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研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
安成　憲一	β遮断薬のすべて（第2版）	荻原俊男、築山久一郎、横山光宏:編集	高血圧における酸化ストレスとβ遮断薬	先端医学社	東京	2004	88-92

雑　誌

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Yasunari K et al.	Effect of Carvedilol on oxidative stress in polymorphonuclear and mononuclear cells in patients with essential hypertension	Am J Med	116/7	460-465	2004
Yasunari K et al.	Comparative effects of valsartan versus amlodipine on left ventricular mass and reactive oxygen species formation by monocytes in hypertensive patients with left ventricular hypertrophy	J Am Coll Cardilol	43/11	2116-2123	2004
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Effects of Carvedilol on Oxidative Stress in Polymorphonuclear and Mononuclear Cells in Patients with Essential Hypertension

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PURPOSE: To compare the effects of carvedilol and propranolol on oxidative stress in leukocytes and C-reactive protein levels in patients with hypertension.

METHODS: Sixty hypertensive patients were randomly assigned to carvedilol (20 mg; n = 30) or propranolol (60 mg; n = 30) for 6 months. Thirty normotensive subjects who were given placebo served as controls. Oxidative stress in polymorphonuclear cells and mononuclear cells were measured by gated flow cytometry. C-reactive protein levels were measured by immunonephelometric assay.

RESULTS: Oxidative stress in polymorphonuclear cells and mononuclear cells was increased significantly in hypertensive patients compared with in normotensive controls. After 6 months of treatment, carvedilol decreased oxidative stress significantly in polymorphonuclear cells by a mean of 45 arbitrary units (95% confidence interval [CI]: 32 to 59 arbitrary units; $P < 0.001$) and propranolol decreased oxidative stress significantly by 20 arbitrary units (95% CI: 7 to 33 arbitrary units; $P < 0.003$; $P = 0.001$ for difference between treatments). Carvedilol also decreased oxidative stress significantly in mononuclear cells by 23 arbitrary units (95% CI: 15 to 31 arbitrary units; $P < 0.001$), whereas propranolol decreased oxidative stress by 2 arbitrary units (95% CI: 7 to 12 arbitrary units; $P = 0.62$; $P = 0.002$ for difference between treatments). Carvedilol decreased C-reactive protein levels significantly by a median of 0.073 mg/dL (interquartile range, 0.034 to 0.112 mg/dL; $P < 0.001$), whereas propranolol decreased levels by 0.012 mg/dL (interquartile range, 0.009 to 0.032 mg/dL; $P = 0.26$; $P = 0.003$ for difference between treatments).

CONCLUSION: These findings suggest that carvedilol inhibits oxidative stress in polymorphonuclear and mononuclear cells, as well as lowers C-reactive protein levels, to a greater extent than does propranolol in hypertensive patients. **Am J Med. 2004;116:460–465.** ©2004 by Excerpta Medica Inc.

Increased oxidative stress has been reported in experimental models of hypertension (1) and patients with essential hypertension (2). If enhanced oxidative stress is involved in the pathogenesis of atherosclerosis in hypertensive patients (3), treatment should include an adequate antioxidant supply, obtained with either concomitant supplementation of antioxidants and antihypertensive compounds, or administration of antihypertensive drugs with antioxidant properties, such as carvedilol (4).

It has been suggested that carvedilol may provide greater benefit than βeta-blockers in the treatment of chronic heart failure because of its antioxidant effects (5). Carvedilol has been shown to inhibit pressure-induced increases in oxidative stress in human coronary smooth muscle cells in culture (6). At a dose of 25 mg/d, carvedilol was observed to have antioxidant effects, as assessed by suppression of ex vivo low-density lipoprotein (LDL) oxidation and reduction of antioxidized LDL antibodies in vivo (7).

Polymorphonuclear leukocytes are one of the main types of inflammatory cells. Once activated, they release reactive oxygen species, including hydrogen peroxide, and mediators of proteolytic tissue degradation, contributing to oxidative stress, inflammation, endothelial damage, and atherosclerosis (8,9). Mononuclear cells are involved in the genesis of atherosclerotic lesions. When stimulated, they increase oxidative stress, induce lipid peroxidation, release cytokines, and induce adhesion to endothelium, which together result in atherosclerosis (10). Indeed, oxidative stress in polymorphonuclear and mononuclear cells might be a link between oxidative stress and inflammation.

Accordingly, the objectives of the present study were to determine the effects of carvedilol, compared with propranolol, on oxidative stress in polymorphonuclear and mononuclear cells in patients with essential hypertension.

METHODS

Study Protocol

Sixty hypertensive patients and 30 normotensive subjects who visited the Osaka City University Hospital during April 2001 to March 2003 were recruited or volunteered

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for the study. The study was approved by the hospital's Institutional Review Board. Written, informed consent was obtained from all subjects.

Hypertension was defined as a blood pressure ≥140/90 mm Hg, measured with the subject in a sitting position on at least three different occasions in the outpatient clinic of the hospital. None of the subjects were taking any medications or supplements, such as nonsteroidal anti-inflammatory drugs, vitamin E, or antioxidants.

Hypertensive patients were given 20 mg of carvedilol (n = 30) or 60 mg of propranolol (n = 30), administered orally for 6 months in a randomized double-blind fashion. Controls were given placebo. No subjects were lost to follow-up during the 6-month study period.

Fasting blood samples were collected at baseline and 6 months. Degree of obesity was estimated by body mass index. Venous blood was used for measurement of plasma insulin, plasma glucose, hemoglobin A_{1C}, plasma cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels. C-reactive protein levels were measured using latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Delaware).

Assay of Intracellular Oxidative Stress

Hydrogen peroxide production by polymorphonuclear and mononuclear cells was measured at baseline and 6 months using a gated flow cytometry technique (11) with some modifications (12); oxidative stress was measured as fluorescence intensity. Fresh blood (1 mL) was collected in tubes containing preservative-free heparin (10 U/mL of blood) and preincubated for 15 minutes with 2',7'-carboxydichlorofluorescein diacetate bis-acetoxymethyl ester (CDCFH bis-acetoxymethyl ester, 100 mol/L; Molecular Probe Co., Eugene, Oregon) in a 37C water bath with gentle horizontal shaking. CDCFH diacetate bis-acetoxymethyl ester is a compound that is converted into a nonfluorescent derivative (CDCFH) by cellular esterases after incorporation into cells. It is oxidized rapidly to the fluorescent carboxydichlorofluorescein in the presence of intracellular hydrogen peroxide. Red cells were lysed, and white blood cells were suspended in 1% paraformaldehyde-phosphate–buffered saline. Fixed samples were kept on ice until flow cytometric analysis was performed on the same day.

Statistical Analysis

All values are expressed as means ± SD, unless otherwise specified. Comparisons were performed with analysis of variance or the Student *t* test. Comparisons of measurements at baseline and 6 months were carried out with the paired *t* test. C-reactive protein level was expressed as medians (interquartile range), and *P* values were computed with the Mann-Whitney *U* test for intergroup comparisons at baseline. The association between oxidative stress and relevant covariates was examined by determination of Pearson correlation coefficients. *P* values <0.05 (two-sided) were considered significant. Analyses were performed using SAS software (Cary, North Carolina).

RESULTS

Hypertensive patients (30 carvedilol, 30 propranolol) and controls (n = 30) were similar in age (Table 1). Forty percent (n = 36) of the cohort was male. Patients in the carvedilol and propranolol groups had similar body mass index and levels of plasma insulin, hemoglobin A_{1C}, triglyceride, HDL cholesterol, and LDL cholesterol at baseline.

Oxidative Stress in Polymorphonuclear and Mononuclear Cells

At baseline, oxidative stress in polymorphonuclear cells was increased significantly in hypertensive patients compared with in normotensive controls (214 ± 30 arbitrary units vs. 179 ± 14 arbitrary units, $P<0.01$; Table 1). After 6 months of treatment, carvedilol decreased oxidative stress in polymorphonuclear cells by 45 arbitrary units ($P<0.001$; Figure 1A; Table 2), whereas propranolol decreased oxidative stress by 20 arbitrary units ($P<0.003$; Figure 1B; Table 2), a mean difference of 25 arbitrary units (95% confidence interval [CI]: 11 to 40 arbitrary units; $P = 0.001$). In controls, oxidative stress in polymorphonuclear cells was relatively unchanged at 6 months ($P = 0.35$; Table 2).

The decrease in mean arterial pressure was similar in the carvedilol and propranolol groups (Table 2). There was a significant relation between decrease in mean arterial pressure and decrease in oxidative stress in polymorphonuclear cells in the carvedilol ($r = 0.38$, $P = 0.04$) and propranolol ($r = 0.81$, $P<0.01$) groups.

At baseline, oxidative stress in mononuclear cells was increased significantly in hypertensive (carvedilol and propranolol groups) patients compared with in controls (80 22 arbitrary units vs. 65± 7 arbitrary units, $P<0.01$). After 6 months of carvedilol, oxidative stress in mononuclear cells decreased by 23 arbitrary units ($P<0.001$; Figure 2A; Table 2), compared with a decrease of 2 arbitrary units with propranolol ($P = 0.62$; Figure 2B; Table 2), a difference of 21 arbitrary units (95% CI: 8 to 33 arbitrary units; $P = 0.002$). In the control group, oxidative stress in mononuclear cells changed little at 6 months ($P = 0.28$; Table 2).

C-Reactive Protein

C-reactive protein levels were significantly higher in hypertensive patients than in normotensive controls at baseline: median, 0.10 mg/dL (interquartile range, 0.05 to 0.20 mg/dL) versus 0.05 mg/dL (interquartile range, 0.05 to 0.13 mg/dL, $P = 0.03$). After 6 months, carvedilol de-

Table 1. Baseline Characteristics of the Participants

Characteristic	Hypertensive Patients (n = 60)		Normotensive Controls (n = 30)
	Carvedilol (n = 30)	Propranolol (n = 30)	
	Number (%), Mean ± SD, or Median (Interquartile Range)		
Age (years)	65 ± 10	66 ± 10	65 ± 11
Male sex (%)	12 (40%)	12 (40%)	12 (40%)
Body mass index (kg/m^2)	24 ± 3	24 ± 4	25 ± 3
Mean arterial pressure (mm Hg)	120 ± 9*	121 ± 9*	101 ± 4†‡
Oxidative stress in polymorphonuclear cells (arbitrary units)	217 ± 23*	210 ± 36*	179 ± 14†‡
Oxidative stress in mononuclear cells (arbitrary units)	80 ± 22*	79 ± 20*	65 ± 7†‡
Insulin (μU/mL)	7 ± 5	8 ± 4	7 ± 3
Hemoglobin A_{1C} (%)	5.5 ± 0.8	5.6 ± 0.4	5.5 ± 1.2
Triglyceride (mg/dL)	119 ± 74	109 ± 42	112 ± 54
HDL cholesterol (mg/dL)	55 ± 14	50 ± 13	55 ± 13
LDL cholesterol (mg/dL)	115 ± 27	115 ± 18	118 ± 33
C-reactive protein (mg/dL)§	0.10 (0.10–0.23)*	0.10 (0.05–0.10)	0.05 (0.05–0.13)‡

* $P < 0.05$ compared with controls.
† $P < 0.05$ compared with propranolol.
‡ $P < 0.05$ compared with carvedilol.
§ P values computed with the Mann-Whitney U test.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

creased levels by 0.073 mg/dL ($P = 0.008$) and propranolol decreased levels by 0.012 mg/dL ($P = 0.26$) (Table 2), a mean difference of 0.061 mg/dL (interquartile range, 0.022 to 0.100 mg/dL; $P = 0.003$). C-reactive protein levels in the control group changed little from baseline to 6 months ($P = 0.83$; Table 2). There was a significant relation between decreases in oxidative stress in mononuclear cells and decreases in C-reactive protein levels in the carvedilol group ($r = 0.46$, $P = 0.01$) but not in the propranolol group ($r = -0.14$, $P = 0.4$).

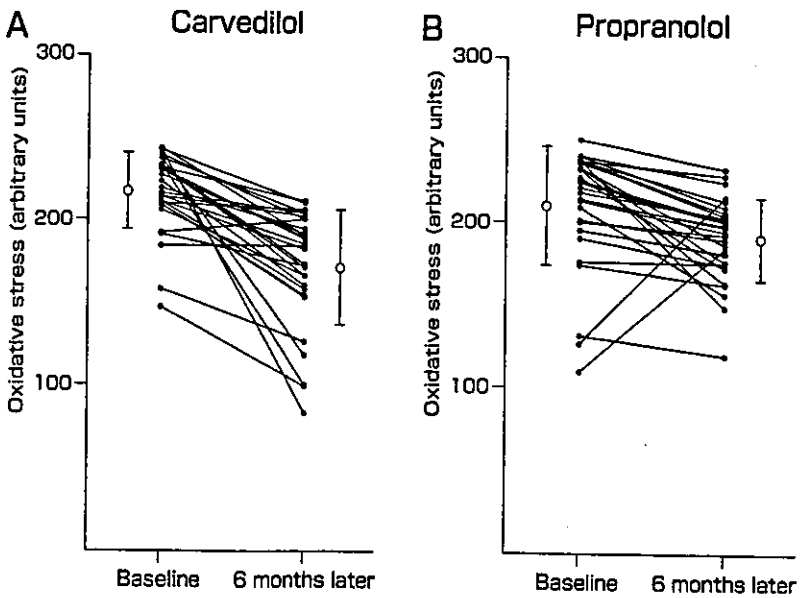

Figure 1. Oxidative stress in polymorphonuclear cells at baseline and after 6 months of treatment with carvedilol 20 mg (A) or propranolol 60 mg (B). The empty circles and bars indicate means ± SD. $P < 0.001$ between values at baseline and 6 months in the carvedilol group; $P < 0.001$ between values at baseline and 6 months in the propranolol group; $P = 0.36$ between values at baseline in the carvedilol and propranolol groups; and $P = 0.02$ between values at 6 months in the carvedilol and propranolol groups.

Table 2. Changes in Measurements from Baseline to 6 Months

Variable	Hypertensive (n = 60)				Normotensive (n = 30)	
	Carvedilol (n = 30)		Propranolol (n = 30)			
	Difference (95% Confidence Interval)	*P* Value*	Difference (95% Confidence Interval)	*P* Value*	Difference (95% Confidence Interval)	*P* Value*
Body mass index (kg/m^2)	0.1 (−0.1 to 0.1)	0.91	0.1 (−0.1 to 0.2)	0.41	−0.3 (−0.1 to 0.6)	0.14
Mean arterial pressure (mm Hg)	−12 (−15 to −9)	<0.001†	−12 (−16 to −9)	<0.001†	1 (−1 to 3)	0.41‡§
Oxidative stress in polymorphonuclear cells (arbitrary units)	−45 (−59 to −32)	<0.001†‡	−20 (−33 to −7)	0.003§	4 (−4 to 12)	0.35‡§
Oxidative stress in mononuclear cells (arbitrary units)	−23 (−31 to −15)	<0.001†‡	−2 (−12 to 7)	0.62†‡	2 (−2 to 6)	0.28§
Insulin ($\mu U/mL$)	0.1 (−0.8 to 1.1)	0.79	0.0 (−0.8 to 0.7)	0.94	0.6 (−0.3 to 1.4)	0.19
Hemoglobin A_{1C} (%)	−0.07 (−0.18 to 0.31)	0.16	−0.01 (−0.11 to 0.09)	0.78	0.2 (−0.12 to 0.15)	0.81
Triglyceride (mg/dL)	7 (−13 to 27)	0.48	7 (−6 to 19)	0.30	−8 (−23 to 7)	0.30
HDL cholesterol (mg/dL)	−1.8 (−4.5 to 0.9)	0.18	0.7 (−1.7 to 3.0)	0.55	−1.7 (−3.8 to 0.5)	0.13
LDL cholesterol (mg/dL)	1.7 (−4.3 to 7.7)	0.56	3.9 (−1.7 to 9.7)	0.16†	−8.1 (−16.7 to 0.3)	0.06‡
C-reactive protein (mg/dL)	−0.073 (−0.112 to 0.034)	0.008†‡	−0.012 (−0.032 to 0.004)	0.26§	0.005 (−0.041 to 0.051)	0.83§

* *P* values are for intragroup comparisons
† $P < 0.05$ compared with controls.
‡ $P < 0.05$ compared with propranolol.
§ $P < 0.05$ compared with carvedilol.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

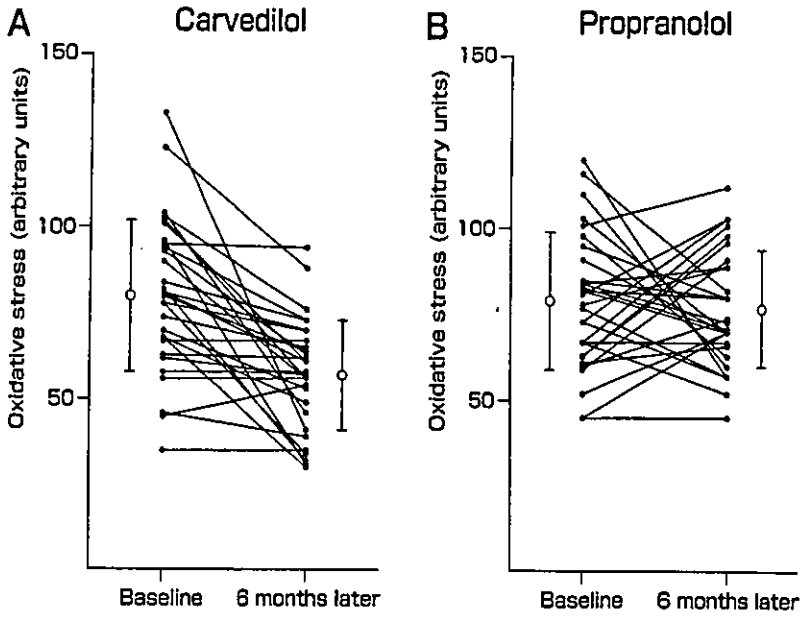

Figure 2. Oxidative stress in mononuclear cells at baseline and after 6 months of treatment with carvedilol 20 mg (A) or propranolol 60 mg (B). The empty circles and bars indicate means ± SD. $P = 0.003$ between values at baseline and 6 months in the carvedilol group; $P = 0.62$ between values at baseline and 6 months in the propranolol group; $P = 0.77$ between values at baseline in the carvedilol and propranolol groups; and $P < 0.001$ between values at 6 months in the carvedilol and propranolol groups.

Other Risk Factors

After 6 months of treatment, there were no statistically significant changes in risk factors, such as body mass index and levels of plasma insulin, hemoglobin A_{1C}, triglyceride, and HDL cholesterol, but LDL cholesterol levels increased significantly in the propranolol group compared with in controls (Table 2).

DISCUSSION

We found that both carvedilol and propranolol inhibited oxidative stress in polymorphonuclear cells. This may be explained partly by the decrease in mean arterial pressure, which correlated significantly with decreases in oxidative stress in polymorphonuclear cells in these patients. Increased oxidative stress in polymorphonuclear cells has been associated with increased blood pressure in spontaneously hypertensive rats (13), and with mean arterial pressure in patients with essential hypertension (12).

Although the decrease in oxidative stress in polymorphonuclear cells was greater in the carvedilol group than in the propranolol group, the decrease in mean arterial pressure was similar in both groups, which suggests that carvedilol may also have direct effects on oxidative stress. It has been reported that carvedilol modulates the NADPH oxidase of polymorphonuclear cells that interfere with the superoxide and free radical generation in vitro (14), as well as inhibits oxidative stress in polymorphonuclear cells in normotensive humans without affecting blood pressure (15), which is consistent with our study.

Although the clinical importance of decreasing oxidative stress in polymorphonuclear cells is not known, a link between increased oxidative stress and altered endothelial vascular reactivity and damage in an experimental model of hypertension has been described (16). It has also been reported that leukocyte count is associated with carotid intima-media thickness (17), and that polymorphonuclear cell count is related to oxidative stress (18).

We also found that carvedilol, but not propranolol, inhibited oxidative stress in mononuclear cells, even though the decrease in mean arterial pressure was similar in both treatment groups. It has been reported that carvedilol inhibits oxidation of LDL and oxidized LDL-induced adhesion of mononuclear cells to endothelial cells (19).

Oxidative stress in mononuclear cells is a possible link between oxidative stress and inflammation in vivo. We have previously shown that C-reactive protein level is related to oxidative stress in mononuclear cells (12). Recently, Shah et al reported that C-reactive protein is a novel marker of cardiovascular risk (20). In the present study, we found that carvedilol decreased C-reactive protein levels, whereas propranolol did not, suggesting that carvedilol may have anti-inflammatory effects, possibly by decreasing oxidative stress in mononuclear cells. Furthermore, there was a significant relation between decreases in oxidative stress in mononuclear cells and C-re-

active protein levels with carvedilol but not with propranolol, and carvedilol is reported to decrease myeloperoxidase activity, a finding indicative of reduced inflammation (21). However, it is possible that carvedilol may have direct effects on C-reactive protein, which would decrease oxidative stress in mononuclear cells, since mononuclear cells have receptors for C-reactive protein (22).

In summary, we observed that oxidative stress in polymorphonuclear and mononuclear cells was increased in hypertensive patients, and that carvedilol decreased oxidative stress, as well as C-reactive protein levels, in these patients. Still, clinicians should bear in mind that there are no data to indicate that antihypertensive agents that reduce oxidative stress provide better outcomes than those that simply lower blood pressure by the same degree.

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Comparative Effects of Valsartan Versus Amlodipine on Left Ventricular Mass and Reactive Oxygen Species Formation by Monocytes in Hypertensive Patients With Left Ventricular Hypertrophy

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OBJECTIVES To compare the effects of the angiotensin receptor blocker (ARB) valsartan versus the calcium channel blocker amlodipine, reactive oxygen species (ROS) formation by monocytes, C-reactive protein (CRP), and left ventricular (LV) mass were studied in 104 hypertensive patients with left ventricular hypertrophy (LVH).

BACKGROUND There is evidence that ARBs have blood pressure (BP)-independent effects on LV mass. Whether regression of LV mass by ARBs is correlated to ROS formation by monocytes and CRP is not fully understood yet.

METHODS A cross-sectional and prospective study was performed. Participants were randomly assigned to either the 80-mg valsartan (n = 52) or 5-mg amlodipine (n = 52) group and were treated for eight months. The left ventricular mass index (LVMI) was calculated from two-dimensional M-mode echocardiography. Formation of ROS by monocytes was measured by gated flow cytometry. In addition, CRP, plasma renin activity, plasma aldosterone, and traditional risk factors were assessed.

RESULTS Multiple regression analysis showed a significant correlation between LVMI and ROS formation by monocytes and between LVMI and CRP. Treatment reduced BP to a similar extent in both groups. Valsartan significantly reduced LVMI after eight months, but amlodipine had less effect (16% vs. 1.2%, n = 50, $p < 0.01$). Formation of ROS by monocytes was reduced to a greater extent with valsartan than with amlodipine (28% vs. 2%, n = 50, $p < 0.01$). Valsartan but not amlodipine reduced CRP levels. A significant correlation between changes in ROS formation by monocytes and LVMI or between CRP and LVMI was observed.

CONCLUSIONS The ARB valsartan has BP-independent effects on LVH, ROS formation by monocytes, and CRP in hypertensive patients with LVH. (J Am Coll Cardiol 2004;43:2116–23) © 2004 by the American College of Cardiology Foundation

Left ventricular hypertrophy (LVH), the most common cardiac consequence of hypertension, is a strong risk factor for cardiovascular complications and morbidity (1,2). In addition to pressure load, LVH appears to be modified by genetic and humoral factors (3,4). Among the most important of such factors is the renin-angiotensin system (5,6). Angiotensin II (ATII) is a powerful stimulator of myocyte growth, and many studies have shown the relationship between plasma ATII and LVH in essential hypertension (7,8).

There is evidence for increased inflammation in some patients with essential hypertension. Evidence for increased inflammation includes increased reactive oxygen species (ROS) formation by monocytes (9) and increased levels of plasma C-reactive protein (CRP) (10). Increased intracellular ROS formation by monocytes can lead to increased expression of cell surface adhesion molecules, which are regarded as markers of inflammation (11). Recently, we demonstrated the relationship between CRP and ROS formation by monocytes (12). It has been reported that CRP stimulates interleukin (IL)-6 release from monocytes (13) and that continuous activation of the IL-6 receptor induces myocardial hypertrophy in mice (14).

Angiotensin receptor blockers (ARBs) are a well-established form of antihypertensive therapy and have recently been shown to have benefits beyond blood pressure (BP) reduction—for example, in microalbuminuria in diabetic subjects (15). In the Losartan Intervention For Endpoint reduction (LIFE) trial (16), the ARB losartan had greater effects on LVH than the comparator substance atenolol for the same reduction in BP. To our knowledge, no study has investigated the possible involvement of

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Abbreviations and Acronyms

ARB	= angiotensin receptor blocker
ATII	= angiotensin II
BP	= blood pressure
CDCFH bis-AM ester	= carboxydichlorofluorescein diacetate bis-acetoxymethyl ester
CRP	= C-reactive protein
IL	= interleukin
LV	= left ventricular
LVH	= left ventricular hypertrophy
LVMI	= left ventricular mass index
ROS	= reactive oxygen species

inflammatory markers such as ROS formation by monocytes or CRP in an ATII-mediated increase in LV mass.

In the present study, we compared the changes in LVH and the changes in inflammatory markers such as monocyte ROS formation and levels of CRP caused by treatment with the ARB valsartan or the calcium channel blocker amlodipine. The possible involvement of inflammatory markers in an ATII-mediated increase in LV mass in hypertensive patients was also studied.

METHODS

Participants. This study consisted of two phases: a cross-sectional analysis of the relationship between the left ventricular mass index (LVMI) and risk of LVH, as well as a prospective, randomized, double-blinded study of hypertensive subjects with LVH who visited Osaka City University Hospital from April 1999 to April 2002. The primary outcome was the change in LVH associated with treatment, and the secondary outcome was the change in inflammatory markers such as oxidative stress in monocytes and CRP associated with treatment. The relationship between LVH and inflammatory markers was also studied.

Subjects who had not been treated for hypertension or who had discontinued antihypertensive agents and who had a BP of ≥140/90 mm Hg after a double-blinded, four-week placebo run-in period were included in the trial. During the run-in period, the presence of LVH was established by echocardiography and defined as LVMI >134 g/m^2 for men and >110 g/m^2 for women and/or septal thickness >12 mm at end diastole (17). None of the subjects were taking any medications, including nonsteroidal anti-inflammatory drugs, vitamin E, or other antioxidants. Randomization was performed by a controller who did not know the results and was using a computer-generated random allocation sequence in a numbered container. The subjects were given oral 80 mg of valsartan or the calcium channel blocker amlodipine (5 mg) for eight months. The protocol was approved by the Institutional Review Board of Osaka City University. Written, informed consent was obtained from all subjects.

Systolic and diastolic BPs were recorded as the average of the second and third rest period, seated, cuff BP measurements, in systole and diastole, respectively, measured after a 5-min rest period. Fasting blood samples were collected, and echocardiography was performed at baseline and at month 8. Obesity was estimated in terms of body mass index. Plasma insulin, plasma glucose, glycosylated hemoglobin, plasma cholesterol, triglyceride, high-density lipoprotein cholesterol, plasma renin and aldosterone concentrations were measured in venous blood. Serum CRP was measured by latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, Delaware), a highly sensitive technique.

Assay of ROS formation by monocytes. Formation of ROS by monocytes was measured using a gated flow cytometric technique, as described in previous studies (18,19), with some modifications (11). Fresh blood (1 ml) was collected from participants into preservative-free heparin (10 U/ml blood). The blood was pre-incubated for 15

Table 1. Baseline Characteristics of Hypertensive Patients

	Valsartan Group (n = 52)	Amlodipine Group (n = 52)	p Value*
Age (yrs)	62 ± 11	64 ± 12	0.6
Gender (M/F)	31/21	31/21	1.0
Body mass index (kg/m^2)	24.1 ± 3.8	24.3 ± 2.8	0.8
Systolic BP (mm Hg)	152 ± 8	152 ± 6	1.0
Diastolic BP (mm Hg)	93 ± 5	92 ± 6	0.4
ROS formation by monocytes (arbitrary units)	91 ± 20	86 ± 24	0.3
C-reactive protein (mg/dl)	0.10 (0.10–0.30)	0.10 (0.05–0.20)	1.0
LVMI (g/m^2)	166 ± 29	161 ± 39	0.5
Glycosylated hemoglobin (%)	5.4 ± 0.5	5.4 ± 1.0	1.0
Triglyceride (mg/dl)	113 ± 59	127 ± 85	0.3
HDL cholesterol (mg/dl)	53 ± 13	54 ± 13	0.7
LDL cholesterol (mg/dl)	118 ± 26	114 ± 34	0.5
Renin (ng/ml/h)	1.67 ± 2.25	1.83 ± 3.45	0.8
Aldosterone (pg/ml)	10.1 ± 3.9	11.7 ± 7.7	0.2

*Computed with the Mann-Whitney *U* test. Data are presented as the mean value ± SD, except for C-reactive protein, expressed as the median value (interquartile range).

BP = blood pressure; HDL and LDL = high- and low-density lipoprotein, respectively; LVMI = left ventricular mass index; ROS = reactive oxygen species.

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Table 2. Multiple Regression Analysis of the Relationship Between Left Ventricular Mass Index and Other Variables for the Entire Group

Variables	Regression Coefficient	Standard Error	Standardized Regression Coefficient	p Value
Age (yrs)	−0.12	0.31	−0.04	0.70
Gender (female)	−8.42	6.96	−0.12	0.23
Body mass index (kg/m²)	2.39	1.03	0.23	0.02
Systolic BP (mm Hg)	1.01	0.48	0.21	0.04
Diastolic BP (mm Hg)	−0.67	0.60	−0.11	0.26
Glycosylated hemoglobin (%)	−6.45	4.17	−0.15	0.13
Triglycerides (mg/dl)	−0.03	0.05	−0.06	0.54
HDL cholesterol (mg/dl)	−0.18	0.29	−0.07	0.55
LDL cholesterol (mg/dl)	−0.03	0.11	−0.02	0.83
C-reactive protein (mg/dl)	21.71	10.23	0.20	0.04
ROS formation by monocytes (arbitrary units)	0.45	0.15	0.29	<0.01
Renin (ng/ml/h)	2.17	1.12	0.19	0.06
Aldosterone (pg/ml)	−0.33	0.58	−0.06	0.57

The dependent variable is left ventricular mass index. Independent variables are age, gender, systolic BP, diastolic BP, glycosylated hemoglobin triglycerides, HDL cholesterol, LDL cholesterol, C-reactive protein, ROS formation by monocytes, renin, and aldosterone.
Abbreviations as in Table 1.

min with 2′,7′-carboxydichlorofluorescein diacetate bis-acetoxymethyl ester (CDCFH bis-AM ester) (100 μmol/l) in a 37°C water bath with gentle horizontal shaking. The CDCFH bis-AM ester is a nonpolar compound that is converted into a nonfluorescent polar derivative (CDCFH) by cellular esterases after incorporation into cells. CDCFH is membrane-impermeable and rapidly oxidized to the highly fluorescent carboxydichlorofluorescein in the presence of intracellular hydrogen peroxide and peroxidase. Red blood cells were lysed, and white blood cells were suspended in 1% paraformaldehyde/phosphate-buffered saline. The fixed samples were kept on ice until flow cytometric analysis on the same day. Formation of ROS by monocytes was measured as the fluorescence intensity by gated flow cytometry. The coefficients of variation of the intra- and inter-assays were 6.6% and 10.2%, respectively.

Echocardiography. Two-dimensional directed and guided M-mode echocardiographic studies were performed in all participants by one experienced investigator. The investigator reading the echocardiograms was blinded as to the treatment group. The LV mass was measured on the M-mode guided echocardiogram, according to the method recommended by the American Society of Echocardiography (20). Left ventricular mass was derived from the formula described by Devereux et al. (17):

$$\text{LV mass (g)} = 0.80 \times 1.04\,([\text{VSTd} + \text{LVIDd} + \text{PWTd}]^3 - [\text{LVIDd}]^3) + 0.6$$

where VSTd is the end-diastolic ventricular septal thickness; LVIDd is the LV end-diastolic internal dimension; and PWTd is the LV end-diastolic posterior wall thickness.

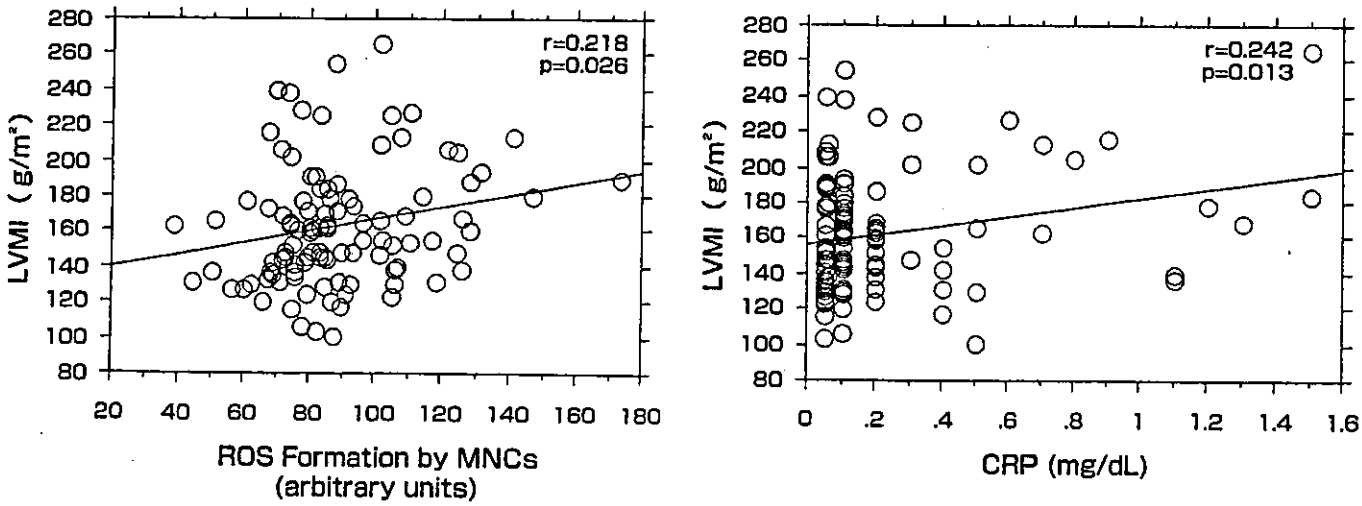

Figure 1. Relationships between reactive oxygen species (ROS) formation by monocytes (MNCs) and left ventricular mass index (LVMI) and between C-reactive protein (CRP) and LVMI (n = 104).

Table 3. Changes in Measurements From Baseline to Six Months

	Valsartan Group (n = 50)			Amlodipine Group (n = 50)			
	Difference	95% CI	p Value (Intragroup)	Difference	95% CI	p Value (Intragroup)	p Value (Intergroup)
Body mass index (kg/m^2)	0.2	(0 to 0.3)	0.4	0.2	(0 to 0.5)	0.1	1.00
Systolic BP (mm Hg)	−12	(−14 to −10)	<0.01	−11	(−13 to −9)	<0.01	0.48
Diastolic BP (mm Hg)	−7	(−8 to −6)	<0.01	−8	(−10 to −5)	<0.01	0.52
Monocyte oxidative stress (arbitrary units)	−26	(−31 to −21)	<0.01	−7	(−15 to 2)	0.1	<0.01
C-reactive protein (mg/dl)	−0.14	(−0.22 to −0.07)	<0.01	0.01	(−0.04 to 0.06)	0.9	<0.01
LVMI (g/m^2)	−28	(−35 to −21)	<0.01	−3	(−7 to 1)	0.1	<0.01
Glycosylated hemoglobin (%)	0	(−0.1 to 0.1)	1.0	0.1	(0 to 0.2)	0.3	0.16
Triglycerides (mg/dl)	1	(−11 to 12)	0.9	−10	(−29 to 10)	0.3	0.27
HDL cholesterol (mg/dl)	−1	(−3 to 1)	0.2	−1	(−3 to 1)	0.2	1.00
LDL cholesterol (mg/dl)	−2	(−8 to 4)	0.5	−5	(−10 to 1)	0.1	0.48
Renin (ng/ml/h)	0.4	(−0.1 to 1.0)	0.1	0	(−0.7 to 0.7)	0.9	0.19
Aldosterone (pg/ml)	−0.5	(−1.5 to 0.5)	0.3	−1	(−2.6 to 0.6)	0.2	0.59

CI = confidence interval; other abbreviations as in Table 1.

Statistical analysis. All data are expressed as the mean value ± SD, unless otherwise specified. Statistical analyses were performed using Statview 5.0 and JMP 4.0 (SAS Institute, Cary, North Carolina). Statistical analysis of the results for intergroup comparisons was performed with the Student *t* test preceded by an *F* test. A comparison of measurements at baseline and six months later was carried out by the paired *t* test (two-sided p value and 95% confidence interval [CI]). C-reactive protein was expressed as the median value (interquartile range), and p values were computed by the Mann-Whitney *U* test for intergroup comparisons at baseline. The relationship between LVMI and relevant covariates was examined by determination of standardized correlation coefficients and linear regression analysis.

RESULTS

Baseline characteristics. During the four-week run-in period, among the 120 patients who underwent randomization, 16 participants withdrew because they became normotensive. Two patients in each treatment group discontinued the study because of adverse events. In the valsartan group, liver dysfunction (n = 1) and headache (n = 1) were

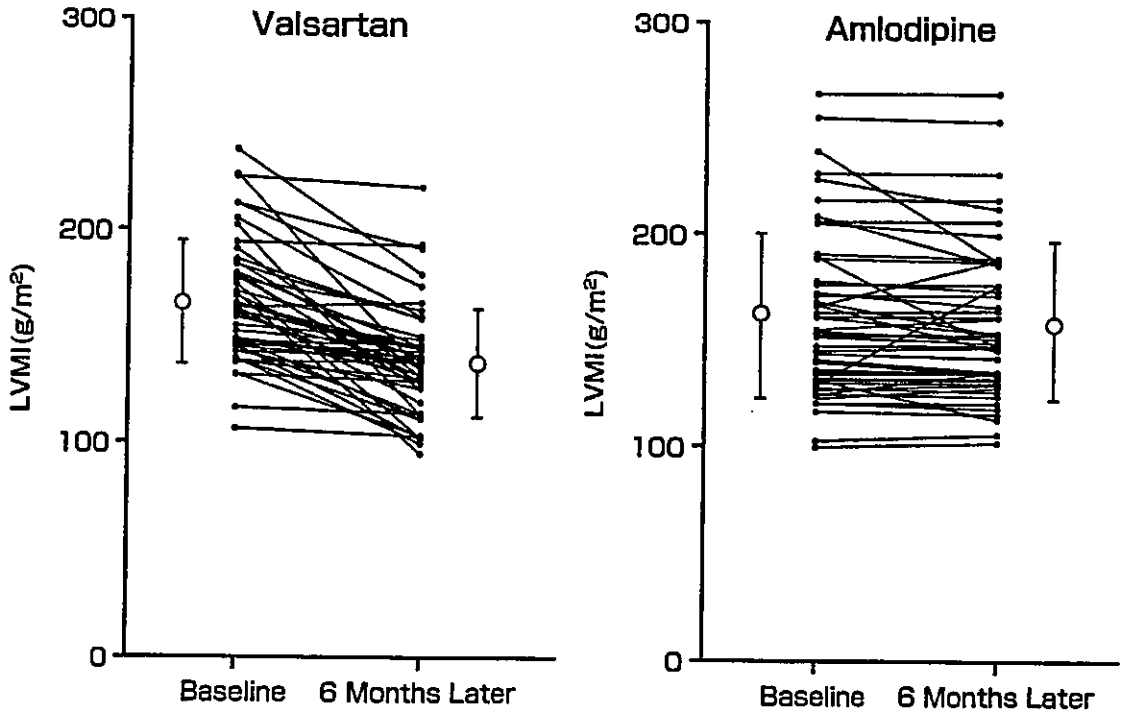

Figure 2. Effect of valsartan (n = 50) and amlodipine (n = 50) on left ventricular mass index (LVMI). The **open circles on the vertical bars** represent the mean value ± SD. $p < 0.01$ for baseline versus six months later in the valsartan group; $p < 0.01$ for baseline versus six months later in the amlodipine group.

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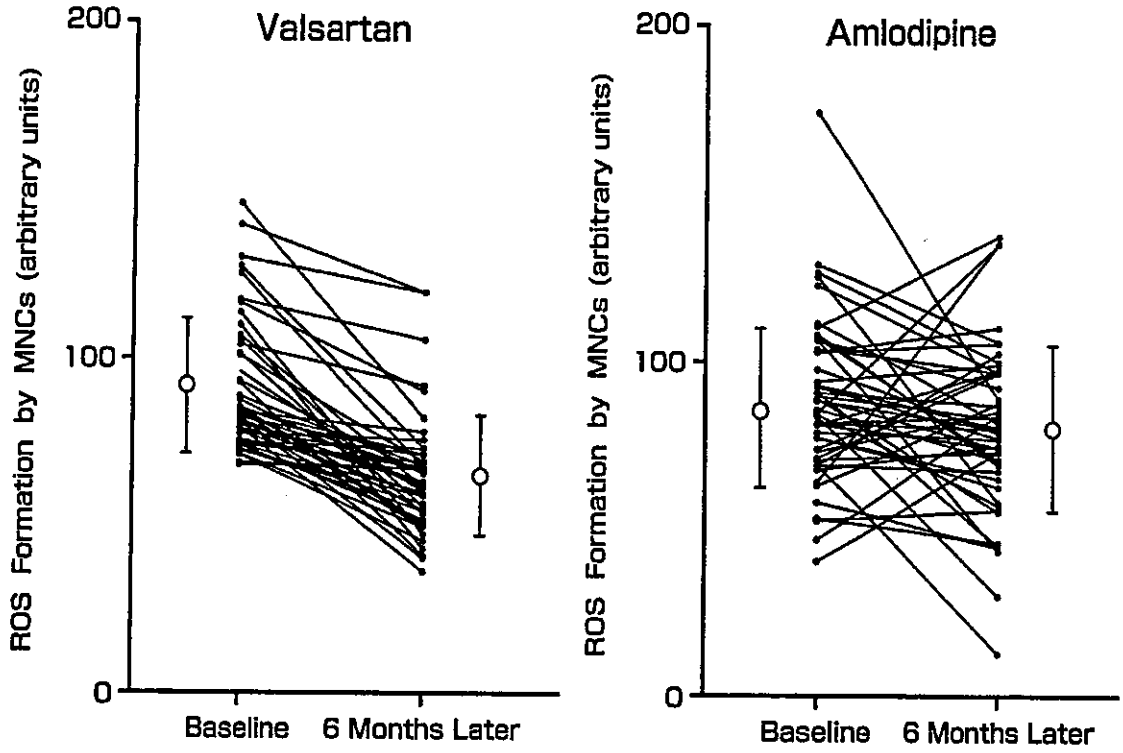

Figure 3. Effect of valsartan (n = 50) and amlodipine (n = 50) on reactive oxygen species (ROS) formation by monocytes (MNCs). The **open circles on the vertical bars** represent the mean ± SD. $p < 0.01$ for baseline versus six months later in the valsartan group; $p = 0.1$ for baseline versus six months later in the amlodipine group.

observed. In the amlodipine group, tachycardia (n = 1) and pretibial edema (n = 1) were observed.

Baseline characteristics in each treatment group are shown in Table 1. Subjects were well matched for age, body mass index, BP, gender, glycosylated hemoglobin, triglycerides, and cholesterol. There were no significant differences between the values for LVMI, CRP, and ROS formation by monocytes.

At baseline, multiple regression analysis was used to quantify the correlation of measured variables to LVMI. The results of the analysis are shown in Table 2. Formation of ROS by monocytes was significantly related to LVMI ($r = 0.29$, $p < 0.01$), and there was a significant correlation between CRP, systolic BP, and body mass index with LVMI (for CRP: $r = 0.20$, $p = 0.04$; systolic BP: $r = 0.21$, $p = 0.04$; body mass index: $r = 0.23$, $p = 0.02$). The relationships between oxidative stress in monocytes and LVMI and between CRP and LVMI are also shown in Figure 1.

Changes in BP. Both valsartan and amlodipine treatment reduced BP to a similar extent. In the valsartan group,

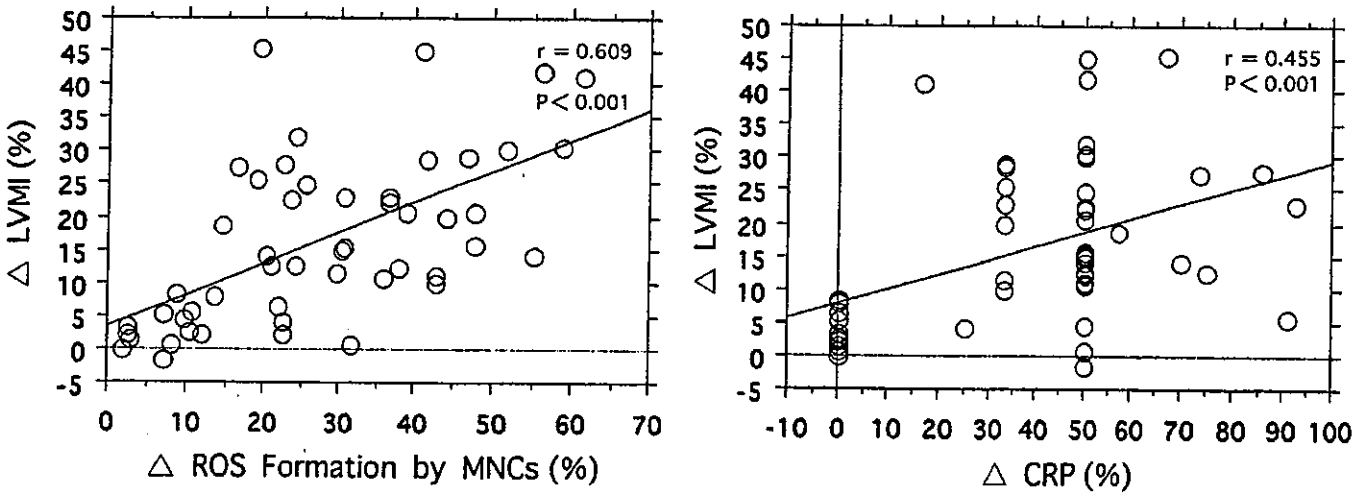

Figure 4. Relationships between the decrease (Δ) in reactive oxygen species (ROS) formation by monocytes (MNCs) and the decrease in left ventricular mass index (LVMI) and between the decrease in C-reactive protein (CRP) and the decrease in LVMI in the valsartan-treated group (n = 50).

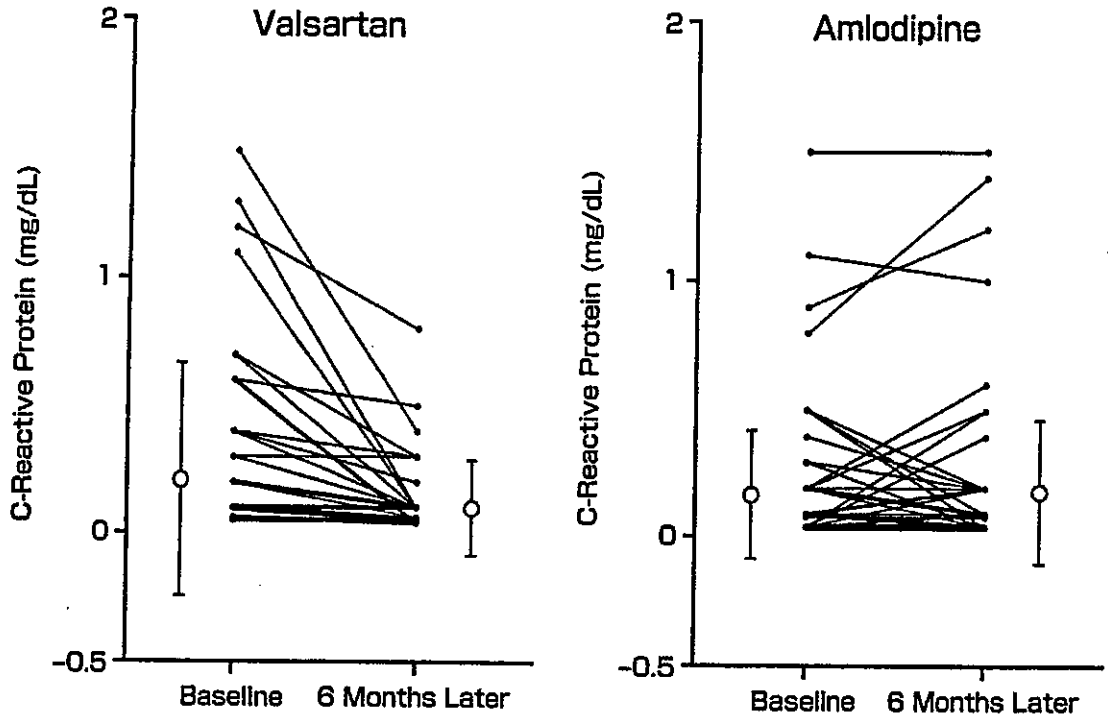

Figure 5. Effect of valsartan (n = 50) and amlodipine (n = 50) on C-reactive protein levels. The **open circles on the vertical bars** represent the mean value ± SD. $p < 0.01$ for baseline versus six months later in the valsartan group; $p = 0.7$ for baseline versus six months later in the amlodipine group.

systolic BP fell from 152 ± 8 mm Hg to 140 ±7 mm Hg and diastolic BP fell from 93 ± 5 mm Hg to 86 ± 5 mm Hg. The reductions in the amlodipine group were from 152 ± 6 mm Hg to 140 ± 6 mm Hg for systolic BP and from 92 ± 6 mm Hg to 84 ± 5 mm Hg for diastolic BP. At the end of the study, no intergroup difference in systolic and diastolic BP was observed (Table 3).

LVMI. Despite the very similar effects on BP, there were highly significant differences between valsartan and amlodipine treatment on LVMI (Table 1, Fig. 2). In the valsartan group, LVMI decreased from 166 ± 29 g/m^2 to 137 ± 26 g/m^2, representing a mean decrease of 16 ± 13% ($p < 0.01$) (Fig. 3, Table 3). In contrast, amlodipine had a lesser effect on LVMI, which was reduced from 161 ± 39 g/m^2 to 158 ± 37 g/m^2, a mean decrease of 1.2 ± 8.1% ($p = 0.14$) (Fig. 3, Table 3). The greater reduction in LVMI with valsartan compared with amlodipine was statistically significant ($p < 0.01$) (Table 3).

Formation of ROS by monocytes. As with LVMI, there were marked differences between the effects of the two treatments on ROS formation by monocytes. In the valsartan group, ROS formation by monocytes was reduced from 91 ± 20 to 65 ± 18 arbitrary units, representing a mean decrease of 28 ± 16% ($n = 50$, $p < 0.01$) (Fig. 3, Table 3). In the amlodipine group, ROS formation by monocytes was reduced from 86 ± 24 to 80 ± 25 arbitrary units, a mean decrease of 2 ± 39% ($n = 50$, $p = 0.11$) (Fig. 3, Table 3). The greater reduction in ROS formation by monocytes with valsartan compared with amlodipine was statistically significant ($p < 0.01$) (Table 3). Linear regression analysis showed a significant correlation between the decrease in LVMI and the decrease in ROS formation by monocytes in the valsartan group ($r = 0.61$, $p < 0.01$) (Fig. 4), but not in the amlodipine group ($r = 0.54$, $p = 0.59$).

CRP. In the valsartan group, CRP levels were reduced significantly, from 0.10 (95% CI 0.10 to 0.30) to 0.08 (95% CI 0.05 to 0.10) mg/dl, a mean decrease of 39 ± 26% ($p < 0.01$) (Fig. 5, Table 3). In contrast, there were no reductions in CRP levels in the amlodipine group (baseline: 0.10 mg/dl [95% CI 0.05 to 0.20]; month 8: 0.05 mg/dl [95% CI 0.05 to 0.20]; $p = 0.94$) (Fig. 5, Table 3). There was a significant correlation between the decrease in CRP and the decrease in LVMI in the valsartan group ($r = 0.46$, $p < 0.01$) (Fig. 4), but not in the amlodipine group ($r = 0.54$, $p = 0.89$). The greater reduction in CRP with valsartan compared with amlodipine was statistically significant ($p < 0.01$) (Table 3). In the valsartan group, there was also a significant correlation between the reduction in CRP and the decrease in ROS formation by monocytes ($r = 0.38$, $p < 0.01$), but no such correlation was observed in the amlodipine group ($r = 0.54$, $p = 0.62$).

Other traditional risk factors. We also examined the treatment-induced changes in other traditional risks factors, such as age, gender, body mass index, glycosylated hemoglobin, triglycerides, and high- and low-density lipoprotein cholesterol, which were measured at baseline and month 8 in both treatment groups. There were no differences in the baseline levels of these factors between the two groups (Table 1). None of these variables was affected significantly by treatment (Table 3).

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DISCUSSION

Summary of results. This study shows that significant differences exist between the effects of ARB with valsartan and calcium channel blockade with amlodipine on LV mass, CRP, and ROS formation by monocytes, and that these effects were unrelated to the effects on BP. Both valsartan and amlodipine produced similar reductions in BP, but the reductions in LVMI (primary outcome) and inflammatory markers such as ROS formation by monocytes and CRP (secondary outcome) were significantly greater with valsartan treatment (Table 3). There were significant correlations at baseline between LVMI and ROS formation by monocytes and between LVMI and CRP, as well as between decreases in the levels of these substances and regression of LVMI.

Effect of valsartan on monocyte oxidative stress and LV mass. In the present study, we found that valsartan inhibited ROS formation by monocytes. It has been previously reported that ATII receptors are expressed in monocytes and that ATII increases ROS formation by monocytes (21). Locally produced ATII might be involved in this increase (5,6). However, in the present study, we can only conclude that endogenous ATII may increase ROS formation by monocytes.

The precise mechanism of ROS formation by monocytes in conjunction with cardiomyocytes and LV mass alteration remains to be elucidated. However, a multiple regression analysis indicated that there is a significant correlation between ROS formation by monocytes and LV mass (Fig. 1, Table 2). In the valsartan group, the reduction in LVMI correlated with the reduction in ROS formation by monocytes (Fig. 4), which suggests that the ATII-induced ROS formation by monocytes may be one of the major causes of increased LV mass in patients with essential hypertension, apart from the increases in LV mass usually attributed to elevated BP. Valsartan has been found to have an antioxidative effect (22), and it has been reported that increased ROS formation by monocytes increases cytokine production, including IL-6 (13), which may cause myocardial hypertrophy (14). Hence, reduced ROS formation by monocytes with valsartan treatment may result in reduced IL-6 production and a corresponding decrease in LVH. In fact, it has been reported that IL-6 production is decreased by valsartan (23).

Effect of valsartan on CRP and LV mass. The most conspicuous differences between the two therapies in the present study were their effects on CRP levels. The CRP reduction with valsartan treatment was significantly greater than that with amlodipine (Table 3). We also observed that the decrease in CRP in the valsartan group significantly correlated to the decrease in LVMI (Fig. 4). C-reactive protein has a direct modulatory effect on monocytes, which promote IL-6 release (13) and may cause cardiac hypertrophy (14). Thus, ATII may interact with CRP, or monocytes, and thus cause hypertrophy.

It is interesting to note that studies with the ARBs losartan and candesartan in patients with coronary artery disease recently reported no effects on CRP levels from treatment (24,25). Whether this is due to differences in study design or differences between the ARBs remains to be established. However, it should be pointed out that some patients in the present study had severe LVH. In such patients, the cardiac renin-angiotensin system may be enhanced (26), which may exacerbate the inflammatory response, including CRP.

Study limitations. A limitation of the present study that may be considered significant is a possible bias due to patient selection. Some patients in the present study had severe LVH. This may call into question the applicability of the results to other patient populations.

Conclusions. The ARB valsartan seems to have effects on CRP, ROS formation by monocytes, and LVMI, unrelated to a reduction in BP. There were also significant correlations at baseline between LVMI and ROS formation by monocytes and between LVMI and CRP, as well as between decreases in the levels of these substances and regression of LVMI, suggesting the possible involvement of inflammatory response such as ROS formation by monocytes and CRP in an ATII-mediated increase in LV mass in hypertensive patients.

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