Candesartan Reduces Oxidative Stress and Inflammation in Patients with Essential Hypertension

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The present study was designed to test the hypothesis that blockade of angiotensin II type-1 receptors reduces oxidative stress and inflammation in patients with essential hypertension. The study population comprised 132 hypertensive patients, some receiving and others not receiving medical treatment. At enrollment their systolic and/or diastolic blood pressures were $\geq 140$ and/or $\geq 90$ mmHg, respectively. The serum concentration of C-reactive protein, and the urine concentrations of 8-epi-prostaglandin F$_2\alpha$ and 8-hydroxydeoxyguanosine were measured at baseline and after 12 weeks of treatment either with an angiotensin II type-1 receptor blocker, candesartan (8 mg daily) (age 64 ± 12 years; male/female 28/39; n = 67), or other antihypertensive agents that do not block the renin-angiotensin system (age 65 ± 10 years, male/female 25/40, n = 65). Candesartan reduced the levels of C-reactive protein (from 0.07 ± 0.04 [median value ± median absolute deviation] to 0.06 ± 0.03 mg/dl, $p < 0.0001$), 8-epi-prostaglandin F$_2\alpha$ (from 210 ± 92 to 148 ± 59 pg/mg creatinine, $p < 0.0001$), and 8-hydroxydeoxyguanosine (from 5.7 ± 1.9 to 4.0 ± 1.3 ng/mg creatinine, $p < 0.0001$), while the levels of these markers were not altered after the treatment with other antihypertensive agents. Blood pressure decreased by a similar amount in both groups, and the reductions in the levels of the markers did not correlate with that of blood pressure. These results suggest that candesartan reduces oxidative stress and inflammation in hypertensive patients independently of its effects on blood pressure. This may provide useful information for determining therapeutic strategies to minimize tissue injury by inflammation and oxidative stress in hypertensive patients. (Hypertens Res 2003; 26: 691–697)

Key Words: angiotensin II type-1 receptor, C-reactive protein, hypertension, inflammation, oxidative stress

Introduction

The production of reactive oxygen species is increased in animal models of hypertension (1–5) and in hypertensive human subjects (6–8). The increased production of reactive oxygen species may play an important role in functional and structural alterations of arteries in hypertension. Indeed, atherosclerosis is an inflammatory disease of the vascular wall initiated and amplified by oxidative stress (9) and hypertension is one of the major risk factors of atherosclerotic cardiovascular diseases, including myocardial infarction, stroke, and peripheral artery disease. Evidence has accumulated over the past decade that angiotensin II may induce oxidative stress in hypertension (2, 7, 10, 11).

In addition to the traditional role of angiotensin II in the regulation of vascular tone and blood pressure, it now appears that the octapeptide is a potent proinflammatory agent (11–13). Although the intracellular signaling events underlying this new effect of angiotensin II following the activation of angiotensin II type-1 receptors are not completely understood, it is known that the activation of the receptors leads to...
the production of reactive oxygen species in the vessel wall via, at least in part, activation of NADH/NADPH oxidase (1, 7, 14–16). This oxidase system is a major source of reactive oxygen species in endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts (4). The excessive production of reactive oxygen species has been referred to as oxidative stress, and has been implicated in many pathophysiological conditions in the cardiovascular system (10–13, 17, 18). There are several untoward events that occur as a consequence of oxidative stress, including oxidative modifications of DNA, lipid oxidation, modification of proteins, and activation of redox-sensitive genes (19). The last of these is particularly important in cardiovascular disease because many of the redox-sensitive cytokines, chemokines, and growth factors are proinflammatory, and play a critical role in the initiation and progression of atherosclerosis (20–22). Recently, it has been reported that inhibition of the angiotensin II type-1 receptor reverses endothelial dysfunction, an initial step of atherosclerosis, in patients with hypertension or atherosclerosis (23, 24). Furthermore, antihypertensive treatment with an angiotensin II type-1 receptor blocker, candesartan, decreases oxidative stress in spontaneously hypertensive rats (25). Thus, the present study was designed to test the hypothesis that a blockade of angiotensin II type-1 receptors may reduce oxidative stress and inflammation in human essential hypertension. Free F2-isoprostanes are synthesized from arachidonic acid through nonenzymatic peroxidation catalyzed by free radicals (26, 27). 8-Epi-prostaglandin F2α (8-epi-PG) is one of the most abundant F2-isoprostanes and is a reliable index of in vivo oxidative stress (28). 8-Hydroxydeoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base, and is a putative biomarker of the total systemic oxidative stress in vivo (29). We measured 8-epi-PG, 8-OHdG, and C-reactive protein (CRP) in patients with essential hypertension, and investigated the effects of candesartan on these markers.

Methods

Subjects and Study Design

The study population comprised 132 patients aged 35–79 years with essential hypertension. The diagnosis of essential hypertension was based on careful evaluation of clinical history, physical examinations, and laboratory and radiological investigations. The duration of hypertension was 3–21 years. Of these patients, 98 were currently on medication, and the remainder had been followed up without medical treatment. None of the patients were receiving angiotensin-converting enzyme inhibitors, angiotensin II type-1 receptor blockers, xanthine oxidase inhibitors, anti-inflammatory drugs including aspirin, or drugs that have an anti-oxidant property such as vitamin C, vitamin E, or probucol. In all patients, systolic blood pressure was ≥90 mmHg on at least three occasions whilst on medication at enrollment or during the follow-up period without medication. Blood pressure was measured in the right arm after a 5-min rest period, following the recommendation of the American Heart Association (30). The average of three consecutive blood pressure measurements was used as the blood pressure. Patients with vascular events or revascularization, diabetes mellitus, acute or chronic inflammatory disease, active liver disease, or renal dysfunction (serum creatinine > 1.2 mg/dl) were excluded from enrollment. Current smokers were also excluded from the study. Patients were assigned to receive 1) additional or new treatment with an angiotensin II type-1 receptor blocker, candesartan (8 mg daily) (ARB group, n = 67) or 2) additional or new treatment with other antihypertensive agents that do not block the renin-angiotensin system (control group, n = 65), according to their hospital ID number. In the control group, diuretics, calcium channel blockers, or α-blockers were used as an additional agent. We measured serum levels of CRP, a sensitive marker of inflammation, and urine concentrations of 8-epi-PG, a reliable index of in vivo oxidative stress, and 8-OHdG, a biomarker of oxidative DNA-base damage, before and after 12 weeks of the additional therapy. During this period antihypertensive or other medication was not changed. All the procedures were approved by the ethics committees of our hospitals, and informed consent was obtained from all patients.

Biochemical Measurements

Blood and urine were sampled in the morning after overnight fasting. The blood samples were centrifuged at 1,000 g for 15 min, and the resulting supernatant was stored at -70°C until use. CRP was measured using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) (31). For the measurement of 8-epi-PG, 5 ml of urine was transferred into a tube containing 0.25 mg of indomethacin and centrifuged at 10,000 g for 10 min, and the resulting supernatant was stored at -70°C until use. The concentration was determined using an enzyme immunoassay kit (8-isoprostane EIA Kit; Cayman Chemical Co., Ann Arbor, USA) (32). For the measurement of 8-OHdG, urine samples were centrifuged at 10,000 g for 10 min, and the resulting supernatant was stored at -70°C until use. The concentration was measured using an enzyme-linked immunosorbent assay kit (Japan Institute for Control of Aging, Fukuroi, Japan) (33). The intraand interassay coefficients of variation were 13.8% and 15.1% for 8-epi-PG, and 2.7% and 5.4% for 8-OHdG, respectively. The urine concentrations of 8-epi-PG and 8-OHdG were corrected for creatinine excretion.

Statistical Analysis

Data are expressed as the means ± SD. All the statistical
analyses were performed using Statview 5.0 (SAS Inst., Cary, USA). Group differences in continuous variables that had a normal distribution were tested by paired or unpaired Student’s t-test. Because the distributions of CRP, 8-epi-PG, and 8-OHdG levels were skewed rightward, median concentrations were computed for these parameters and are expressed as median value ± median absolute deviation. The significance of any difference in medians was assessed by the Mann-Whitney or Wilcoxon signed rank test. The levels of CRP and 8-OHdG, but not CRP, were lower in patients receiving 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (161 ± 73 [median value ± median absolute deviation] pg/mg creatinine, 5.0 ± 1.7 ng/mg creatinine, and 0.06 ± 0.03 mg/dl, respectively) than in those without the inhibitors (228 ± 111 pg/mg creatinine, p < 0.01; 6.2 ± 2.1 ng/mg creatinine, p < 0.01; and 0.07 ± 0.03 mg/dl, NS; respectively). Other medication did not affect the levels of CRP, 8-epi-PG, or 8-OHdG. At baseline, the two groups were closely matched with respect to demographic and clinical characteristics, and medical history (Table 1). Some patients had abnormal lipid profiles and were under medical treatment with HMG-CoA reductase inhibitors, but serum lipid levels were the same in both groups (Table 1). Medication at baseline was not different in the two groups.

### Results

Table 1 shows the baseline characteristics of the subjects. The levels of CRP, 8-epi-PG, and 8-OHdG were not different between patients with (n = 98) and without medication at enrollment (n = 34) (data not shown). At baseline, the levels of CRP, 8-epi-PG, and 8-OHdG were not correlated with blood pressure in hypertensive patients (Table 2). Serum levels of CRP were correlated with those of uric acid, high-density lipoprotein cholesterol, and triglyceride, but not with total cholesterol (Table 2). Although a significant correlation was detected between the markers of oxidative stress (i.e., 8-epi-PG and 8-OHdG), CRP was not correlated with either 8-epi-PG or 8-OHdG (Table 2). The levels of 8-epi-PG and 8-OHdG, but not CRP, were lower in patients receiving 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (161 ± 73 [median value ± median absolute deviation] pg/mg creatinine, 5.0 ± 1.7 ng/mg creatinine, and 0.06 ± 0.03 mg/dl, respectively) than in those without the inhibitors (228 ± 111 pg/mg creatinine, p < 0.01; 6.2 ± 2.1 ng/mg creatinine, p < 0.01; and 0.07 ± 0.03 mg/dl, NS; respectively). Other medication did not affect the levels of CRP, 8-epi-PG, or 8-OHdG. At baseline, the two groups were closely matched with respect to demographic and clinical characteristics, and medical history (Table 1). Some patients had abnormal lipid profiles and were under medical treatment with HMG-CoA reductase inhibitors, but serum lipid levels were the same in both groups (Table 1). Medication at baseline was not different in the two groups.

Analyses of the effects of candesartan administered over 12 weeks revealed marked reductions in the levels of CRP, 8-epi-PG, and 8-OHdG in the ARB group, but not in the control group (Figs. 1 and 2), though the reduction in blood pressure produced by the additional medical treatment was the same in the ARB (150.1 ± 15.2/87.5 ± 9.9 mmHg) and
The changes in the levels of the markers were not affected by the differences in gender, age, duration of hypertension, or medication at baseline (data not shown). Other clinical data were not altered after 12 weeks of the treatment either in the ARB or control group (data not shown). Percentage changes in mean blood pressure did not correlate with those in CRP (\( \rho = 0.119, \text{NS} \)), 8-epi-PG (\( \rho = 0.065, \text{NS} \)), or 8-OHdG (\( \rho = 0.015, \text{NS} \)). A significant correlation between the changes in 8-epi-PG and 8-OHdG was observed (\( \rho = 0.433, \ p < 0.0001 \)), but the changes in the levels of the markers of oxidative stress were not correlated with those of CRP (8-epi-PG, \( \rho = -0.100; 8\text{-OHdG}, \rho = -0.082; \text{NS} \)).

### Discussion

In the present study performed among patients with essential hypertension, 12 weeks of treatment with candesartan, an an-
candesartan, did not affect CRP levels in the control group. Furthermore, the change in CRP levels was not correlated with that in blood pressure. These observations suggest that candesartan has important anti-inflammatory effects in addition to the lowering of blood pressure. Low-grade inflammation is present among patients at risk from future atherothrombotic disease (34), and an increase in CRP levels is predictive of vascular events, including myocardial infarction, stroke, and peripheral artery disease (34–38). Moreover, CRP itself has direct inflammatory effects at the endothelial level—CRP can induce the expression of several cellular adhesion molecules that are critical to early atherogenesis (34). Thus, candesartan may attenuate the excess vascular risk associated with low-grade, systemic inflammation.

The specific pathway by which angiotensin II type-1 receptor blockers reduce CRP and exert anti-inflammatory effects remains uncertain. However, in the past few years it has become apparent that one of the most important consequences of angiotensin II type-1 receptor activation is the production and release of reactive oxygen species (10–12). Expression of inflammatory molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and monocyte chemotactic protein-1, which play a critical role in the initiation and progression of atherosclerosis, is redox-sensitive (20–22). In the present study, the decrease in CRP levels by candesartan was accompanied by decreases in urine levels of 8-epi-PG and 8-OHdG. A significant correlation was observed between the decrease in these two markers of oxidative stress, indicating that in vivo oxidative stress was decreased by candesartan. Thus, the reduction of inflammation may be attributed to, at least in part, the reduction of oxidative stress. However, other mechanisms must also be involved, as changes in the levels of the markers of inflammation and oxidative stress induced by the blocker did not correlate with each other. The present results strongly suggest that mechanisms other than reduction in blood pressure must be important in the effect of candesartan. This is consistent with previous studies showing that angiotensin II induces oxidative stress via a pressure-independent mechanism (39) and that candesartan reduces hypercholesterolemia-associated oxidative stress (40).

Our cross-sectional analyses at enrollment failed to detect any correlation between the levels of blood pressure and CRP, which is inconsistent with the report of Bautista et al. (41). However, it should be noted that the current study, which was designed for intervention, was limited for cross-sectional analysis by the small number of the subjects. Furthermore, most of the patients studied were under medical treatment at enrollment, and this might have affected the correlation between CRP and blood pressure. The correlations between the markers of inflammation and the levels of uric acid, high-density lipoprotein, and triglyceride observed in the present study may have some pathophysiological implications, but further investigation is required to elucidate the relationship.

**Fig. 2.** Mean blood pressure (MBP) and levels of C-reactive protein (CRP), 8-epi-prostaglandin F2α (8-epi-PG), and 8-hydroxydeoxyguanosine (8-OHdG) at baseline (filled circles) and after 12 weeks of treatment (open circles) with candesartan (ARB) or other antihypertensive agents (Control). Only candesartan reduced the levels of CRP, 8-epi-PG, and 8-OHdG. Vertical bars indicate the mean ± SD (MBP) and median ± median absolute deviation (CRP, 8-epi-PG, and 8-OHdG). *p < 0.0001 (MBP, by paired Student’s t test; CRP, 8-epi-PG, and 8-OHdG, by Wilcoxon signed rank test) vs. baseline.
Cardiovascular complications associated with hypertension are, at least in part, caused by the progression of atherosclerosis; thus, the optimal therapeutic strategy is to minimize this progression. Accumulating evidence supports the hypothesis that vascular inflammation and oxidative stress play a key role in atherosclerosis, and the present study shows that these factors can be reduced by antihypertensive treatment with candesartan. This provides a possible theoretical background for the use of angiotensin II type-1 receptor blockers in hypertension.

References

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