Antiinflammatory Effects of Angiotensin II Subtype 1 Receptor Blockade in Hypertensive Patients With Microinflammation

Danilo Fliser, MD; Konrad Buchholz, MD; Hermann Haller, MD; for the EUropean Trial on Olmesartan and Pravastatin in Inflammation and Atherosclerosis (EUTOPIA) Investigators

Background—Experimental studies revealed proinflammatory properties of angiotensin II. We evaluated antiinflammatory effects of the angiotensin II subtype 1 receptor antagonist olmesartan medoxomil alone and in cotherapy with the HMG-CoA reductase inhibitor pravastatin in patients with essential hypertension and microinflammation.

Methods and Results—We measured a panel of vascular inflammation markers, including high-sensitivity C-reactive protein, and lipid levels during 12 weeks of therapy with olmesartan (n = 100) or placebo (n = 99) in a prospective double-blind multicenter study. Pravastatin was added to the double-blind therapy at week 6 in both treatment arms. Blood pressure control was achieved with addition of hydrochlorothiazide. Olmesartan treatment had already significantly reduced serum levels of high-sensitivity C-reactive protein (−15.1%; P < 0.05), high-sensitivity tumor necrosis factor-α (−8.9%; P < 0.02), interleukin-6 (−14.0%; P < 0.05), and monocyte chemotactic protein-1 (−6.5%; P < 0.01) after 6 weeks of therapy, whereas placebo treatment (ie, blood pressure reduction) had no major effect on inflammation markers. After 12 weeks of therapy, high-sensitivity C-reactive protein (−21.1%; P < 0.02), high-sensitivity tumor necrosis factor-α (−13.6%; P < 0.01), and interleukin-6 (−18.0%; P < 0.01) decreased further with olmesartan and pravastatin cotherapy, but treatment with pravastatin alone (ie, cotherapy with placebo) did not significantly alter inflammation markers. In contrast, addition of pravastatin led to a significant (P < 0.001) reduction in LDL cholesterol serum concentrations in the olmesartan and placebo treatment groups (−15.1% and −12.1%, respectively).

Conclusions—Angiotensin II receptor blockade significantly reduces vascular microinflammation in patients with essential hypertension by as early as week 6 of therapy. This antiinflammatory action of angiotensin II receptor antagonists may contribute to their beneficial cardiovascular effects. (Circulation. 2004;110:1103-1107.)

Key Words: angiotensin ■ atherosclerosis ■ cholesterol ■ hypertension ■ inflammation
we have measured a panel of inflammation biomarkers, including hsCRP serum levels, before and during 3 months of therapy with olmesartan. Furthermore, we have evaluated the effect of a combination therapy with the HMG-CoA reductase inhibitor pravastatin.

Methods

Patients

The present phase IIIb trial was a randomized, placebo-controlled, double-blind, parallel-group study conducted between July 2001 and July 2003 at 26 investigator sites in Germany, Poland, and the Czech Republic. The local ethics committees of all study centers approved the study protocol, and all participants gave written informed consent. Eligible for inclusion were male and female patients >18 years of age with essential hypertension, any diagnosed atherosclerotic disease (eg, coronary or peripheral artery disease), type 2 diabetes mellitus (HbA1c level between 8% and 12%), and/or LDL cholesterol serum concentration between 3.89 and 6.48 mmol/L. Patients had a mean sitting diastolic blood pressure (DBP), ie, phase V Korotkoff sounds of conventional BP measurement, between 95 and 110 mm Hg inclusive at screening (newly diagnosed hypertensives without previous treatment) or at the end of a 2-week taper-off period (patients already receiving antihypertensive treatment) before entry into the placebo run-in phase. In addition, patients must have had an hsCRP serum concentration >3 mg/L and detectable interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1) serum levels. Patients’ blood samples at screening were immediately transported to the Medical School Hannover, where these inflammation markers were measured and from which the results were communicated to the study centers within 48 hours.

Exclusion criteria were any type of secondary hypertension; malignant hypertension; renovascular occlusive disease; renal transplant; serum creatinine >150 μmol/L and/or proteinuria >100 mg/dL; any other type of chronic inflammatory disease (eg, rheumatoid arthritis); an acute inflammatory disease and/or an hsCRP level >8 mg/L; and or/and drug abuse). Furthermore, patients with sitting DBP >110 mm Hg or sitting systolic BP (SBP) >200 mm Hg, patients who had taken any statins within 3 months before screening, and patients with significantly elevated liver enzyme levels were excluded. Finally, patients with known hypersensitivity or contraindication to Ang II subtype 1 receptor antagonists, statins, hydrochlorothiazide (HCTZ), or related drugs were also excluded. Female patients of childbearing potential were enrolled if they had a negative pregnancy test within 48 hours before starting the active treatment period and routinely used adequate contraception before and during the study.

Protocol and Measurements

After a taper-off period of 2 weeks for pretreated patients and an obligatory 2-week placebo run-in phase for all patients, those who were eligible for the trial were randomized to 1 of the 2 treatment groups receiving either 20 mg olmesartan or olmesartan matching placebo once daily in the morning. For the purpose of consistency, conventional blood pressure measurements with mercury sphygmomanometers and appropriate cuff sizes were performed in all study centers throughout the study. For each patient, blood pressure measurements were performed by the same person between 7 and 11 AM on all visits. Baseline sitting DBP was defined as the mean of 3 measurements before the first administration of double-blind medi-
cation. Patients with no reasonable response to treatment, ie, sitting DBP >90 mm Hg, additionally received either 12.5 or 25 mg HCTZ. After week 6 of double-blind medication, all patients received 20 mg pravastatin once daily (in the evening at bedtime) as an add-on to the double-blind treatment. Treatment compliance was assessed by tablet counts. Patients who proved not to have complied with dispensing instructions during the placebo run-in phase were excluded from the trial. During the study, patients were not allowed to receive any other antihypertensive drugs, other lipid-lowering agents, tricyclic antidepressants, or long-acting nitrates. The dose of comedication was not changed throughout the trial. Regular laboratory examinations, ECGs, and adverse event recordings were performed to assess safety parameters.

The primary study objective was to evaluate the antiinflammatory effect of olmesartan using a panel of inflammation markers: hsCRP, high-sensitivity tumor necrosis factor-α (hsTNF-α), IL-6, ICAM-1, and monocyte chemotactic protein-1 (MCP-1). In addition, the effect of combination therapy of olmesartan and pravastatin on these inflammation markers was investigated. For this purpose, inflammation markers and total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride serum concentrations were analyzed at baseline (ie, after the placebo run-in period) and after 6 and 12 weeks of treatment. Blood samples were taken in the morning after an overnight fast of ≥12 hours. They were immediately cooled on ice and centrifuged at 1500g and 4°C for 10 minutes, and the supernatants were stored in 1-mL aliquots at −80°C until further analyses. All samples were labeled with a code, and the analysts were not aware of the status of the samples. Measurements of routine chemistry were performed in a central study laboratory using standard equipment and certified methods. Inflammation markers were measured in the laboratory of the Division of Nephrology, Medical School Hannover. Serum concentration of hsCRP was measured with a clinically validated high-sensitivity nephelometric assay (Dade Behring).22 Serum concentrations of hsTNF-α, IL-6, MCP-1, and ICAM-1 were measured with an ELISA according to manufacturer’s instructions (R&D Systems). The intra-assay coefficients of variation for these tests ranged between 4.9% and 6.8%, respectively. To eliminate interassay variability, all samples from one patient were tested in a single assay.

Statistical Analysis

To compare demographic and laboratory parameters between treatment groups at baseline, we used a χ2 test for categorical variables (eg, gender) and the Wilcoxon rank-sum test for continuous variables (ie, age). ANCOVA was used to compare changes from baseline to weeks 6 and 12 in the level of circulating inflammation markers, BP, and lipid parameters in the olmesartan and placebo treatment arms, taking treatment and trial center effects into account. Analyses were performed on the full analysis set (intention-to-treat approach). For this approach, last-observation-carried-forward methods were applied for the replacement of missing values. All statistical tests were 2-sided tests with a 5% level of significance. Data are presented as mean±SD.

Results

After a screening of 507 individuals, 226 patients with essential hypertension fulfilling the above inclusion criteria were enrolled on an intention-to-treat basis (total set); 211 patients were finally randomized and completed the trial after 12 weeks of treatment. Twelve patients were excluded from the final analysis because of major protocol violations or violation of at least 1 criterion of schedule compliance. Thus, the final full analysis set comprised 199 patients, of whom 100 were treated with olmesartan and 99 were in the placebo treatment group. The demographic data of these patients at baseline are presented in Table 1. Both treatment groups were comparable with regard to age, gender, and body mass index,
but the placebo treatment group comprised significantly more patients with known type 2 diabetes mellitus.

The absolute baseline values of hsCRP, hsTNF-α, IL-6, and MCP-1 are shown in Table 1, whereas percent changes in inflammation markers during 12 weeks of treatment are presented in the Figure. A statistically significant decrease was observed for hsCRP (Figure, A), hsTNF-α (Figure, B), IL-6 (Figure, C), and MCP-1 (Figure, D) after 6 weeks of treatment with olmesartan. Serum levels of hsCRP, hsTNF-α, and IL-6 decreased even further in the olmesartan treatment arm after 12 weeks of therapy. In contrast, in the placebo treatment group, the only significant decrease was observed for IL-6 after 6 weeks but not after 12 weeks of treatment. With the addition of pravastatin to the placebo treatment arm, hsCRP decreased by 8.0%, but this decrease was not statistically significant. Moreover, a significant difference (P<0.05) between treatment groups was found for hsTNF-α at week 12 of the double-blind treatment (Figure, B). No significant changes in ICAM-1 serum levels were found in the olmesartan (baseline, 248±67 ng/mL; week 6, 246±92 ng/mL; week 12, 248±89 ng/mL) and placebo (baseline, 266±71 ng/mL; week 6, 258±64 ng/mL; week 12, 256±61 ng/mL) treatment groups.

We did not find significant changes in lipid levels after 6 weeks of olmesartan and placebo treatment (Table 2). In contrast, total and LDL cholesterol levels significantly decreased in both treatment groups after 12 weeks of therapy without any significant differences between treatments. Thus, the observed decreases reflect the effect of pravastatin cotherapy. Blood pressure significantly decreased in both treatment groups, with a more distinct decrease observed with olmesartan (Table 2). The differences between treatments were statistically significant except for the difference observed for SBP at week 12. In the placebo group, more

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*P<0.05, olmesartan vs placebo.

Changes in serum concentrations of hsCRP (A), hsTNF-α (B), IL-6 (C), and MCP-1 (D) in patients with essential hypertension after 6 and 12 weeks of therapy with olmesartan (n=100) or placebo (n=99). Pravastatin was added to both treatment arms at week 6. *P<0.05, **P<0.02, #P<0.01 vs baseline; $P<0.05$, olmesartan vs placebo.
patients needed coadministration of HCTZ (42.4% versus 21.0% of patients treated with olmesartan).

### Discussion

The results of the present prospective, double-blind, placebo-controlled study document that the Ang II subtype 1 receptor antagonist olmesartan medoxomil significantly reduces the level of vascular microinflammation in patients with essential hypertension. Treatment with olmesartan significantly reduced a panel of inflammation markers currently used to characterize vascular inflammation such as hsCRP, hsTNF-α, and IL-6. Among these markers, hsCRP has attracted the greatest attention in cardiovascular medicine because of its strong predictive power for cardiovascular events in a variety of populations. It is probably due to its long-term stability during storage, long half-life, lack of diurnal variation, and lack of age and sex dependence. As a consequence, it has been proposed that the level of vascular microinflammation in subjects at risk could be monitored by using hsCRP serum concentrations.

Our patients with essential hypertension were a population at high risk, like that studied in the Heart Outcomes Prevention Evaluation trial. In most of them, atherosclerotic disease and/or the key components of the metabolic syndrome (eg, type 2 diabetes mellitus and/or hyperlipidemia) were present. This was reflected by a rather high level of microinflammation and a mean baseline hsCRP serum concentration of >4.5 mg/L in both treatment groups. After 12 weeks of therapy with olmesartan, the hsCRP level was reduced by >20%, whereas blood pressure reduction in the placebo treatment group had only minor or no effect on inflammation markers. Thus, the beneficial cardiovascular effects documented for inhibitors of the renin-angiotensin system (eg, ACE inhibitors and Ang II receptor antagonists) could be attributed, at least in part, to their antiinflammatory action independent of the blood pressure-lowering effect.

This seems an attractive hypothesis because Ang II mediates a variety of proinflammatory effects. The conclusion that long-term reduction in blood CRP levels by Ang II receptor blockade may have therapeutic benefits in patients at high risk is supported by current experimental work that revealed that CRP not only is an excellent biochemical indicator of inflammation but also possesses proatherogenic properties. For example, CRP activates endothelial cells to express adhesion molecules, selectins, cytokines, and the chemokine MCP-1. It also induces the secretion of IL-6 and decreases the bioavailability of nitric oxide in human endothelial cells. The concentration of CRP that elicits these proinflammatory responses in vitro is >5 μg/mL, ie, far above the mean hsCRP serum concentration encountered in the general population. However, in subjects in whom a cluster of cardiovascular risk factors such as smoking, hypertension, and diabetes mellitus is present, hsCRP levels may be higher and approach the level observed in our cohort. In contrast to the significant decrease in hsCRP and other inflammation markers, we did not observe a change in ICAM-1 levels with olmesartan treatment. This unexpected finding may point to a specific interruption of the inflammation pathway by Ang II receptor blockade and deserves further (experimental) studies.

Surprisingly, coadministration of pravastatin to the placebo treatment arm significantly reduced LDL cholesterol levels but had no significant effect on inflammation markers. On first glance, our finding seems to contradict results of recent clinical studies with pravastatin and simvastatin. These results were obtained by post hoc analysis of a large outcome trial and in smaller short-term studies, and may therefore have been confounded by other therapies usually given to cardiovascular risk patients such as aspirin. In the primary prevention cohort of the Pravastatin Inflammation CRP Evaluation trial comprising 1702 subjects with no prior history of cardiovascular disease, treatment with 40 mg pravastatin significantly reduced hsCRP by 16.9%, but the absolute decrease in mean hsCRP serum concentration was modest, ie, 0.20 mg/L. In the present study, pravastatin add-on therapy in the placebo treatment group led to a nonsignificant decrease in hsCRP serum levels by 8.0%, but the absolute decrease was 0.36 mg/L. Large prospective trials in patients with elevated hsCRP serum concentrations are on the way to elucidate this issue. However, the absence of a major effect of pravastatin on the panel of inflammation markers in the placebo treatment arm permits the notion that the significant and persistent decreases in hsCRP, hsTNF-α, and IL-6 serum concentrations of populations.2,7,18
concentrations during 12 weeks of olmesartan therapy were primarily the result of continuous Ang II receptor blockade.

In conclusion, treatment with the Ang II receptor antagonist olmesartan significantly reduces biochemical markers of (vascular) inflammation in patients with essential hypertension by as early as week 6 of therapy. These antiinflammatory properties of Ang II receptor antagonists may have beneficial cardiovascular effects in addition to their blood pressure-lowering action.

Appendix

Acknowledgment
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