Combination therapy based on the angiotensin receptor blocker olmesartan for vascular protection in spontaneously hypertensive rats

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Abstract. For hypertension, combination therapies are recommended to acheive a low target blood pressure. In this study, the efficacy of combination therapies for preventing organ damage was investigated in spontaneously hypertensive rats (SHR). Twenty-week-old male SHR were orally administered olmesartan (Olm) (5 mg/kg/day) for the first 4 weeks. Subsequently, rats were randomly divided into 5 groups and administered add-on drugs for another 4 weeks as follows: Olm+Olm (5 mg/kg/day), Olm+azelnidipine (Aze) (30 mg/kg/day), Olm+temocapril (Tem) (10 mg/kg/day), Olm+atenolol (Ate) (5 mg/kg/day), Olm+hydrochlorothiazide (HCTZ) (5 mg/kg/day). Blood pressure and heart rate were measured at weeks 0, 4 and 8 by the tail-cuff method. Heart and kidney weights were determined, and endothelial function was assessed by evaluating the dilator response to acetylcholine. In comparison to untreated control SHR, a significant reduction in systolic blood pressure was observed at weeks 4 and 8 in all groups ($p<0.05$), while heart rate was significantly reduced at week 8 in only the Olm+Aze and Olm+Ate groups ($p<0.05$). In all groups, heart but not kidney weight was significantly decreased ($p<0.05$), and endothelial function was significantly improved ($p<0.05$) compared to the control SHR. In the Olm+Olm, Olm+Tem and Olm+Aze groups, endothelial function was significantly improved as compared to the other treatment groups ($p<0.05$). Thus, when using an angiotensin receptor blocker as a first-line therapy, an antihypertensive in the form of an angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, or calcium channel blocker, such as azelnidipine, should be used as a second-line drug to protect against vascular damage.

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Introduction

Data from the largest meta-analysis of hypertensive patients conducted to date clearly indicate that increased systolic blood pressure (BP) in any age group is associated with a significant increase in cardiovascular disease (1). Several studies have confirmed the significant cardiovascular risk associated with hypertension, and the impressive health benefits that can be derived from treatment of this disease (2). Despite these findings, worldwide epidemiological data show that less than one-third of hypertensive patients achieve a BP <140/90 mmHg (3). Notably, among patients receiving antihypertensive medication and follow-ups by a physician, less than 50% have a BP <140/90 mmHg (4,5).

Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs) and long-acting calcium channel blockers (CCBs) are widely recognized as the most effective drugs for the treatment of hypertension. Recently published clinical trials, such as CASE-J and VALUE, suggest that an ARB is best used as the first-line drug to achieve a low target BP in patients with diabetes, ischemic heart disease or chronic kidney disease (3,6,7).

The importance of combination therapy in the treatment of hypertension is well established. Clinically, combination therapy for hypertension using two or more drugs from different classes can result in improved drug efficacy (3,8). However, it remains to be determined which drugs are most effective when used as second-line therapy to protect against the organ damage induced by hypertension. In particular, the combination of a CCB and either an ACE inhibitor or an ARB is popular in the treatment of hypertension. On the other hand, diuretics have been established worldwide as potent hypertensive drugs. In this study, we compared the efficacy of various ARB-based combination therapies (ARB/ARB, ARB/ACE inhibitor, ARB/CCB, ARB/β-blocker and ARB/diuretic) in spontaneously hypertensive rats (SHR) by measuring heart and kidney weights and assessing endothelial function in the aorta.

Materials and methods

Experimental design. Male SHR (15 weeks old) were purchased from Charles River Breeding Laboratories (Osaka, Japan). The rats were given free access to water and standard laboratory rat chow (11.3 mEq Na^+/100 g, 32.6 mEq K^+/100 g, 24.6% protein by weight; Oriental Yeast Co., Osaka, Japan), and were maintained in a 12-h light to 12-h dark photoperiod. Rats were divided into an untreated control group and 5 groups treated for 4 weeks with olmesartan (Olm). Subsequently, Olm treatment was continued in the treatment groups for another 4 weeks with the addition of i) Olm, ii) temocapril (Tem), iii) azelnidipine (Aze), iv) atenolol (Ate) or v) hydrochlorothiazide (HCTZ). Drugs were administered by gavage. After a total treatment period of 8 weeks, the rats were sacrificed by decapitation, and the hearts and kidneys were harvested and weighed. The experimental protocol of the study was approved by the Osaka University Ethics Committee of Animal Experiments.

Drugs. Olm, Tem and Aze were obtained from Daiichi-Sankyo Co. Ltd. (Tokyo, Japan). Ate and HCTZ were purchased from Sigma Chemical Co. (St. Louis, MO).

Measurement of body weight, blood pressure and heart rate. At weeks 0, 4 and 8, body weight (BW) was measured. Systolic BP and heart rate (HR) were also measured at weeks 0, 4 and 8 using the tail-cuff method in conscious rats with a sphygmomanometer (Softron Co. Ltd., Tokyo, Japan) as previously described (9).

Evaluation of vasodilator properties in response to acetylcholine. Freshly harvested aortas were cleaned of fat and connective tissues, cut into helical strips and mounted in 30-ml organ baths containing Krebs-Henseleit buffer (120 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, pH 7.4), then maintained at 37˚C and oxygenated with 95% O_2, 5% CO_2 (10-12). Vessels were equilibrated for 60 min with changes in the bathing fluid every 15 min. Isometric tension studies were performed using a Grass model 7D polygraph. Optimal resting tension was determined in baseline studies, then the response to vasoactive drugs was determined as previously described (10-12). Cumulative dose-response curves to phenylephrine (10^{-9} to 10^{-4} M) were established. The vessels were then submaximally pre-contracted with phenylephrine (typically $3x10^{-6}$ M), and endothelial function was evaluated by means of vascular relaxation to acetylcholine (10^{-9} to 10^{-4} M). Nitric oxide mediation of acetylcholine responses was confirmed by blocking acetylcholine-induced relaxation using N ω-nitro-L-arginine methyl ester (L-NAME) (1 mM), a specific competitive inhibitor of nitric oxide synthase. Contractile responses were measured from the polygraph chart and expressed as a percentage of the maximal contraction, and relaxation was expressed as a percentage of the precontracted tension.

Statistical analysis. All values are expressed as the mean ± SEM. Analysis of variance followed by the Bonferroni/Dunnet's test was employed to determine the significance of differences in multiple comparisons. P-values <0.05 were considered statistically significant.

Table I. Changes in body weight (g).

Group	Week 0	Week 4	Week 8
WKY	351.2±4.2	385.9±6.1	417.2±6.6
SHR	358.9±5.6	392.3±6.3	409.2±7.3
Olm+Olm	363.0±2.5	391.6±3.3	404.2±3.6
Olm+Aze	357.8±5.3	388.4±5.1	400.1±5.7
Olm+Tem	352.1±5.8	382.3±5.6	398.5±5.9
Olm+Ate	362.6±2.8	394.0±3.7	401.1±4.2
Olm+HCTZ	356.6±5.0	388.2±5.1	387.6±5.7

Measurements were taken at week 0 (before the start of treatment) and at weeks 4 and 8 after the start of treatment. WKY, untreated Wistar Kyoto rats; SHR, untreated spontaneously hypertensive rats; Olm, olmesartan; Aze, azelnidipine; Tem, temocapril; Ate, atenolol; HCTZ, hydrochloro-thiazide. n=8 per group. Values are the mean ± SE.

Table II. Changes in systolic blood pressure (mmHg).

Group	Week 0	Week 4	Week 8
WKY	115±2	114±1	114±1
SHR	211±3	211±2	215±2
Olm+Olm	210±3	194±2	183±2
Olm+Aze	210±3	194±1	180±1
Olm+Tem	210±3	193±1	177±1
Olm+Ate	210±3	191±2	180±1
Olm+HCTZ	211±3	193±2	174±2

Measurements were taken at week 0 (before the start of treatment) and at weeks 4 and 8 after the start of treatment. WKY, untreated Wistar Kyoto rats; SHR, untreated spontaneously hypertensive rats; Olm, olmesartan; Aze, azelnidipine; Tem, temocapril; Ate, atenolol; HCTZ, hydrochloro-thiazide. n=8 per group. Values are the mean ± SE.

Results

Body weight, blood pressure and heart rate. Table I shows the changes in BW. None of the treatments affected BW, as no significant differences were observed when comparing the BW of the treatment groups. Table II shows the changes in systolic BP. In all the treated groups, there were significant reductions in BP at 4 weeks after treatment with olmesartan. At week 8, a further significant reduction in BP was observed. There were no significant differences between the groups at weeks 4 or 8. Table III shows the changes in HR. No significant changes were observed among the treated groups at week 4. However, at week 8, HR was significantly reduced in the Olm+Aze and Olm+Ate groups compared to untreated control SHR ($p<0.05$).

Effect on cardiac and kidney weights. Fig. 1 shows heart and left ventricle (LV) weights corrected for BW in SHR after 8 weeks of treatment. The corrected heart and LV weights were significantly decreased by each treatment compared to

Table III. Changes in heart rate (beats/min).

Group	Week 0	Week 4	Week 8
WKY	394±8	382±8	376±8
SHR	385±5	381±72	377±5
Olm+Olm	409±6	396±5	394±5
Olm+Aze	400±7	392±10	373±8
Olm+Tem	393±12	3943±10	388±9
Olm+Ate	395±11	385±8	364±8
Olm+HCTZ	396±9	375±9	381±7

Measurements were taken at week 0 (before the start of treatment) and at weeks 4 and 8 after the start of treatment. WKY, untreated Wistar Kyoto rats; SHR, untreated spontaneously hypertensive rats; Olm, olmesartan; Aze, azelnidipine; Tem, temocapril; Ate, atenolol; HCTZ, hydrochlorothiazide. n=8 per group. Values are the mean ± SE.

the heart and LV weights of the control SHR. The corrected heart and LV weights of the control SHR were significantly increased compared to those of untreated Wistar Kyoto (WKY) rats, consistent with previous reports (10). Among the treatment groups, there were no significant differences between the corrected heart and LV weights.

As shown in Fig. 2, the corrected kidney weight to BW of the SHR was significantly increased compared to that of the WKY rats. None of the treatments had an effect on kidney weight, as no significant differences were observed when comparing the corrected kidney weights of the treatment groups.

Effect on endothelial function. It is known that hypertension induces the impairment of endothelial function. In animal models, this is commonly assessed by the dilator response to acetylcholine. As previously reported, the dilator response of the aorta in the SHR was impaired compared to that observed in WKY control rats (10). Antihypertensive treatments are known to improve impaired endothelial function. There were significant improvements in impaired endothelial function in all the treatment groups of the present study (Fig. 3A). Strong inhibition of the renin-angiotensin system (RAS) by Olm+Olm and Olm+Tem resulted in increased improvement of endothelial function as compared to the groups administered a β-blocker or diuretic as the second-line therapy. Notably, the Olm+Aze group showed almost the same improvement in endothelial function as the Olm+Olm and Olm+Tem groups. Moreover, acetylcholine at a concentration of $10^{-6.5}$ M resulted in a significant improvement in impaired endothelial function in the Olm+Olm, Olm+Aze and Olm+Tem groups compared to the Olm+Ate and Olm+HCTZ groups ($p<0.05$) (Fig. 3B). The endothelium-dependent dilatation of the aorta in the SHR treated with each drug was consistent with the finding that this increase in dilation was completely abolished by the administration of L-NAME (data not shown). The endothelium-dependent dilation of the aorta in untreated control SHR was also completely abolished by the administration of L-NAME (data not shown).

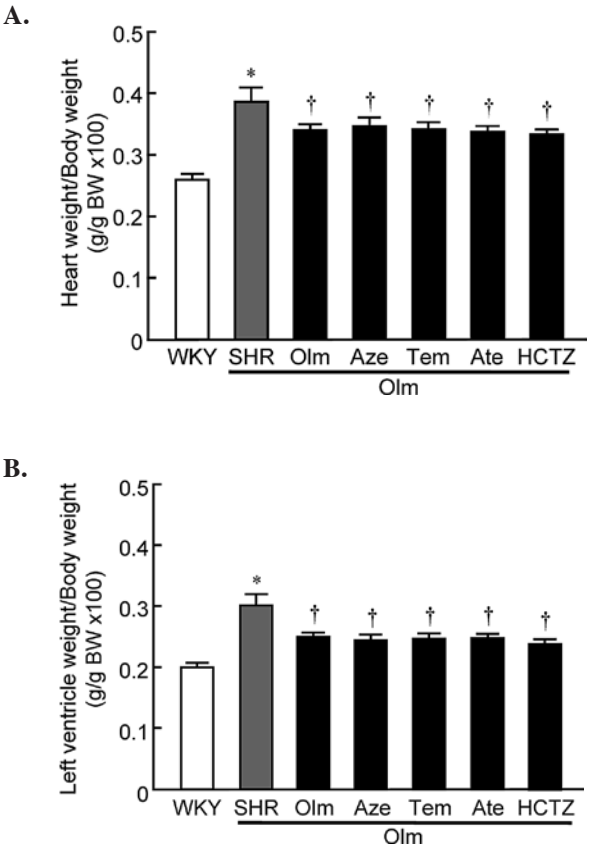

Figure 1. Protective effect of olmesartan (Olm), azelnidipine (Aze), temocapril (Tem), atenolol (Ate) and hydrochlorothiazide (HCTZ) on cardiac hypertrophy. Cardiac hypertrophy in spontaneously hypertensive rats was assessed by measuring the heart and left ventricular weights. Values are the mean ± SE of 6 animals, expressed as the ratio of heart weight (A) and left ventricle weight (B) to body weight. *$p<0.01$ and †$p<0.05$ vs. untreated Wistar Kyoto rats.

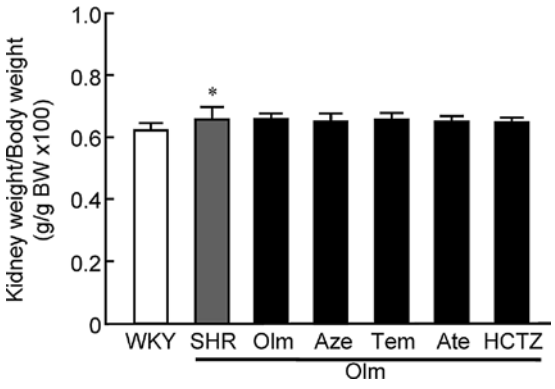

Figure 2. Protective effect of olmesartan (Olm), azelnidipine (Aze), temocapril (Tem), atenolol (Ate) and hydrochlorothiazide (HCTZ) on kidney weight. As one of the phenotypic changes associated with kidney damage, kidney weight was measured in spontaneously hypertensive rats. Values are the mean ± SE of 6 animals, expressed as the ratio of kidney weight to body weight. *$p<0.01$ vs. untreated Wistar Kyoto rats.

Discussion

In clinical studies and experimental models, RAS inhibition has been shown to improve endothelial dysfunction and fibrinolytic activity, and to decrease vascular inflammation and oxidative stress (13-17). Moreover, in clinical trials, RAS

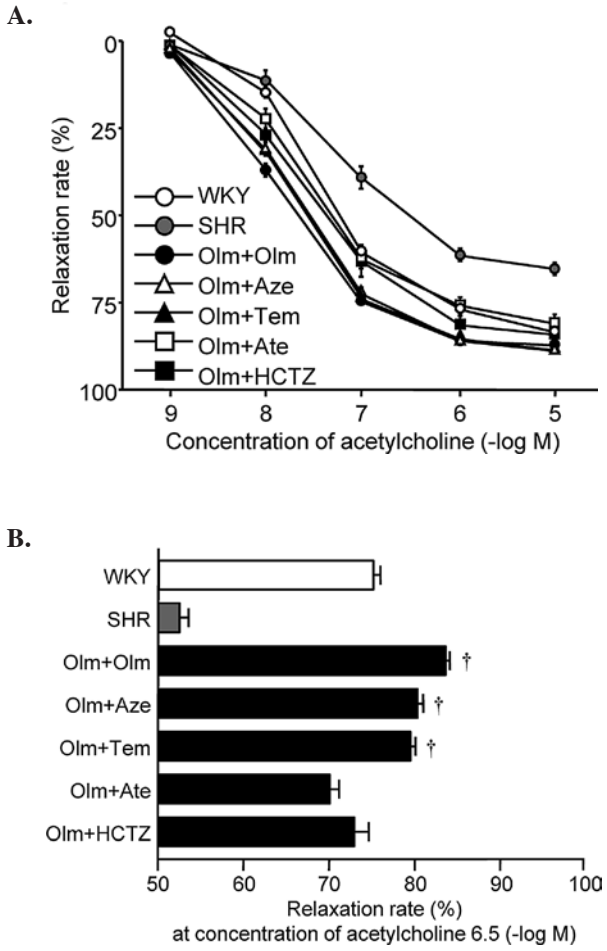

Figure 3. Vasodilator response of the aorta to acetylcholine 8 weeks after each antihypertensive treatment. Values are expressed as percentages of maximum relaxation (100%) induced by 10^{-4} M papaverine. Points and vertical bars represent the mean ± SE of 6 preparations (A). WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; Olm, olmesartan; Aze, azelnidipine; Tem, temocapril; Ate, atenolol; HCTZ, hydrochlorothiazide. Data shown are at the $10^{-6.5}$ M concentration of acetylcholine (B). Values are the mean ± SE of 6 preparations. †p<0.05 vs. untreated WKY, Olm+Ate and Olm+HCTZ rats.

inhibition has been shown to reduce the risk of cardiovascular events and mortality and to demonstrate benefits for patients with left ventricular hypertrophy, stroke, heart failure and non-diabetic and diabetic renal diseases (18-21). RAS mediates adaptive and maladaptive responses to cell and tissue injuries, and thereby plays a central role in the pathophysiology of cardiovascular and renal disease through its main effecter, angiotensin (Ang) II (22). The Ang II type 1 (AT_1) receptor mediates numerous deleterious effects of Ang II, including vasoconstriction, sympathetic nervous system activation, smooth muscle cell growth and proliferation, vascular inflammation, generation of reactive oxygen species and endothelial dysfunction (23). ARBs counteract the effects of Ang II via distinct pathways by selectively antagonizing all AT_1 receptor effects and stimulating AT_2 receptors. This may counteract the negative effects of AT_1. Based on extensive evidence, ARBs are considered the essential component for the treatment of patients with hypertension and heart failure, diabetes, stroke or chronic kidney disease (6,7).

Hypertension is the most commonly diagnosed disease worldwide. Despite concerted efforts, BP remains poorly controlled, particularly in high risk populations. The most recent age-adjusted estimates show that BP control increased by up to approximately 60% in treated patients (24). However, BP control was only 30% in hypertensive patients overall, based on targets for patients with uncomplicated hypertension (<140/90 mmHg) (25). It is critical that clinicians worldwide focus on achieving recommended target BP values in these patients. One solution is the use of combination therapy. In terms of efficacy, using two complementary antihypertensive agents in combination consistently results in greater efficacy than high-dose monotherapy. However, it is still unclear which combination or high-dose monotherapy is best from the point of view of organ protection. The present study aimed to elucidate this issue.

We measured BW, BP and HR, and examined heart and kidney weights and endothelial function in seven groups of rats. ARB monotherapy in the first 4 weeks significantly reduced BP but not HR. At week 8 after treatment with an ARB plus various antihypertensive agents, BP was further reduced compared to the significantly reduced BP noted at week 4. However, no differences were observed among the groups. In contrast, at week 8, HR was significantly reduced in only two groups, Olm+Ate and Olm+Aze. It was expected that treatment with Olm+Ate would reduced HR, as atenolol is known to suppress HR by inhibiting sympathetic nerve activation. In contrast to atenolol, CCBs generally increase HR by sympathetic nerve activation due to a rapid decrease in BP. Our results were consistent with previous reports, which indicate that the CCB used in this study, azelnidipine, suppresses HR (26). In keeping with the activity of CCBs, azelnidipine has been reported to suppress the increase in HR by the activation of sympathetic nerve activity induced by a rapid decrease in BP (27). This suppression may be due to the rate of BP lowering (28), or to the direct inactivation of sympathetic nerve activity (29). Further experiments are needed to clarify this mechanism.

Complications associated with hypertension, including stroke, heart failure and renal failure, are often lethal. End-organ damage, including cardiac hypertrophy and arteriosclerosis, occurs during the early phases of these complications. Thus, the prevention of end-organ damage is crucial in the treatment of hypertension (30,31). Based on this consideration, we examined the effect of various antihypertensive treatments on cardiac hypertrophy. Antihypertensives have been reported to have an effect on cardiac hypertrophy in SHR (32,33). In this study, there were no significant differences between the corrected heart and LV weights; the level of cardiac hypertrophy prevention in the treatment groups was almost the same. This may be due to the fact that, though the reduction of BP is key to preventing cardiac hypertrophy, RAS also plays an important role in its development. As each treatment was based on the same ARB, Olm, the inhibition of RAS was potentially sufficient on its own for the prevention of cardiac hypertrophy in the SHR.

Endothelial cells are known to secrete various substances. Among these are many anti-proliferative factors, including nitric oxide and vascular natriuretic peptides. It has thus been hypothesized that endothelial cells modulate vascular growth

(34-36). Endothelial dysfunction may therefore promote abnormal vascular growth, leading to end-organ damage. In experimental hypertensive models, the activation of vascular RAS has been reported in blood vessels (37,38). In other words, vascular protective effects may be mediated by the blockade of Ang II. In this study, we observed significant improvements in endothelial function in all the treatment groups (Fig. 2). This may be due to the fact that the groups were all subjected to the same ARB-based treatment, resulting in the suppression of RAS. However, the Olm+Olm and Olm+Tem groups showed further significant improvement compared to the Olm+Ate and Olm+HCTZ groups (Fig. 3b). The stronger inhibition of RAS by the addition of an ARB or an ACE inhibitor may therefore induce further improvements. Notably, the Olm+Aze group showed a significant improvement in endothelial function compared to the Olm+Ate and Olm+HCTZ groups. As mentioned above, the activation of RAS may play a role in the development of endothelial dysfunction induced by hypertension. The AT_1 receptor has been shown to mediate the overproduction of reactive oxygen species via NAD(P) H oxidase in blood vessels in hypertensive models (17,39), and azelnidipine has been reported to suppress the production of reactive oxygen species (40-44). In the present model, azelnidipine may have exerted this anti-oxidative effect, leading to further significant improvements in endothelial function. Though there are few reports on the anti-oxidative effect of atenolol or hydrochlorothiazide, it may explain the observed difference in the improvement of endothelial function.

The potential of ARBs in the prevention of organ damage has been reported in experimental models and clinical studies. ARBs have been proven to have benefits beyond BP lowering effects. This fact warrants the use of ARBs in clinical practice worldwide. Hypertensive patients undergoing treatment seldom achieve the target BP. Thus, combination therapy based on ARBs is recommended in several guidelines. The second-line choice of therapy is generally a CCB, β-blocker or diuretic. In special cases, a high dose of an ARB or an ARB+ACE inhibitor is necessary. Due to its prohibitively high cost, this stronger inhibition of RAS is only recommended in diabetic or chronic kidney disease patients. The present study suggests that the protective effect against end-organ damage differs depending on the choice of secondary antihypertensive drug.

In conclusion, combination therapy based on an ARB is effective in reducing blood pressure. Moreover, compared to β-blockers or diuretics, the CCB azelnidipine, which has unique effects on heart rate and oxidative stress, may be suitable in terms of vascular protection.

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