Fosinopril Decreases Levels of Soluble Vascular Cell Adhesion Molecule-1 in Borderline Hypertensive Type II Diabetic Patients With Microalbuminuria

Slobodan Gasic, Oswald F. Wagner, Peter Fasching, Christine Ludwig, Mario Veitl, Stylianos Kapiotis, and Bernd Jilma

Angiotensin converting enzyme inhibitors (ACE-I) are a mainstay for the treatment of heart failure, and of diabetic microalbuminuria. Recently ACE-I have been found to decrease plasma levels of circulating vascular cell adhesion molecule-1 (cVCAM-1) in patients with congestive heart failure. As increased cVCAM-1 levels are pathognomonic for diabetics with microangiopathy, we investigated the effects of ACE-I on plasma levels of cVCAM-1, intercellular adhesion molecule (cICAM-1), and cE-selectin in microalbuminuric diabetics. In addition, the effects of ACE-I on plasma levels of plasminogen activator inhibitor (PAI-1) and of tissue plasminogen activator (TPA) were studied. Fosinopril (10 mg/day) was administered over 12 weeks to 11 microalbuminuric patients with non-insulin-dependent diabetes mellitus (NIDDM). As expected, baseline plasma concentrations of cE-selectin, cICAM-1, and cVCAM-1 were markedly higher in patients than in healthy control subjects (n = 82; P < .001). PAI-1 levels in NIDDM were similar to those in control subjects, whereas TPA levels were about 25% lower in patients than in control subjects (P = .013). Serum levels of cVCAM-1 decreased by −19% (CI: −25% to −13%) after treatment with fosinopril (P = .003) and were no longer different from those of the control group. In contrast, plasma levels of cE-selectin, cICAM-1, PAI-1, and TPA were unaffected. As expected microalbuminuria decreased by −44% (CI: −65 to −22; P = .004). In conclusion, fosinopril lowered cVCAM-1 levels along with microalbuminuria in NIDDM. This may represent a novel mechanism of action of ACE-I in diabetes-associated endothelial dysfunction. Whether decreased VCAM-1 expression is responsible for the observed reduction in microalbuminuria, deserves further investigation. Am J Hypertens 1999;12:217–222 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Diabetic microalbuminuria, fosinopril, angiotensin converting enzyme inhibitor, vascular cell adhesion molecule-1, E-selectin.
Activation of the renin-angiotensin system predicts cardiovascular risk in hypertensive patients.\(^1\) Conversely, angiotensin converting enzyme inhibitors (ACE-I) are first line therapeutics in hypertension, and have become a mainstay for the secondary prevention after myocardial infarction, for the treatment of heart failure,\(^2\) as well as of diabetic microalbuminuria.\(^3\)

Angiotensin converting enzyme inhibitors have pleiotropic effects on the cardiovascular system,\(^2\) and recently ACE-I have been found to decrease plasma levels of the vascular cell adhesion molecule-1 (VCAM-1) in patients with congestive heart failure.\(^4\) The expression of VCAM-1, intercellular adhesion molecule-1 (ICAM-1), and E-selectin is preferentially found at inflammatory sites such as atherosclerotic lesions,\(^5\) where they mediate the recruitment of monocytes into the plaque.\(^6\) As activated endothelial cells release adhesion molecules in vitro\(^7\) and in vivo,\(^8\) serum concentrations of circulating adhesion molecules (cAM) are generally considered to reflect endothelial activation in vivo.\(^9\) Because these molecules are shed from activated endothelium, increased cAM levels have been found in atherosclerosis, ischemic heart disease,\(^10,11\) and diabetes mellitus.\(^9,12,13\) Thus the ACE-I induced decrease in cVCAM-1 levels may reflect a novel beneficial mechanism of action of ACE-I on the cardiovascular system.

As high cVCAM-1 levels appear to be pathognomonic for patients with diabetic microalbuminuria,\(^14,15\) we were particularly interested in investigating the effects of ACE-I on plasma levels of cVCAM-1, cICAM-1, and cE-selectin in such patients. In addition, growing evidence suggests that angiotensin II increases the production or release of plasminogen activator inhibitor (PAI-1),\(^16\) which is an established independent marker for cardiovascular risk.\(^17\)

Thus, therapeutic modulation of PAI-1, the main inhibitor of the fibrinolytic system, has become of interest. An increased PAI-1 activity was found in patients with NIDDM.\(^18–20\) However, there have been diverging reports on the capability of ACE-I to lower PAI-1 concentrations in patients with heart disease,\(^21,22\) and there is no information as to the influence of ACE-I on fibrinolytic activity in diabetes mellitus. Hence, the second aim of this trial was to investigate the effects of ACE-I on plasma levels of PAI-1 and of tissue plasminogen activator (TPA).

**MATERIAL AND METHODS**

**Study Design** The study protocol was approved by the Ethics Committee of the University Hospital. A prospective longitudinal study was conducted in 11 patients with microalbuminuria due to non-insulin-dependent diabetes mellitus (NIDDM). To determine the sample size, we assumed that the expected effect of ACE-I on cVCAM-1 would be similar to the approximately 20% decrease previously observed in patients with congestive heart failure.\(^4\) We deemed that detecting a 20% decrease would be of clinical relevance as most of the cardiovascular diseases including hypertension and diabetes are associated with a 20% to 30% increase in cAM.\(^10,11,13,23,24\) Based on previous findings on the intrasubject variability of cAM\(^25,26\) and the expected standard deviation we calculated\(^27\) that 10 subjects were required in our study. In addition, 82 healthy subjects served as control subjects.

**Subjects** Patients Eleven patients (six men and five women with a body mass index of 28.1 ± 6.5 kg/m\(^2\), aged 54 ± 8 years, with diabetes type II for 14 ± 8 years) were enrolled. Four patients received insulin treatment, four were on oral antidiabetics, and three were managed by dietary measures only. Inclusion criteria were microalbuminuria > 200 mg/day and an age between 30 and 65 years. Exclusion criteria were clinically manifest heart failure (New York Heart Association [NYHA] class III to IV), microalbuminuria > 250 \(\mu\)g/min, hyperthyroidism, a history of any non-cardiovascular disease associated with increased levels of adhesion molecules, anemia, and comedication with \(\beta\)-adrenergic antagonists or glucocorticoids.

Control Subjects Eighty two subjects (37 men and 45 women) aged 54.4 ± 9.0 years with an average body mass index of 25.5 ± 4.2 kg/m\(^2\) served as control subjects. All were free of any medication and had no relevant medical history.

**Intervention** After written informed consent had been obtained, a baseline blood sample was drawn. All patients were treated for 12 weeks with 10 mg of fosinopril every day, a dose known to almost completely inhibit the ACE activity.\(^28\) All subjects were instructed to take the drug in the morning. Drug compliance was checked every 3 weeks during subsequent visits at the outpatient ward, and was > 90% as determined by blister control.

**Assays** All samples were determined as duplicates. TPA (Coaliza, t-PA, Chromogenix, Mölndal, Sweden) and PAI-1 (Technolone, Actibind PAI-1) were determined with established enzyme immunoassays.\(^29,30\) All soluble adhesion molecules were determined by commercially available enzyme immunoassays for soluble E-selectin, ICAM-1, and VCAM-1 (R&D Systems, Oxfordshire, England) as described previously.\(^25,26\) Glucose, glycosylated hemoglobin (HbA\(_1c\)), microalbuminuria, and serum lipids were determined as described previously.\(^14,31,32\)

**Statistics** As data were nonnormally distributed, nonparametric statistics were applied. A two-tailed \(P\) value of < .025 was considered significant, to account for the two main outcome variables (cVCAM-1 and...
Changes in measured endpoints after initiation of fosinopril treatment were compared with baseline values with the Wilcoxon signed ranks test. Comparisons between patients and healthy control subjects were made with the Mann-Whitney U test. Correlations were calculated with the Spearman ranks correlation test.

RESULTS

Blood Pressure Baseline systolic and diastolic blood pressure averaged 140 ± 13 and 81 ± 7 mm Hg before fosinopril treatment and fell by −6% (−14% to 8%) and −4% (−16% to 12%). These changes did not reach statistical significance.

Metabolic Parameters Fosinopril treatment did not alter blood glucose, HbA1c, or lipid values (Table 1).

cE-selectin, cICAM-1, and cVCAM-1 Baseline Values Serum concentrations of cE-selectin, cICAM-1, and cVCAM-1 were markedly higher in patients than in control subjects ($P < .0001$; Figure 1).

After ACE-I Serum levels of cVCAM-1 decreased by −19% (CI: −25% to −13%) after 12 weeks of treatment with fosinopril (Figure 2; $P = .003$) and were no longer different from those of the control group (Figure 1). Plasma levels of cE-selectin and cICAM-1 demonstrated no apparent decrease (Figure 1).

Microalbuminuria After fosinopril treatment microalbuminuria decreased by −44% (CI: −65% to −22%; $P = .004$; Figure 2). The decrease in microalbuminuria did not correlate with the decrease in cVCAM-1 ($r^2 = 0.12; P = .71$).

TPA and PAI-1 Baseline Values TPA levels were significantly less in patients (6.1 ng/mL; CI: 4.5 to 7.6) than those observed in healthy subjects (8.9 ng/mL; CI 7.8 to 10.2; $P = .013$). PAI-1 levels in NIDDM averaged 16.1 ng/mL (CI: 8.4 to 23.8), whereas those of control subjects were 20.1 ng/mL (CI: 15.4 to 24.7; $P > .05$; Figure 1).

After ACE-I TPA levels of 7.4 ng/mL (5.1 to 9.7) and PAI-1 levels of 20.7 ng/mL (CI: 7.5 to 34) were not significantly different from those initiation of therapy ($P > .025$).

DISCUSSION

The aim of this trial was to investigate the effects of 12 weeks of fosinopril treatment (10 mg/day) on serum levels of cAM, PAI-1, and TPA in type II diabetic patients with microalbuminuria.

### Table 1. Metabolic Parameters of Patients with Type II Diabetes and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>NIDDM Patients Before and After Fosinopril</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>143 ± 45</td>
<td>153 ± 46</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.0 ± 1.1</td>
<td>7.7 ± 35</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>229 ± 68</td>
<td>226 ± 75</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>46 ± 22</td>
<td>45 ± 23</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>123 ± 27</td>
<td>128 ± 35</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>247 ± 360</td>
<td>199 ± 226</td>
</tr>
</tbody>
</table>

Data are given as means ± SD.

HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycosylated hemoglobin.

* $P < .0001$ patients v control subjects.
The major finding of our trial was that fosinopril reduced cVCAM-1 levels by 19% to concentrations seen in healthy subjects. This is in good agreement with the report that ACE-I decreased cVCAM-1 by about 20% in patients with congestive heart failure.4 In that trial only a subgroup of patients, in whom ACE-I increased the acetylcholine induced forearm blood flow, responded with a decrease in cVCAM-1. This supports the concept that enhancement of endothelium dependent relaxation by ACE-I was associated with the decrease in cVCAM-1. Inhibition of ACE (kininase II) increases the concentration of bradykinin, which stimulates nitric oxide (NO) production. Nitric oxide, in turn, down-regulates the expression of VCAM-1.33–35 Whether an ACE-I induced increase in NO production contributes to the decrease in cVCAM-1 in patients suffering from microalbuminuria, however, remains to be determined.

Other potential effector pathways explaining the observed decrease in cVCAM-1 would include a lower generation of angiotensin II, which, however, had no acute effects on cAM levels when infused at doses that increased mean arterial blood pressure by 20%.36 In addition, VCAM-1 is increased by advanced glycosylation end products37,38 that are formed in response to chronic hyperglycemia.15 Thus, ACE-I could possibly block the stimulatory actions of these molecules by a mechanism that is, as yet, unknown.

The observed ACE-I induced decrease in cVCAM-1 may be of clinical relevance. First, serum levels of cAM are elevated by about 20% to 30% in atherosclerosis,11 and cVCAM-1 levels appear to correlate with the extent of atherosclerosis.39 Thus, any decrease in cVCAM-1 may be beneficial with regard to the cardiovascular system. Second, cVCAM-1 is also increased in diabetic patients14 and appears to be pathognomonic for microangiopathy.15,31,40 Renal VCAM-1 expression also seems to play a role in the pathogenesis and propagation of a variety of kidney diseases by increasing leukocyte recruitment and hence the local production of inflammatory mediators.41 In view of the high sensitivity of the renal vasculature to angiotensin II effects, it is tempting to speculate that elevated VCAM-1 may contribute to the development of microalbuminuria. Finally, evidence is growing that soluble E-selectin and cVCAM-1 induce angiogenesis,42 and that their plasma levels are related to the presence14 and degree of diabetic retinopathy.40 The latter study also showed that antibodies against either cAM partially inhibit retinal endothelial cell migration in response to diabetic serum.

In contrast to cVCAM-1, plasma levels of E-selectin and ICAM-1 did not significantly decrease, albeit the differences in the plasma levels between patients and control subjects were more pronounced than that of cVCAM-1 (Figure 1). Although all the examined cAM are upregulated by a variety of cytokines, other stimuli may specifically regulate individual adhesion molecules. E-selectin appears to be under direct metabolic control in type II diabetes.24,31,43 The failure of ACE-I to affect plasma glucose, concentrations of insulin, or HbA1c (Table 1) may explain unchanged E-selectin levels.

As with ICAM-1 and E-selectin, fosinopril treatment did not change plasma levels of PAI-1 or TPA. This is in good agreement with previous studies in
obese men and in patients with congestive heart failure. However, it is partly at variance with other trials that demonstrated an ACE-I induced decrease of PAI-1 by 18% and 44% in patients after myocardial infarction. First, this discrepancy in results could be due to a different mechanism regulating PAI-1 release in patients suffering from diabetes. Platelets may account for > 90% of blood PAI-1, and platelet PAI-1 levels correlate with metabolic control in type II diabetes. As metabolic control was unaffected by fosinopril therapy, this may be a possible explanation for the lack of ACE-I effects on PAI-1 in our study. Second, plasma levels of PAI-1 in our patients were not different from those of control subjects, indicating limited room for improvement. Third, although an exact power calculation is not possible because of the non-normal distribution of PAI-1 data, the small sample size limits the power of this trial with regard to detecting a decrease in PAI-1 levels.

In conclusion, ACE-I therapy lowered cVCAM-1 levels along with microalbuminuria in patients with NIDDM (Figure 2). This is a new potential mechanism of action of ACE-I in diabetes associated endothelial dysfunction, which is characterized by a plethora of pathophysiologic changes. Whether decreased VCAM-1 expression is responsible for the observed reduction in microalbuminuria deserves further investigation.

REFERENCES