Time-course changes in parameters were analyzed by two-way ANOVA with post-hoc multiple comparisons using the Bonferroni-Dunn test. Non-parametric data were analyzed by the Kruskal-Wallis H-test followed by Tukey's method for post-hoc between-group comparisons. A p value less than 0.05 was considered to indicate statistical significance.

RESULTS

Physical Measurements and Organ Weights

All the rats have survived and completed the 12-week study period. Figure 1 shows the time-course changes in body weight (panel A), blood pressure (panel B) and pulse rate (panel C) during the study period. The values of body weight, blood pressure and pulse rate at 12 weeks were listed in Table 1. The body weight increases were similar among the control, the AZL, the OLM and the combination groups. The blood pressures were comparably lowered in the AZL, the OLM and the combination groups as compared with the control group throughout the study period. The pulse rate was significantly reduced in the AZL group than in the control group, while it was not significantly changed in the OLM or the combination group as compared with the control group.

Figure 2 depicts the weights of cardiovascular organs such as the heart and the aorta. The weights of cardiac ventricles were significantly reduced in the AZL group, the OLM group and the combination group as compared with the control group. The reductions in cardiac weights were not significantly different between the 3 antihypertensive treatments. The weight

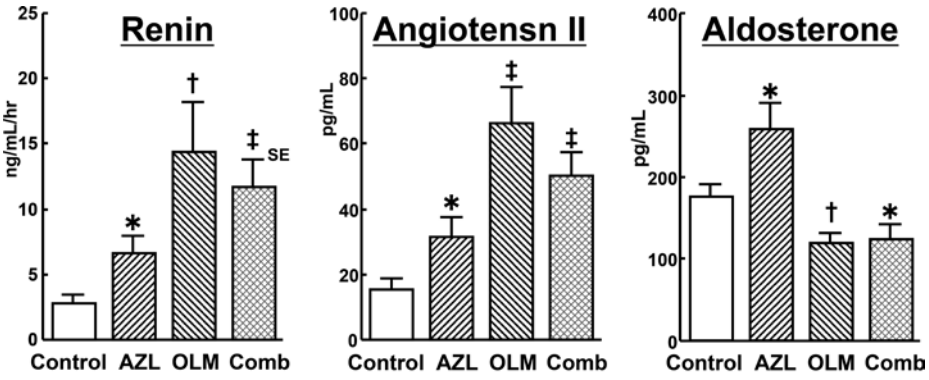

Figure 3 Circulating levels of renin-angiotensin-aldosterone system in control untreated SHR and SHR treated with azelnidipine (AZL), olmesartan (OLM) or both (Comb) for 12 weeks. ＊p＜0.05, †p＜0.01, ‡p＜0.001 vs. control untreated SHR.

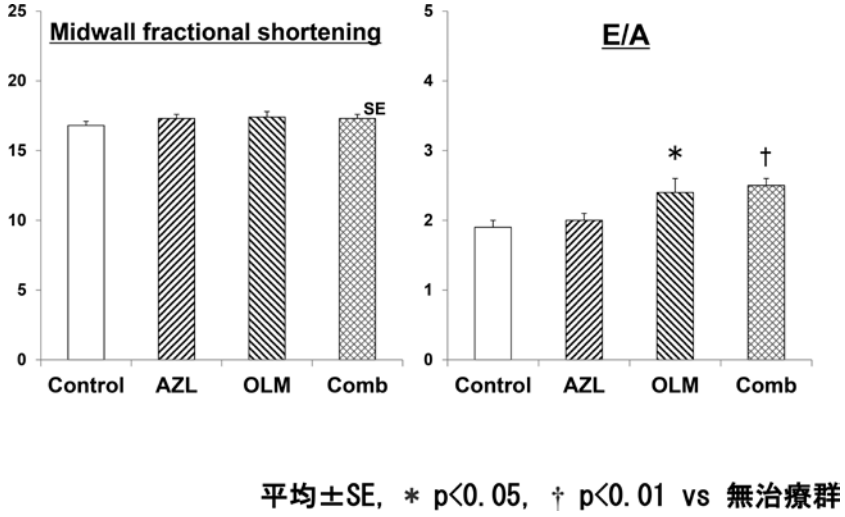

Figure 4 Parameters of systolic and diastolic function of left ventricle in control untreated SHR and SHR treated with azelnidipine (AZL), olmesartan (OLM) or both (Comb) for 12 weeks. ＊p＜0.05, †p＜0.01 vs. control untreated SHR.

of descending thoracic aorta per unit area was also significantly reduced in the 3 treatment groups to similar extents as compared with the control group.

Circulation Levels of renin-Angiotensin-Aldosterone System

Figure 3 presents the plasma levels of renin, angiotensin II and aldosterone in the 4 experimental groups. Plasma renin activity and plasma angiotensin concentration were significantly increased in the SHR groups treated with antihypertensive drugs as compared with the control untreated SHR. Especially, the increases were prominent in the groups given OLM. Regarding the aldosterone in plasma, the level was significantly increased in the AZL group while it was significantly decreased in the OLM and the combination groups as compared with the control group.

Evaluation of Cardiac Injury

Echocardiographic data obtained at the end of the 12-week study period are shown in Figure 4. The midwall fractional shortening, an index of left ventricular

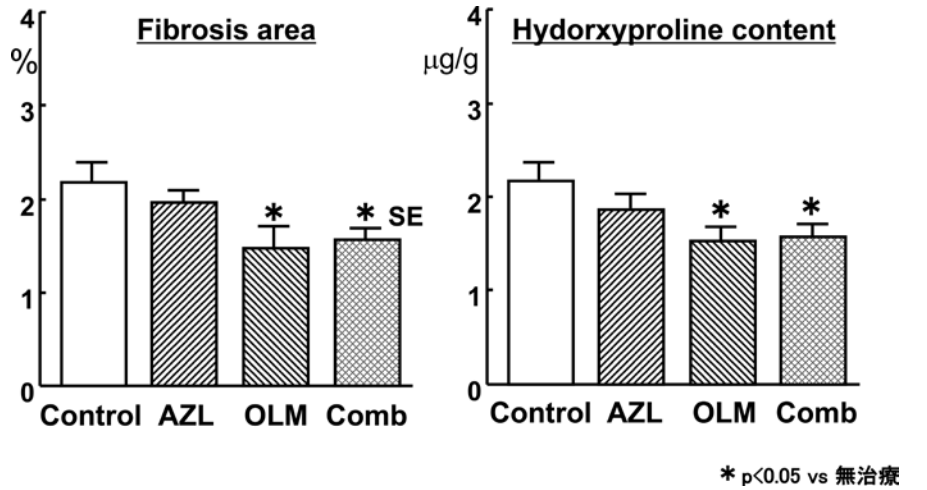

Figure 5 Parameters of left ventricular fibrosis in control untreated SHR and SHR treated with azelnidipine (AZL), olmesartan (OLM) or both (Comb) for 12 weeks. ＊p＜0.05 vs. control untreated SHR.

systolic function, was not significantly different between the control untreated SHR and the 3 groups of SHR given AZL, OLM or the combination therapy. With regard to the index of left ventricular diastolic function, the E/A ratio of transmitral flow velocity was improved in the OLM and the combination groups as compared with the control group. However, this parameter of left ventricular diastolic function did not differ in the AZL group as compared with the control group.

The bar graphs of Figure 5 compare the extents of left ventricular wall tissue fibrosis between the 4 groups of rats. As shown in the left panel, the fibrosis area stained in the Masson trichrome sections was significantly reduced in the OLM and the combination groups as compared with the control group. However, the fibrosis area observed in the AZL group was not significantly different with the control group. The measurements of hydroxyproline in the left ventricular tissue showed the results similar to the histological findings (right panel, Figure 5). The content of this amino acid composing collagen was lower in the OLM and the combination groups than in the control group, however, the content was not significantly different between the AZL and the control groups.

DISCUSSION

Several studies have investigated the protective effects of antihypertensive drugs against cardiovascular organ injuries using various animal models of hypertension, including mineralocorticoid-salt administration[9,10], renovascular hypertension[11~13] and renal ablation[14,15], in which hypertensive organ injuries progress rapidly over a period of several weeks to months. However, these are more likely the experimental models of secondary hypertension and the rapid temporal course of organ damage does not accurately reflect the organ damage that occurs over a span of decades in humans with essential hypertension. In this context, the pathophysiology of organ damage in SHR may more closely parallel to that which occurs in humans with essential hypertension[16~18]. Thus, it seems more appropriate to evaluate the effects of antihypertensive therapy using SHR in order to apply the findings to clinical medicine, considering that the majority of hypertensive patients are essential hypertension. SHR exhibit cardiac hypertrophy and arterial wall thickening with the development of hypertension although the organ injury, such as renal dysfunction does not occur until a later stage of life. Therefore, SHR is thought to be a suitable model to evaluate the therapeutic effects against hypertensive injuries in the heart and the arteries.

It has been suggested that the enhancement of renin-angiotensin-aldosterone system (RAAS) is involved in the progression process of cardiovascular tissue and organ injuries[19,20]. Particularly, angiotensin II promotes hypertrophy of cardiovascular cells, and aldosterone induces fibrosis of the cardiovascular tis-

sues[19,20]. Fibrosis and deposition of intercellular matrices, such as collagen, in the cardiac tissue cause reduction in left ventricular distensibility[21]. Therefore, antihypertensive drugs suppressing the RAAS, such as ACE inhibitors and ARBs, may be expected to protect cardiovascular organs from hypertensive injury independent of their effects on blood pressure[1,2]. It has been indicated that the growth of cardiovascular cells is enhanced in SHR and hypertrophy of cardiovascular tissues and organs develops before blood pressure elevation[22~25]. In the present study, the antihypertensive therapy including ARB mitigated the increase in left ventricular mass and fibrosis of left ventricular tissue. This mitigation of cardiac fibrosis, combined with inhibition of cardiac hypertrophy, may account for the observed preservation of myocardial contractility and distensibilty by ARB as compared with CCB alone.

Considerable amount of clinical evidence indicates that inhibitors of the RAAS improve the prognosis of patients with myocardial infarction or heart failure[26~29]. However, with regard to the primary prevention, it is not necessarily clear if there is an advantage to RAAS inhibitors over other classes of antihypertensive drugs in the prevention of coronary artery disease and heart failure in hypertensive patients. In CASE-J (Candesartan Antihypertensive Survival Evaluation in Japan) trial[30], Japanese hypertensive patients with high risks of cardiovascular diseases were treated with an ARB (candesartan) or a CCB (amlodipine) for an average of 3.2 years, which produced no significant differences in cardiovascular morbidity or mortality. ALLHAT (Antihypertensive and Lipid-Lowering treatment to Prevent Heart Attack Trial)[31] is a large-scale clinical study in which the effects of a diuretic (chlorthalidone), a CCB (amlodipine), and an ACE inhibitor (lisinopril) were compared in hypertensive patients with high risks of cardiovascular diseases. The trial investigators reported that the incidence of heart failure showed a nonsignificant trend towards lower incidence in the ACE inhibitor group than in the CCB group. The VALUE (Valsartan Antihypertensive Long-term Use Evaluation) trial[32], which compared the effects of an ARB, valsartan, and a CCB, amlodipine, in high-risk hypertensive patients, also reported a nonsignificant trend towards lower incidence of heart failure in the ARB group than in the CCB group. Further, the meta-analyses of large-scale clinical trials of hypertension treatment by the Blood Pressure Lowering Treatment Trialists' Collaboration[33,34] suggested that ACE inhibitors were more effective than CCBs in preventing heart failure and ARBs have comparable effects to ACE inhibitors in this respect. Thus, it is suggested that inhibitors of RAAS are advantageous for the primary prevention of cardiac injury in hypertension.

Epidemiological studies have indicated that an increased heart rate is associated with a higher incidence of cardiovascular diseases and a worse prognosis[35~37]. Tachycardia due to reflex activation of the sympathetic nervous system is one of the major adverse effects of CCB therapy. In this respect, unlike other dihydropyridine CCB, azelnidipine has been shown to inhibit sympathetic nerve activity[38]. In the present study, the pulse rate of SHR was reduced in the AZL group. This property of azelnidipine may be beneficial for the inhibition of cardiovascular events in hypertensive patients. However, the pulse rate was not significantly changed in SHR given lower dose of AZL in combination with OLM. Therefore, the inhibitory effect of AZL on sympathetic nerve activity seems dose-dependent.

Considering the physiological actions of angiotensin II, it is quite natural that the plasma renin and angiotensin II levels were increased and plasma aldosterone is decreased by ARB such as OLM in the present study. In addition, OLM has been reported to suppress plasma aldosterone more prominently than other ARB[39]. The reduction in blood pressure and renal perfusion pressure by CCB is also expected to enhance plasma renin and angiotensin II as observed in this study. Regarding the effect on aldosterone secretion, the in vitro study has shown that AZL rather inhibits aldosterone synthesis in cultured adrenocortical cells[40]. However, this effect does not seem potent enough to counteract the stimulation of RAAS by blood pressure reduction in vivo because plasma aldosterone was increased in SHR given AZL in the present study.

The present study demonstrated that the antihypertensive drug therapy including ARB such as OLM is superior to the monotherapy using CCB in inhibiting the myocardial fibrosis of left ventricle and preserving its diastolic function. Thus, long-term antihypertensive therapy using the inhibitors of RAAS may be advanta-

geous in preventing the occurrence of heart failure in hypertensive patients.

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