Effects of enalapril and losartan on circulating adhesion molecules and monocyte chemotactic protein-1

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ABSTRACT

At least four independent studies in different clinical settings showed that angiotensin-converting enzyme inhibitors (ACE-Is) such as enalapril effectively decrease plasma levels of circulating adhesion molecules (cAMs). To examine whether this effect may be mediated by the decreased action of angiotensin, we compared the effects of enalapril with the direct angiotensin-II antagonist, losartan, on plasma levels of cAMs, and monocyte chemotactic protein-1 (MCP-1). In a randomized trial, we recruited 32 untreated patients (19 male, aged 59 ± 13 years) with hypertension, who received either enalapril (mean dose 17 mg/day) or losartan (mean dose 77 mg/day) at equipotent doses. Enalapril decreased plasma levels of all cAMs after 8 weeks of treatment: cE-selectin levels decreased by 13% (P < 0.007), intercellular adhesion molecule-1 (cICAM-1) by 15% (P = 0.002) and vascular cell adhesion molecule-1 (cVCAM-1) by 19% (P = 0.003). Similarly, enalapril decreased plasma levels of MCP-1 by 13% (P < 0.001). Losartan did not significantly change cAM or MCP-1 plasma concentrations after 8 weeks of treatment: cE-selectin levels decreased by 3%, cICAM-1 by 5%, cVCAM-1 by 8%, whereas MCP-1 increased by 2% (all P = NS; not significant). The enalapril effect on percentage changes of cVCAM-1 was significantly different from losartan (P = 0.0429). Eight weeks of antihypertensive treatment with enalapril but not losartan, significantly decreased plasma levels of cAMs and MCP-1 in hypertensive patients. The beneficial effects of ACE-Is on cAMs may have implications for atherogenesis and the reduction of cardiovascular events, which cannot be fully explained by their antihypertensive effects alone.

INTRODUCTION

Angiotensin-converting enzyme inhibitors (ACE-Is) are well-established drugs for patients with hypertension, heart failure or diabetic microalbuminuria, with beneficial effects on morbidity and mortality, which cannot be fully explained by their antihypertensive effects [1,2]. Well-known mechanisms of action of ACE-Is include inhibition of angiotensin II generation and decreased degradation of bradykinin. The latter is pertinent since...
several animal models have shown that some of the cardioprotective effects of ACE-Is could be attributed to bradykinin [3]. Bradykinin stimulates NO production by endothelial cells, and may thereby help to ‘correct’ endothelial dysfunction [4]. In line with this mechanism of action, ACE-Is have been shown to improve endothelial vasodilator function in patients suffering from hyperlipidaemia, coronary artery disease and/or heart failure [5–7].

Circulating vascular cell adhesion molecule-1 (VCAM-1) is a cell activation marker that is released into the circulation when inflammation is induced [8,9], but is down-regulated at the cellular level by NO [10]. A study by Drexler et al. [6] showed that an ACE-I, perindopril, significantly decreased plasma levels of VCAM-1 in a subgroup of patients with congestive heart failure, where forearm vasodilator function also significantly improved. Fosinopril and recently enalapril have been shown to down-regulate VCAM-1 levels in diabetics [11,12]. Similar effects are seen with enalapril in hypertensive patients with metabolic abnormalities [13], and with captopril in critically ill patients [14]. The latter studies also showed that ACE-Is suppressed other adhesion molecules such as E-selectin and intercellular adhesion molecule-1 (ICAM-1). This is of interest because these leucocyte/endothelial adhesion molecules appear to play an important role in the pathogenesis of atherosclerosis [15].

Furthermore, plasma levels of these cell adhesion molecules are increased in metabolic syndromes such as diabetes, hypertension and atherosclerosis [16–21], and predict adverse outcomes in patients at cardiovascular risk [22,23]. Nevertheless, the mechanism(s) by which ACE-Is lower CAMs are not known, and could involve decreased angiotensin II formation, lowered bradykinin breakdown or increased prostaglandin production. We hypothesized that if the effects of the ACE-Is in lowering CAMs are mediated by a decrease in angiotensin II levels, this will be mimicked by a direct angiotensin II antagonist such as losartan. We therefore compared the effects of the ACE-I, enalapril, with a direct angiotensin II receptor antagonist, losartan, on the plasma levels of VCAM-1, cCAM and E-selectin in hypertensive patients. In addition, we compared the effects of these two drugs on plasma levels of the chemokine monocyte chemotactic protein-1 (MCP-1). This is of interest, because MCP-1 is expressed at high levels in atherosclerotic plaques [24], its expression is increased by angiotensin II [25,26], and enalapril has recently been shown to lower MCP-1 plasma levels in patients with myocardial infarction [27].

Further, we have recently found significantly higher plasma levels of MCP-1 in healthy young men than in pre-menopausal women [28]. Thus, MCP-1 may not only serve as a marker of atherosclerosis, but higher plasma levels in men compared with women are compatable with the well-known sex difference in cardiovascular diseases. We were thus interested in whether the observed down-regulation of MCP-1 in patients with coronary artery disease could be reproduced in another patient population.

**METHODS**

**Study population**

We recruited 32 untreated patients with hypertension (M/F = 19/13; mean age 59 ± 13 years), who were defined as those with elevated multiple blood pressure readings in general practice or the hospital outpatient clinic, and again in a research clinic. Blood pressures were measured in a quiet room after 5 min rest, using a conventional mercury sphygmomanometer; the mean of three readings was taken, with diastolic blood pressures at Korotkoff phase V. We excluded patients on aspirin or warfarin, those with associated cardiovascular or cerebrovascular disease, diabetes, connective tissue disease, malignancy and chronic inflammatory conditions that may alter these parameters. For descriptive purposes, baseline levels of CAMs and MCP-1 were compared with those from 32 normotensive age- and sex-matched healthy controls (58 ± 14 years) drawn from amongst healthy hospital staff. All controls were free of diabetes, and were without any signs or symptoms of cardiovascular or connective tissue disease, malignancy or hypertension. For further descriptive comparison, an additional control group consisted of 32 young healthy volunteers aged 19–35 years (mean age, 27 years), because in elderly control subjects subtle atherosclerotic changes cannot be excluded with certainty.

In a prospective randomized double-blind parallel group manner, the untreated hypertensive patients received either enalapril (10–20 mg once daily) or losartan (50–100 mg once daily) for 8 weeks, to achieve a target blood pressure of below 160/90 mmHg. The study protocol was approved by the West Midlands district ethics committee and informed consent was obtained from patients entered into the study. Blood samples were collected in the morning hours to avoid potential circadian variation of adhesion molecules [29] prior to treatment and also after 4 and 8 weeks of antihypertensive monotherapy.

Drugs were started at a test dose of 2.5 mg of enalapril at night and the dose increased over 1 week to 5 mg, followed by 10 mg, and then 20 mg; at a 4 week review, the dose increased to 40 mg if needed. For losartan, 50 mg was given to start, which was increased to 100 mg at 4 weeks, if needed.

**Blood samples and assays**

Blood samples were drawn from the antecubital vein by venepuncture, after 10 min rest and without application
of a tourniquet. Samples were anticoagulated with trisodium citrate (0.11 M, 9:1, v/v) and separated using a refrigerated centrifuge at 3000 g for 15 min. The platelet-poor plasma was immediately separated and frozen at −80 °C. Plasma E-selectin, ICAM-1, VCAM-1 and MCP-1 were assayed by enzyme immunoassays purchased from R&D Systems (Oxon, U.K.) [28,30]. Intra-assay coefficients of variation for all ELISA assays were < 5%, and inter-assay variances were < 10%.

Statistical analysis
An a priori sample size calculation [31], which was based on the intraindividual coefficient of variation of all cAMs (6–8%) [30] and the expected decrease in cVCAM-1 (19%), indicated that 16 subjects in each group would be sufficient to detect an approx. 15% change from baseline with a power of 80%. Baseline values of blood pressures are expressed as means ± S.D. Treatment effects are expressed as the 95% confidence interval of the percentile change from baseline. Paired comparisons pre- and post-treatment were performed using the Friedman’s repeated measures ANOVA and the paired Wilcoxon test. Com-}

RESULTS
We studied 32 patients with untreated hypertension (mean blood pressure of 169±11/94±8 mmHg): 17 patients were treated with enalapril (mean dose 17 mg/day) and 15 patients were treated with losartan (mean dose 77 mg/day), with all patients completing the study.

Table 1   Effects of enalapril and losartan on blood pressure
sBP/dBP: systolic/diastolic blood pressure. Data are means ± S.D. **P < 0.01 versus baseline; †P > 0.05 enalapril versus losartan for baseline blood pressure or treatment effects.

<table>
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<th>Before treatment</th>
<th>After 4 weeks of treatment</th>
<th>After 8 weeks of treatment</th>
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<tr>
<td><strong>Enalapril</strong></td>
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<tr>
<td>sBP (mmHg)</td>
<td>168 ± 13</td>
<td>151 ± 14**</td>
<td>146 ± 15**</td>
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<tr>
<td>dBP (mmHg)</td>
<td>92 ± 9</td>
<td>84 ± 9**</td>
<td>81 ± 8**</td>
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<td><strong>Losartan</strong></td>
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<tr>
<td>sBP (mmHg)</td>
<td>169 ± 9</td>
<td>157 ± 10**</td>
<td>149 ± 13**</td>
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<tr>
<td>dBP (mmHg)</td>
<td>98 ± 7</td>
<td>92 ± 8**</td>
<td>88 ± 6**</td>
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The antihypertensive effects of enalapril and losartan are summarized in Table 1, with similar blood pressure reductions in both treatment groups. There were no significant differences in mean age, sex ratio and baseline variables between treatment groups (results not shown).

At baseline, there were no significant differences in mean blood pressure and measured indices between the two treatment groups. The hypertensive patients had similar baseline plasma levels of the cAMs compared with those of healthy, age- and sex-matched controls (Figure 1; P > 0.05 between hypertensives and normotensives). However, they had higher levels of cE-selectin and cICAM-1 compared with normotensive and aged controls (P < 0.001 and P = 0.013 respectively; Figure 1).

Enalapril decreased plasma levels of all cAMs and MCP-1 after 8 weeks of treatment: cE-selectin levels decreased by 13% [95% confidence interval (95% CI): 5–20%; P = 0.007], cICAM-1 by 15% (95% CI: 8–21%, P = 0.002), cVCAM-1 by 19% (95% CI: 10–27%, P = 0.003) and MCP-1 levels by 13% (95% CI: 5–22%, P < 0.001; Figure 2). cAM levels were significantly lower after 8 weeks compared with 4 weeks of treatment (Figure 2; P < 0.017 for all parameters).

Losartan did not significantly lower cAM or MCP-1 levels after 8 weeks of treatment (Figure 2): cE-selectin levels decreased by 3% (95% CI: −11 to 4%), cICAM-1 by 5% (95% CI: −14 to 5%) and cVCAM-1 by 8% (95% CI: −17 to 1%), whereas MCP-1 increased by 2% (95% CI: −15 to 19%) (ANOVA: P > 0.6 for cE-selectin or cICAM-1, P > 0.15 for cE-selectin or cVCAM-1).

The enalapril effect on percentage changes of cVCAM-1 was significantly different from losartan (P = 0.0429). A similar trend was observed for percentage changes in cE-selectin (P = 0.0621).
Figure 2  Comparison of the effects of enalapril and losartan on cAMs and MCP-1 in hypertensive patients

Only enalapril (filled bars) significantly decreased all parameters, although both drugs were equipotent in terms of lowering blood pressure. Data are means ± 95% CIs; ** P < 0.01 versus baseline.

DISCUSSION

We have demonstrated in a prospective randomized clinical trial that an ACE-I (enalapril) but not an angiotensin II antagonist (losartan) significantly lowered levels of all examined cAMs and MCP-1. Indeed, treatment with enalapril decreased plasma levels of cE-selectin and cICAM-1 to levels observed in young healthy controls, and even decreased cVCAM-1 levels below the control values.

A recent study by Ferri et al. [13] reported a 60–70% decrease in all cAMs after 12 weeks of enalapril treatment in hypertensive patients with and without metabolic abnormalities. The present trial confirms these observations with tighter 95% CIs in the present trial and in our previous study in diabetic patients with borderline hypertension [12]. In agreement with our findings, Andersen et al. [11] reported in a recent letter that 2 months of enalapril treatment reduced cVCAM-1 and cE-selectin levels by approx. 10%. The differences in the magnitude of effects cannot be due to different assays (since the same enzyme immunoassays were used in all studies), but they may be attributable to differences in the study designs. The group of our patients was comparable to subgroup IV studied by Ferri et al. [13]. Whilst Ferri et al. [13] reported that either 6 or 12 weeks of enalapril therapy lowered cICAM-1 and cVCAM-1 by a similar degree, we found that 8 weeks (but not 4 weeks) of treatment was required to significantly lower cAM levels. In a small trial in five hypertensive patients [32] and in the trial by Ferri et al. [13], losartan decreased cAM levels by approx. 8–15% in hypertensive patients without associated metabolic disorders after 12 weeks. The sequential treatment trial design does not allow a direct comparison between losartan and enalapril in that previous trial [13]. Yet, it is noteworthy that the data by Ferri et al. [13], and also the data by Andersen et al. [11], are in keeping with findings in the present study, in that enalapril more effectively decreased cAM levels than losartan.

The difference between treatment with ACE-Is and an angiotensin II antagonist suggests that the cAM-lowering effects of ACE-Is are at least in part due to a mechanism other than inhibition of angiotensin generation and action. Interestingly, angiotensin infusion does not directly stimulate the release of soluble cVCAM-1 or cE-selectin [33], whereas different results have been observed for cICAM-1 [32]. Whilst ACE-Is increase the sensitivity to exogenously administered bradykinin [34], inhibition of endogenous bradykinin breakdown does not appear to contribute to forearm vasodilatation in patients with heart failure [35]. Nonetheless, it is possible that decreased bradykinin breakdown and hence a potential increase in NO formation could play a role in the down-regulation of cAM levels, because NO decreases adhesion molecule expression [10]. Alternatively, ACE-Is could lower cAM and MCP-1 levels by increasing prostaglandin production. Both increased NO and prostaglandin synthesis are potential effects of ACE-Is but not angiotensin receptor antagonists. However, as our study was only designed to examine the relative contribution of angiotensin action to cAM and MCP-1 production, the precise mechanism by which ACE-Is lower cAM and MCP-1 levels still deserves further investigation.

While blood pressure had decreased already after 4 weeks of drug administration, an effect on plasma levels of cAMs was not seen until after 8 weeks of treatment. Since blood pressure was lowered in a time-dependent fashion as dosages of both drugs were increased (Table 1), there are two, not mutually exclusive, explanations for the time lag in the enalapril-induced decrease of cAM levels. First, a threshold dose of ACE-Is, and therefore a threshold concentration of ACE-Is, may be needed to
effectively lower cAM levels. Alternatively, the effect of ACE-Is on cAM levels is indirect and may require considerable time for adaptive responses.

The superiority of enalapril compared with losartan is of interest for the treatment of cardiovascular diseases because studies have shown that adhesion molecules and MCP-1 play an important role in the pathogenesis of atherosclerosis [25,26]. Furthermore, plasma levels of cAMs have been shown to predict adverse outcomes in patients at cardiovascular risk [22,23,36]. Hence, any beneficial effects of ACE-Is on hard endpoints, such as mortality and cardiovascular events, may not be easily extrapolated to angiotensin receptor antagonists. Along these lines, the Losartan Heart Failure Survival Study (ELITE-II) [1,22] showed some tendency of a survival advantage in certain subgroups of patients who took captopril rather than losartan. Since the ELITE-II study compared only a rather old ACE-I to losartan, further studies in even larger populations are required.

In the Valsartan Heart Failure Trial (Val-HeFT) study there again appeared to be a more favourable effect of ACE-Is compared with angiotensin II antagonism on outcome in certain groups. However, the Valsartan Heart Failure Trial study also showed that valsartan had a beneficial effect on outcome particularly in those patients not receiving an ACE-I at baseline. How can this study be interpreted in light of our findings? Firstly, patients with congestive heart failure are certainly different from patients with primary hypertension. Secondly, a recent study demonstrated that levels of cAMs were not predictive of outcome in coronary heart disease [37]. Thus, a study of surrogate biochemical markers need not necessarily reflect cardiovascular outcome or mortality.

There are several limitations of the current trial. Firstly, we felt that withholding effective antihypertensive treatment from patients for months was not ethically justified for study purposes, hence, we cannot tell whether losartan may have prevented detection of small effects of losartan. The 95% CIs of the current trial and results of the 95% CIs of the current trial and results of the EXPERT study demonstrated that levels of soluble TNF-receptors and soluble adhesion molecules ICAM-1, E-selectin and VCAM-1 under systemic rhTNF alpha therapy. Eur. J. Clin. Invest. 26, 404–410

In conclusion, we found that 8 weeks of antihypertensive treatment with enalapril but not losartan, significantly decreased cAM levels and MCP-1 in hypertensive patients. The beneficial effects of the ACE-Is on cAMs may have implications for atherogenesis and for the reduction of cardiovascular events, which cannot be fully explained by their antihypertensive effects alone.

**ACKNOWLEDGMENTS**

We are indebted to Christa Drucker for her technical assistance. F. L. L. -S. -H. was supported by a non-promotional research fellowship from Merck Sharpe and Dome.

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Received 7 September 2001/2 January 2002; accepted 16 April 2002