*Original Article*

# Candesartan Decreases Carotid Intima-Media Thickness by Enhancing Nitric Oxide and Decreasing Oxidative Stress in Patients with Hypertension

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**Candesartan has been reported to produce nitric oxide (NO) and to decrease oxidative stress in animal studies. We investigated candesartan's effect on the production of NO and oxidative stress as well as on carotid intima-media thickness (IMT) in hypertensive patients. One-hundred age-matched hypertensive patients were enrolled into an angiotensin II receptor blocker (ARB) group (*n*=50) or a non-ARB group (*n*=50). The ARB group was treated with candesartan 8 mg and, when needed, Ca channel blockers, angiotesin-converting enzyme (ACE) inhibitors, and/or *β*-blockers. The non-ARB group was treated with drugs other than ARB. Carotid IMT was assessed by echocardiography before and 12 and 24 months after treatment. The urine levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), an indicator of oxidative stress, and the serum levels of NOx, an indicator of NO, were measured. Blood pressure decreased to below 140/90 mmHg to the same extent in both groups. Carotid IMT decreased significantly in the ARB group, but not in the non-ARB group, at 12 and 24 months after treatment. The urine levels of 8-OHdG decreased significantly at 6 and 12 months after treatment in the ARB group but did not decrease in the non-ARB group. The serum levels of NOx increased significantly at 6 and 12 months after treatment in the ARB group but not in the non-ARB group. In conclusion, candesartan decreases carotid IMT by enhancing NO production and decreasing oxidative stress in patients with hypertension. (*Hypertens Res* 2008; 31: 271–279)**

***Key Words*: candesartan, nitric oxide, oxidative stress, intima-media thickness**

## Introduction

Hypertension is one of the risk factors for atherosclerosis (*1*, *2*). The renin-angiotensin system plays an important role in the development and maintenance of hypertension and accelerates atherosclerosis (*3*, *4*). Angiotensin II's effect on vascular smooth muscle cells causes vessel wall remodeling, characterized by decreased lumen diameter and increased media thickness (*5*, *6*). The stimulation of angiotensin II receptors has been reported to produce oxidative stress in the vessels and to damage the vessel walls (*7*, *8*). On the other hand, nitric oxide (NO) has been reported to protect against atherosclerosis (*9*). Antihypertensive drugs, angiotensin II receptor blockers (ARBs), have been reported to produce NO and to decrease oxidative stress in animal studies (*10*, *11*). Therefore, we hypothesized that the blockade of angiotensin II receptors will decrease oxidative stress and increase NO

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**Table 1. Patient's Characteristics**

| | ARB group ($n$=50) | Non-ARB group ($n$=50) |
|---|---|---|
| Age, years old | 59.8±10.2 | 58.8±9.8 |
| Men, *n* (%) | 23 (46) | 21 (42) |
| Systolic BP, mmHg | 181.26±17.85 | 179.46±18.93 |
| Diastolic BP, mmHg | 100.58±11.47 | 101.32±11.40 |
| Smoking, *n* (%) | 13/50 (26) | 10/50 (20) |
| Diabetes mellitus, *n* (%) | 4/50 (8) | 4/50 (8) |
| Hyperlipidemia, *n* (%) | 18/50 (36) | 19/50 (38) |
| IHD, *n* (%) | 5/50 (10) | 2/50 (4) |
| CVD, *n* (%) | 11/50 (22) | 6/50 (12) |
| Added drugs (mean number) | 2.30±0.81 | 2.14±0.69 |
| CCB, *n* (%) | 29 (58)* | 46 (92) |
| ACEI, *n* (%) | 21 (42)* | 22 (44) |
| β-Blocker, *n* (%) | 10 (20) | 26 (52) |
| α-Blocker, *n* (%) | 3 (6) | 8 (16) |
| Diuretics, *n* (%) | 2 (4) | 5 (10) |
| Mean-IMT, mm | 0.867±0.175 | 0.848±0.251 |
| PS | 1.70±1.95 | 1.66±2.39 |
| 8-OHdG, ng/mg Cr | 10.99±4.67 | 12.21±5.14 |
| NOx, μmol/L | 43.80±20.28 | 44.37±21.81 |
| Mean-LD, mm | 7.816±0.839 | 7.561±0.802 |

*$p$<0.01. ARB, angiotensin II receptor blocker; BP, blood pressure; IHD, ischemic heart disease; CVD, cerebrovascular disease; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor; IMT, intima-media thickness; PS, plaque score; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; NOx, nitrogen oxides; LD, lumen diameter.

production, thereby improving atherosclerosis in patients with hypertension. We investigated the effect of candesartan, an ARB, on the production of NO and oxidative stress as well as on carotid intima-media thickness (IMT) in patients with hypertension.

## Methods

### Subjects

One-hundred patients with high blood pressure were enrolled into either an ARB group ($n$=50) or a non-ARB group ($n$=50). This study was a prospective open-label, randomized study that started in April 2002 and ended in October 2005. The subjects were consecutively selected from among outpatients who had systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg. None of the subjects had been treated with antihypertensive drugs for at least 6 months at the time of their first visit to our hospital.

The ARB group was treated with candesartan 8 mg and, when needed, Ca channel blockers, angiotensin-converting enzyme inhibitors (ACEI), β-blockers, α-blockers, and/or

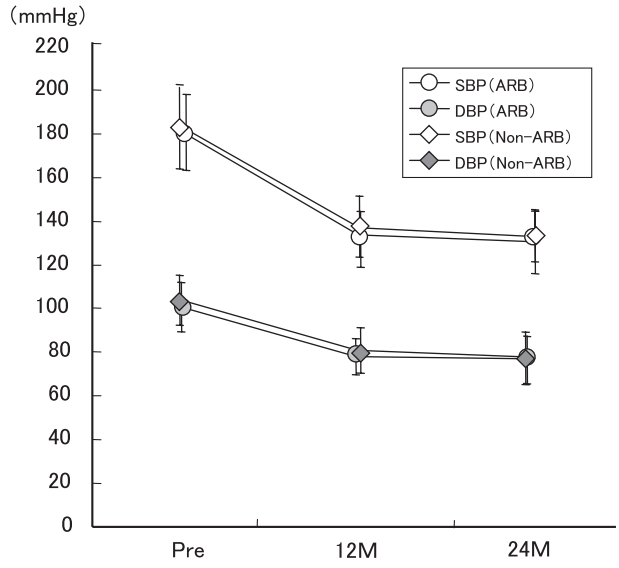


**Fig. 1.** *Time course changes in systolic and diastolic blood pressure in each group.*

diuretics were added according to Japanese Society of Hypertension (JSH 2000) guidelines until systolic and diastolic blood pressures fell within 140 and 90 mmHg, respectively. The non-ARB group was treated with at least one of the antihypertensive drugs mentioned above other than ARB. According to the guidelines, patients with systolic blood pressure of more than 180 mmHg and/or diastolic blood pressure of more than 110 mmHg, as well as high-risk patients with moderate hypertension and risk factors and/or complications, were immediately treated with antihypertensive drugs and given urine and blood examinations, in addition to carotid echocardiography, on the same day that these blood pressures were obtained.

Patients with diastolic blood pressure above 120 mmHg, HbA1c above 9%, serum creatinine above 2.0 mg/dL, a cancer-bearing state, or severe hepatic damage were excluded. Also excluded were patients whom the doctor considered inappropriate for enrollment.

The study protocol was approved by the Ethics Committee of Ibi Kosei Hospital. Written informed consent was obtained from each patient before starting the study.

### Follow-Up

All subjects were followed-up by doctors at the outpatient clinic of Ibi Kosei Hospital and were treated with the aim of reducing systolic blood pressure and diastolic blood pressure to below 140/90 mmHg by standard lifestyle modification and pharmacological intervention according to JSH 2000 guidelines. We aimed to reach this goal within 3 months after entry and to maintain blood pressure strictly below 140/90 mmHg throughout the 2-year follow-up period. Blood pres-

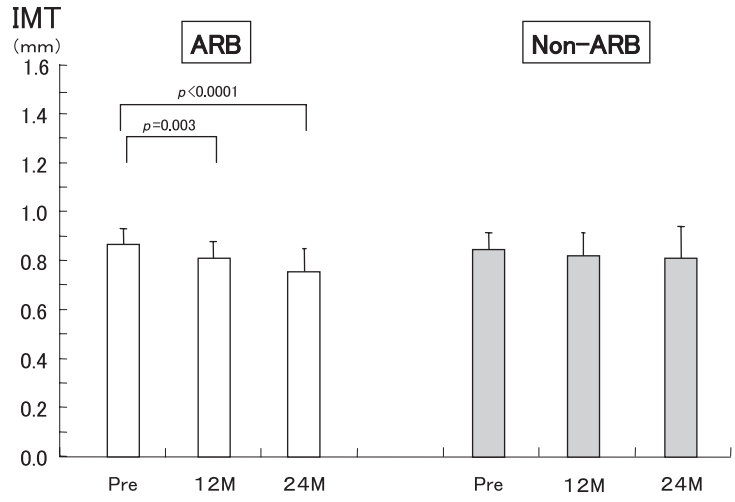


**Fig. 2.** *Time course changes in carotid intima-media thickness (IMT) in each group.*

sure was measured using a sphygmomanometer once a month at the outpatient office, and echocardiography was undertaken before and after 2 years of follow-up. None of the patients experienced any cardiovascular events during the 2-year follow-up period.

### Blood Pressure Measurement

Blood pressure was measured with a standard mercury sphygmomanometer after the subject had been seated for at least 10 min. Three consecutive measurements were obtained, and the mean of the last two was regarded as the blood pressure.

### Measurement of Plaque Score, Carotid IMT, Carotid Lumen Diameter, and Levels of Oxidative Stress and Nitric Oxide

The carotid IMT, plaque score, and carotid lumen diameter (LD) were assessed by echocardiography before and at 12 and 24 months after treatment. Carotid IMT was defined as the mean of IMT measurements at six sites on the carotid arteries using B-mode ultrasound according to the method performed in the ARIC study (*12*). Carotid LD was measured at 1 cm proximal to the bifurcation of the internal carotid arteries using B-mode ultrasound. The plaque score was counted by summing the maximum thickness of the intima-media complex (plaque thickness) measured in mm on the near and far walls at each of four divisions on both sides of the carotid arteries. This scoring system was taken from Handa *et al*. (*13*). The normal ranges of IMT and LD in our hospital were 0.5–1.0 mm and 6.1–7.9 mm, respectively. The coefficient of variation (CV) value of carotid IMT was 3.7%.

The urine levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), an indicator of oxidative stress, and the serum levels of NOx, an indicator of NO, were measured before and at 12 and 24 months after treatment. 8-OHdG was measured using an ELISA kit (Japan Institute for the Control of Aging, Nikken SEIL, Shizuoka, Japan), and NOx (nitrite/nitrate; $NO_2$-/$NO_3$-) was measured using an ELISA kit (Nitrite/Nitrate Colorimetric Assay Kit, Cayman Chemical, Ann Arbor, USA). The normal ranges of urine 8-OHdG levels and serum NOx levels in our hospital were 7.50–21.3 ng/mg Cr and 0.79–70.7 μmol/L, respectively. The CV value of urine 8-OHdG levels was 7.1%, and that of serum NOx levels was 2.3%. On the day 8-OHdG and NOx were measured, patients did not eat breakfast but took their medications.

### Statistical Analysis

All the data obtained are presented as means±SD. All statistical analyses were conducted with SPSS version 12.0 (SPSS, Chicago, USA). The effects of the drugs were analyzed by two-way analysis of variance with repeated measures (repeated ANOVA) followed by Dunnett's post hoc test. In Figs. 5 and 7, the data were analyzed by ANOVA followed by Scheffe's test. A $p$ value less than 0.05 was considered significant.

## Results

Table 1 shows the subjects' baseline characteristics. There were no differences in any of the parameters, such as smoking, diabetes mellitus, hyperlipidemia, ischemic heart disease (IHD), or cerebrovascular disease (CVS), between the groups. The patients were distributed among all ages from the 40s to the 80s, and there was no difference in the distribution of ages between the groups. There was no significant difference in systolic or diastolic blood pressure between the groups at baseline. There was no difference in the number of patients treated with ACEI and the number of drugs combined

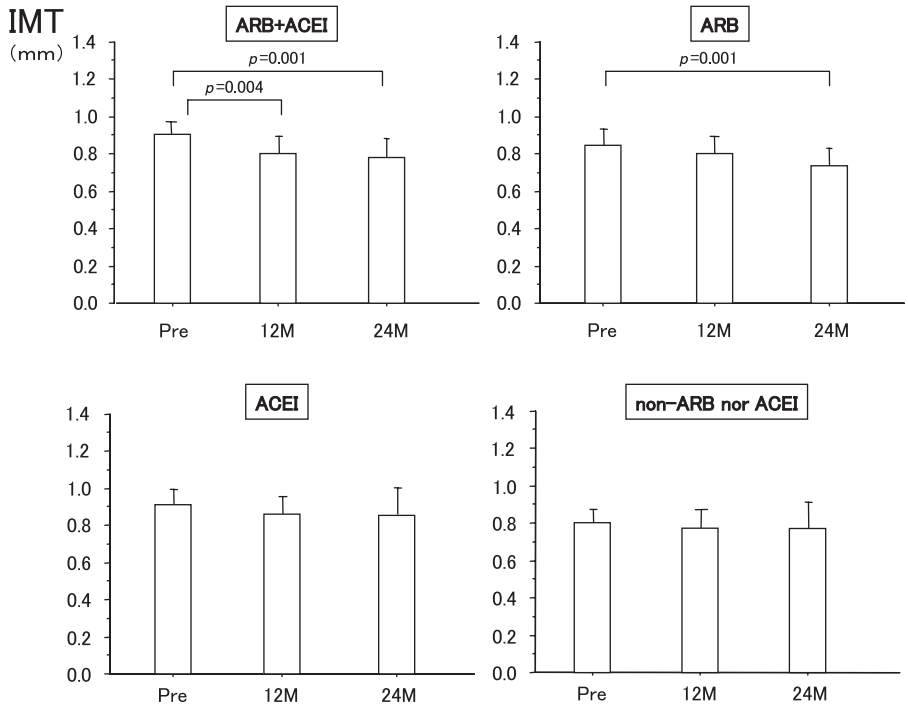


**Fig. 3.** *Time course changes in the carotid intima-media thickness (IMT) in the ARB+ACEI group, the ARB-alone group, the ACEI-alone group, and the group with neither ARB nor ACEI.*

between the two groups.

The number of subjects with systolic blood pressure above 180 mmHg and/or diastolic blood pressure above 110 mmHg was 24 (48%) in the ARB group and 25 (50%) in the non-ARB group, and the number of high-risk patients with moderate high blood pressure and risk factors and/or complications was 5 in the ARB group and 5 in the non-ARB group.

Since the percentage of patients receiving ACEI was 42 and 44% in the ARB and non-ARB groups, respectively, we attempted to assess the effects of ARB only, of ACEI only, and of the two together. We therefore performed a sub-analysis, dividing the patients into four groups (ARB+ACEI, ARB-alone, ACEI-alone, and neither).

## Effect on Blood Pressure

Time course changes in blood pressure in response to antihypertensive therapy regimens are shown in Fig. 1. At 12 and 24 months after treatment, systolic blood pressure significantly decreased in the ARB group relative to the baseline, from 181.26±10.76 to 131.68±12.39 mmHg and to 129.60±16.90 mmHg, respectively; in the non-ARB group, the decreases at 12 and 24 months were from 179.46±9.76 to 131.50±10.79 mmHg and to 126.73±15.25 mmHg. At the same time points, diastolic blood pressure significantly decreased, from 101.38±7.12 to 77.17±8.20 mmHg and to 76.22±11.19 mmHg in the ARB group; in the non-ARB group the decreases were from 102.52±7.71 to 76.96±8.53 mmHg and to 74.28±12.05 mmHg. There were no significant differences between these time points for both systolic and diastolic blood pressure.

## Effect on Plaque Score

The plaque score decreased from 1.70±0.78 to 1.29±0.86 (12M, $p=0.395$) and to 0.93±1.18 (24M, $p=0.015$) in the ARB group; in the non-ARB group, the decreases were from 1.66±0.70 to 1.29±0.76 (12M, $p=0.235$) and to 1.01±1.09 (24M, $p=0.617$). Relative to baseline, the plaque score was significantly decreased in the ARB group at 24 months after treatment.

## Effect on Carotid IMT

Figure 2 shows the time course changes in the carotid IMT. The values decreased significantly from 0.867±0.066 to 0.795±0.076 mm (12M, $p=0.003$) and to 0.735±0.102 mm (24M, $p<0.0001$) in the ARB group, whereas in the non-ARB group the decreases were not significant: from 0.848±0.087 to 0.811±0.097 mm (12M, $p=0.280$) and to 0.790±0.138 mm (24M, $p=0.819$) at 12 and 24 months after treatment, respectively.

When the patients were divided into four groups—ARB+ACEI ($n=21$), ARB alone ($n=29$), ACEI alone ($n=22$), and neither ($n=28$) the carotid IMT decreased from 0.902±0.063 to 0.795±0.070 mm (12M, $p=0.004$) and to

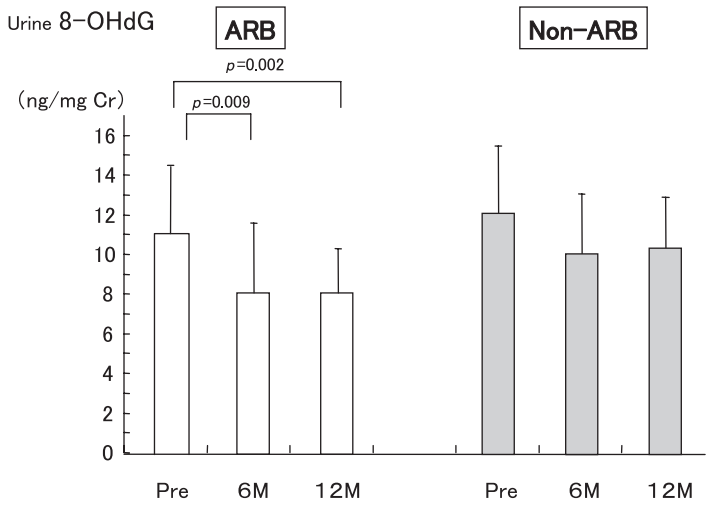


**Fig. 4.** *Time course changes in the urine levels of 8-OHdG in each group.*

0.727±0.098 mm (24M, $p=0.001$) in ARB+ACEI, and from 0.842±0.072 to 0.795±0.080 mm (12M, $p=0.236$) and to 0.738±0.104 mm (24M, $p=0.001$) in the ARB group; but no decreases were found in the other two groups (Fig. 3).

### Effect on Carotid Artery LD

The carotid LDs were 7.82±0.23 mm at 0 month, 7.93±0.31 mm at 12 months, and 7.80±0.44 mm at 24 months ($p=0.386$) in the ARB group, and 7.56±0.15 mm at 0 month, 7.73±0.21 mm at 12 months and 7.72±0.35 mm at 24 months ($p=0.081$) in the non-ARB group. Neither group showed any significant change in LD during the course of treatment, although LD tended to increase in the non-ARB group.

### Effect on Urine 8-OHdG Levels

The urine levels of 8-OHdG decreased significantly, from 10.96±3.26 to 7.61±3.74 ng/mg Cr (6M, $p=0.004$) and to 7.89±2.33 ng/mg Cr (12M, $p=0.009$) at 6 and 12 months after treatment in the ARB group, but the decreases were not significant in the non-ARB group: from 12.00±3.27 to 9.89±3.55 ng/mg Cr (6M, $p=0.095$) and to 10.34±2.23 ng/mg Cr (12M, $p=0.068$), as shown in Fig. 4.

When the patients were further divided into four groups, the urine levels of 8-OHdG at 6 months were 8.421±3.283 in ARB+ACEI, 7.083±2.558 in the ARB alone, 7.827±3.891 in ACEI alone, and 13.971±5.169 in neither. At 12 months, the levels were 7.659±3.305 in ARB+ACEI, 8.273±4.311 in ARB alone, 8.360±2.917 in ACEI alone, and 11.433±3.339 in neither (Fig. 5). The urine levels of 8-OHdG were significantly lower in ARB+ACEI ($p=0.030$, 6M; $p=0.008$, 12M), ARB alone ($p=0.024$, 6M; $p=0.018$, 12M), and ACEI ($p=0.019$, 6M; $p=0.045$, 12M) than in neither.

### Effect on Serum NOx Levels

The serum levels of NOx increased significantly, from 37.09±25.20 to 62.88±28.89 μmol/L (6M, $p=0.009$) and to 66.29±17.98 μmol/L (12M, $p=0.003$) at 6 and 12 months after treatment in the ARB group, but they did not increase in the non-ARB group: from 44.84±12.76 to 44.14±13.87 μmol/L (6M, $p=0.557$) and to 44.17±8.71 μmol/L (12M, $p=0.997$) (Fig. 6). When the patients were further divided, the levels at 6 months were 72.279±36.292 in ARB+ACEI, 53.100±29.586 in ARB, 44.418±22.214 in ACEI alone, and 35.071±17.993 in neither, and the levels at 12 months were 73.518±30.420 in ARB+ACEI, 59.436±26.959 in ARB, 45.127±23.248 in ACEI alone, and 43.372±13.890 in neither (Fig. 7). At 12 months, the levels were significantly higher in ARB+ACEI than in ACEI ($p=0.019$, 12M) and in neither ($p=0.007$, 12M).

### Side Effects

No serious side effects of the drugs were observed in either group during the study period.

## Discussion

The present study demonstrated that, at both 12 and 24 months after administration, candesartan significantly decreased blood pressure to the same extent in the ARB group as in the non-ARB group. The plaque score was decreased in the ARB group at 24 months after treatment. At both 12 and 24 months, carotid IMT decreased significantly in the ARB group but not in the non-ARB group. The urine levels of 8-OHdG were significantly lower than baseline at 6 and 12 months in the ARB group, but not in the non-ARB group. The serum levels of NOx, an indicator of nitric oxide, were signif-

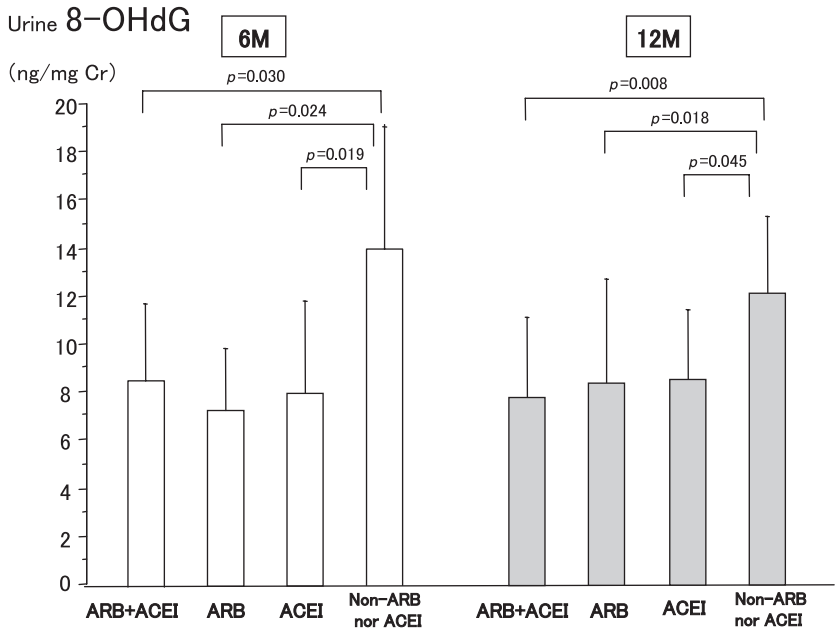


**Fig. 5.** *The urine levels of 8-OHdG at 6 and 12 months in the ARB+ACEI group, the ARB-alone group, the ACEI-alone group, and the group with neither ARB nor ACEI.*

icantly higher at 6 and 12 months in the ARB group, but not in the non-ARB group.

Hypertension is one of the risk factors for cardiovascular disease (*14*). Reduction in blood pressure reduces the likelihood of cardiovascular disease (*15*). Under the conditions where blood pressure decreased to the same extent in both the ARB and non-ARB groups, carotid IMT decreased significantly in the former but not in the latter at both 12 and 24 months after treatment. This suggests that candesartan treatment decreased carotid IMT independent of the decrease in blood pressure, since carotid IMT did not decrease in the non-ARB group despite the decrease in blood pressure.

When the patients were divided into four groups—ARB+ACEI, ARB alone, ACEI alone, and neither—the carotid IMT at 24 months decreased significantly in ARB+ACEI and ARB alone, tended to decrease in ACEI alone, but did not decrease in the fourth group (Fig. 3). This may suggest that the blockade of the renin angiotensin system by ARB and/or ACEI decreases carotid IMT and that the combination of ARB and ACEI especially strongly decreases carotid IMT.

Meta-analysis of the previous large-scale clinical trials has revealed that Ca channel blockers, but not ACEI, significantly decreased the carotid IMT (*16*). However, two prospective trials—INSIGHT (nifedipine) and MIDAS (isradipine)—showed that Ca channel blockers did not reduce carotid IMT, although they did prevent the thickening of IMT compared with diuretics (*17*, *18*). Indeed, in the present study Ca channel blockers were given to 58% of the patients in the ARB group and to 92% of the patients in the non-ARB group. The non-ARB group did not show a reduction of carotid IMT, consistent with the results of INSIGHT and MIDAS. The discrepancy between the results of the meta-analysis by Wang *et al*. (*16*) and the present study may be due to the duration of treatment with Ca channel blockers, the kinds of Ca channel blockers used (in the present study, amlodipine, nifedipine, nicardipine, benidipine, cilnidipine, and diltiazem), and the extent of the decrease in blood pressure.

There seem to be several mechanisms by which carotid IMT is decreased significantly in the ARB group. One possible mechanism may be enhanced production of NO. While ARBs block the angiotensin II type 1 (AT1) receptors, plasma levels of angiotensin II increase, and then increased angiotensin II stimulates angiotensin II type 2 (AT2) receptors (*10*). It has been reported that NO is produced *via* the activation of AT2 receptors and bradykinin B2 receptors (*19*, *20*). As a matter of fact, in the present study, the serum levels of NOx, an indicator of NO, were significantly increased at 6 and 12 months after treatment relative to baseline in the ARB group, but not in the non-ARB group. Since NO has been reported to improve atherosclerosis (*9*), it is possible that NO produced by candesartan treatment may be related to the decrease in carotid IMT in the present study. Vascular oxidative stress limits the bioavailability of endothelial NO and promotes atherosclerosis; while NO itself exerts antioxidative effects (*21*). It has been reported that NO donor isosorbide mononitrate could inhibit the increase in vascular bioavailability of superoxide and prevent IMT of the thoracic aorta in the cholesterol-fed rabbits (*21*). In the present study, we used the serum level of NOx as an indicator of NO. However, since the serum

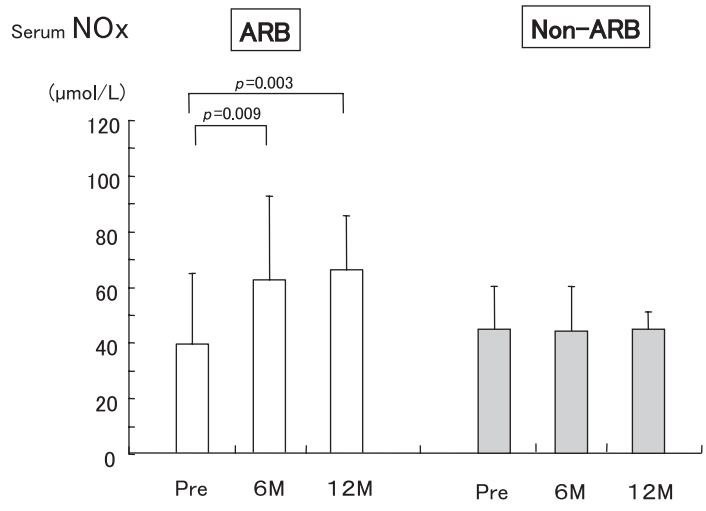


**Fig. 6.** *Time course changes in the serum levels of NOx in each group.*

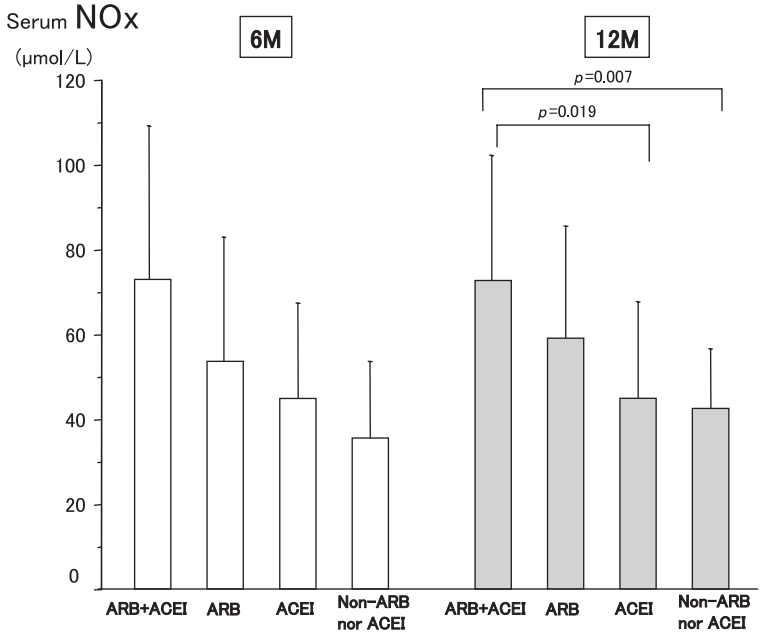


**Fig. 7.** *The serum levels of NOx at 6 and 12 months in the ARB+ACEI group, the ARB-alone group, the ACEI-alone group and the group with neither ARB nor ACEI.*

level of NOx is determined by the balance between the production and clearance of NOx, the serum level of NOx does not necessarily represent the production of NO (*22*). The serum level of NOx is reported to be influenced by renal function, diet (*23*), age, and smoking (*24*). However, in the present study, serum NOx may not have been influenced, because there were no subjects with abnormal renal function and the subjects fasted more than 12 h, and there was no significant difference in the age and smoking rate between the groups. When the patients were divided into the four groups, the serum levels of NOx at 12 months were higher in the order ARB+ACEI, ARB alone, ACEI alone, and neither (Fig. 7). The serum NOx levels were significantly higher in ARB+ACEI than in ACEI or in neither. These results suggest that the blockade of the renin angiotensin system by ARB and/or ACEI leads to the production of NO. The mechanism by which carotid IMT is more decreased in the ARB group than in the non-ARB group may be related to the greater increase in serum NOx levels by the combination of ARB and ACEI in the ARB group.

Another possible mechanism by which carotid IMT is decreased in the ARB group may be decreased vascular oxidative stress. Oxidative stress has been known to damage vessel walls and to be a cause of atherosclerosis (*7*, *8*). It has been reported that the activation of AT1 receptors leads to the release of vascular superoxide (*25*). One of the important consequences of increased superoxide production induced by activation of AT1 receptors is the inactivation of nitric oxide (*26*). The two radicals, superoxide and nitric oxide, reacts with one another, resulting in the formation of peroxinitrite (*26*). The endogenous superoxide dismutase and other scavengers of superoxide keep this reaction to a minimum. However, this reaction proceeds when cellular levels of superoxide are increased upon activation of AT1 receptors. Loss of nitric oxide *via* this mechanism leads to endothelial dysfunction and then promotes atherosclerosis. AT1 receptor antagonists or ACEIs normalize oxidative stress and endothelial dysfunction and reduce the progression of atherosclerosis (*27*, *28*).

Therefore, the blockade of AT1 receptors by ARBs would decrease the production of oxyradicals in the vessel walls. In the present study, treatment with candesartan in the ARB group but not the non-ARB group significantly decreased the urine levels of 8-OHdG, an indicator of oxidative stress, at 6 and 12 months after treatment. When the patients were divided into four groups the urine levels of 8-OHdG were lower in ARB+ACEI, ARB alone, and ACEI alone than in the group with neither (Fig. 5). These results suggest that the blockade of the renin angiotensin system by ARBs and/or ACEIs leads to the inhibition of oxidative stress. This also suggests that the mechanism by which carotid IMT is decreased more in the ARB group than in the non-ARB group may be due to the more decreased urine levels of 8-OHdG by the combination of ARB and ACEI in the ARB group.

In addition, consistent with our study, it has been reported that the combination of an ARB and an ACEI exerts an additive inhibitory effect against neointima formation *via* nitric oxide production and suppression of oxidative stress in rats (*29*). The renin-angiotensin system plays an important role in the development and maintenance of hypertension and the development of atherosclerosis (*3*, *4*). In the present study, candesartan, an AT1 receptor blocker, not only decreased the blood pressure but also decreased the carotid IMT, suggesting that candesartan has effects beyond lowering blood pressure, such as decreasing carotid IMT, *i.e.*, the regression of atherosclerosis. This may be due to enhanced NO production and decreased oxidative stress by candesartan treatment.

In conclusion, candesartan decreases carotid IMT *via* a mechanism involving enhanced NO production and decreased oxidative stress in patients with essential hypertension.

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