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Lymphocyte-suppressing action of angiotensin-converting enzyme inhibitors in coronary artery disease patients with normal blood pressure

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Abstract:
The clinical effectiveness of angiotensin-converting enzyme (ACE) in the prevention and treatment of cardiovascular disorders partially results from its anti-inflammatory action. No previous study has investigated the effect of any ACE inhibitor on lymphocyte cytokine release. In this study, we compared the effects of serum- and tissue-type angiotensin-converting enzyme inhibitors on systemic inflammation and lymphocyte secretory function in normotensive patients with stable coronary artery disease. The study included 134 patients with coronary artery disease who were randomized into one of three groups and treated with enalapril (20 mg/d, n = 47), perindopril (4 mg/d, n = 45) or placebo (n = 42), respectively. The control group included 40 age-, sex- and weight-matched healthy subjects. The plasma lipid profile, glucose metabolism markers, hsCRP and lymphocyte cytokine release were examined at the beginning of the study and after 30 and 90 days of treatment. Phytohemagglutinin-stimulated T cells released significantly more interleukin-2, interferon-γ and TNFα than the lymphocytes of control subjects. Neither enalapril nor perindopril treatment was associated with any significant changes in blood pressure. Perindopril treatment inhibited lymphocyte cytokine release and systemic inflammation, while the effect of enalapril was insignificant. Perindopril, and, to a lesser extent, enalapril, strongly reduced lymphocyte cytokine release in insulin-resistant but not insulin-sensitive subjects. Our results indicate that perindopril is superior to enalapril in producing lymphocyte-suppressing and systemic anti-inflammatory effects in normotensive coronary artery disease patients. These effects may contribute to a reduction in the vascular risk of this group of patients, particularly in those subjects who are resistant to insulin, when these patients are treated with tissue-type angiotensin-converting enzyme inhibitors.

Key words:
angiotensin-converting enzyme inhibitors, coronary artery disease, lymphocytes, proinflammatory cytokines, risk factors

Abbreviations: ACE – angiotensin-converting enzyme, CRP – C-reactive protein, ELISA – enzyme-linked immunosorbent assay, EUROPA – European Trial on Reduction of Cardiac Events With Perindopril in Stable Coronary Artery Disease Study, HDL – high density lipoproteins, hsCRP – high sensitivity C-reactive protein, HOPE – Heart Outcomes Prevention Evaluation Study, ICAM-1 – intercellular adhesion molecule-1, LDL – low density lipoproteins, MCP-1 – monocyte chemoattractant protein-1, MMP-9 – matrix metalloprotease 9, PAI-1 – plasminogen activator inhibitor-1, PEACE – Prevention of Events with Angiotensin-Converting Enzyme Inhibition Study, PROGRESS – Perindopril Protection Against Recurrent Stroke Study, TNFα – tumor necrosis factor-α, VCAM-1 – vascular cell adhesion molecule-1

Introduction

Recently, three large clinical trials have shown that angiotensin-converting enzyme (ACE) inhibitors reduce mortality and fatal and non-fatal cardiovascular and cerebrovascular events in atherosclerotic patients without heart failure and left ventricular systolic dysfunction [4, 20, 29]. The fact that perindopril-induced reduction in cardiovascular events was more pronounced than expected for the small reduction in blood pressure [2, 4] indicates that clinical benefits of ACE inhibitors result not only from their hypotensive properties but also from pleiotropic effects, which include the regulation of smooth muscle cell proliferation and migration, cytoprotection of vascular endothelium and anti-inflammatory and antioxidative action, in addition to their beneficial effects on coagulation, fibrinolysis and platelet activities [9, 17, 18, 24]. Despite producing an anti-inflammatory effect in various cell culture studies, to the best of our knowledge, no previous study has examined the effect of any ACE inhibitor on cytokine release by human lymphocytes.

Various ACE inhibitors differ in their solubility, oral bioavailability, hepatic extraction, elimination half-life of active compounds and protein binding capacity [3], and therefore, it is still unclear whether the beneficial action of these agents on inflammation results from their "class effect" or differs from drug to drug. So far, few studies have been conducted to compare the effects of various ACE inhibitors on cytokine production, and the results of these studies are inconclusive. Captopril, enalapril and cilazapril, but not ramipril, lisinopril, perindopril or spirapril, decreased the synthesis of interleukin-1 and TNFα by stimulated human peripheral blood mononuclear cells (PBMCs) [22]. Constantinescu et al. [1] revealed that both captopril and lisinopril significantly inhibited interleukin-12 and interferon-γ production by activated PBMCs. A single supraphysiological dose of captopril, delapril and cilazapril reduced TNFα production in human PBMCs and *in vivo* in mice [5]. Recently, we have found that perindopril was superior to enalapril in exhibiting antioxidant, antithrombotic and profibrinolytic activities in normotensive subjects with coronary artery disease (CAD) [11]. Moreover, in the same group of patients, the former drug more markedly reduced plasma leptin levels and increased plasma adiponectin, suggesting a stronger action on the hormonal activity of human adipose tissue [13].

In this study, we investigated whether the systemic anti-inflammatory action of ACE inhibitors, observed in our previous study [11], is related to their impact on lymphocyte secretory function in normotensive patients with CAD. We also assessed whether physicochemical and pharmacokinetic differences between tissue-type (perindopril) and plasma-type (enalapril) ACE inhibitors determine the strength of the lymphocyte-suppressing effect of these agents. Interleukin-2, interferon-γ and TNFα were selected for study because they are important products of T cells and increased levels are associated with a higher risk of the development and progression of atherosclerosis [23, 26]. Moreover, our team has long-term experience in their assessment [10, 14]. To assess systemic inflammation, we measured hsCRP plasma levels, which are considered to be a highly sensitive marker of low-grade vascular inflammation [8, 21]. Using very strict inclusion criteria in this study enabled us to minimize the impact of concurrent diseases and concomitant therapies.

Materials and Methods

Subjects

Patients (aged 40–70 years) were eligible for the study if they had stable CAD with the presence of clinical symptoms of this disorder despite treatment with acetylsalicylic acid, a β-blocker and a statin. The diagnosis of CAD was established on the basis of clinical symptoms and/or an exercise test performed using a bicycle ergometer with electric brakes. A positive result on the exercise stress test was defined as a horizontal or downsloping ST-segment depression of at least 1 mm at 80 ms after the J point. Subjects were excluded if they met at least one of the following criteria: (1) any form of acute coronary syndrome or a previous history of acute coronary syndromes; (2) chronic CAD as an indication for coronarography; (3) other acute ischemic conditions (presently or in the past); (4) diabetes mellitus; (5) obesity (BMI > 30 kg/m^2); (6) symptomatic congestive heart failure; (7) arterial hypertension (according to ESC/ESH); (8) any acute and chronic inflammatory processes; (9) impaired renal or hepatic function; (10) malabsorption syndromes; (11) previous treatment with ACE inhibitors or the existence of contraindications to the administra-

tion of ACE inhibitors; and/or (12) poor patient compliance.

Study design

One hundred thirty-four subjects fulfilled all entry criteria and were eligible for the study. All patients were fully informed of the purpose and the possible risks of the study and provided written informed consent. The study was performed in accordance with the 1964 Helsinki Declaration, and the protocol was accepted by the local ethics committee. These patients were randomly assigned in a double-blind fashion to receive enalapril (20 mg daily; n = 47), perindopril (4 mg daily; n = 45) or placebo (n = 42) according to a computer-generated randomization procedure. Because enalapril was administered twice daily while perindopril was given only once in the morning, the evening dose of perindopril was replaced with placebo. In turn, placebo was administered both in the morning and evening. No change in dosage was made throughout the 90-day study period. Each treatment group was divided into two subgroups with normal or disturbed insulin sensitivity, respectively. Normal insulin sensitivity was arbitrarily defined as a homeostasis model assessment (HOMA) index less than 2.0. If the HOMA index exceeded this threshold value, the patient was considered to be insulin-resistant. CAD patients were compared with 40 age-, sex- and weight-matched control subjects with no evidence of CAD, selected among individuals screened sonographically for the presence of asymptomatic atherosclerosis.

During the treatment, systolic and diastolic blood pressure were monitored at each visit in a sitting position using standard cuff equipment. They were determined during Korotkoff sounds 1 and 5. The values used in statistical analyses were the means of 3 measurements taken at intervals of at least 5 min, starting 15 min after the patient had sat down. All measurements were made on the left arm. Compliance with and the safety of the treatment were assessed twice monthly. Withdrawal criteria included symptomatic hypotonia, acute renal insufficiency, angioneurotic edema, elevation of plasma creatinine > 1.5 mg/dl or potassium > 5.3 mmol/l, neutropenia or thrombocytopenia.

Laboratory assays

Laboratory assays were performed at the following three times: at the beginning of the study and after 30 and 90 days of perindopril or enalapril treatment. Venous blood samples were drawn from the antecubital vein in a quiet, temperature-controlled room (24–25°C) after the patients had been in a recumbent position for at least 15 min. Samples, taken 12 h after the last meal, which occurred between 8:00 and 9:00 a.m. (to avoid circadian fluctuations of the parameters studied), were immediately coded so that the person performing laboratory assay was blinded to subject identity and study sequence. To minimize analytical errors, all assays were performed in duplicate.

The plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were assessed colorimetrically using commercially available kits purchased from bioMerieux (Marcy l'Etoile, France). LDL levels were measured directly. Plasma glucose content was determined using the glucose oxidase method with a glucose analyzer (Beckman, Palo Alto, CA, USA). Plasma insulin was assessed using commercially available radioimmunoassay kits (Linco Research Inc, St. Charles, MO, USA), which do not cross-react with human proinsulin. The HOMA index was calculated by the following formula: HOMA ratio = fasting blood glucose (mg/dl) × immunoreactive insulin (μU/ml)/405. Plasma levels of CRP were measured using a high-sensitivity monoclonal antibody assay (MP Biomedicals, Orangeburg, NY). Cytokine release from blood T lymphocytes stimulated with phytohemagglutinin was assessed as described previously [19]. Interleukin-2, interferon-γ and tumor necrosis factor-α (TNFα) were determined using commercial ELISA kits (R&D Systems, McKinley Place N.E. Minneapolis, MN) according to the manufacturer's instructions. The minimum detectable levels for hCRP, interleukin-2, interferon-γ and TNFα were 0.1 ng/ml, 8 pg/ml, 15 pg/ml and 4.4 pg/ml, respectively. The intraassay coefficients of variation for hCRP, interleukin-2, interferon-γ and TNFα were 4.7%, 3.8%, 5.5% and 4.6%, respectively.

Statistical analyses

Results are expressed as the means ± SD. The groups were compared using a one-way ANOVA followed by the *post-hoc* Bonferroni test (blood pressure, lipid profile, pre- and post-OGTT plasma glucose) or the Kruskall-Wallis test followed by the Mann-Whitney U test (insulin, HOMA, hsCRP, interleukin-2, interferon-γ and TNFα). Student's paired *t* test (blood pressure, lipid profile, pre- and post-OGTT plasma

glucose) or the Wilcoxon test (insulin, HOMA, hsCRP, interleukin-2, interferon-γ and TNFα) were applied to compare pre-, inter- and post-treatment data within the same group. To compare pre-, inter- and post-therapy data within the same treatment group, Student's paired *t* test (lipid profile and plasma glucose) or the Wilcoxon test (HOMA, hsCRP and cytokines) was applied. For categorical variables, the χ^2 test was used. Correlations were assessed using Kendall's tau test. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were made using GraphPad Prism 2.01 software (GraphPad Software Inc., San Diego, CA, USA) and Statistica 6.1 (StatSoft, Tulsa, OK, USA).

Results

Baseline characteristics

All groups were similar as regard to age, sex, weight, medical background and clinical characteristics (Tab. 1). Compared to the control group, normotensive patients with CAD exhibited higher plasma levels of hsCRP and increased lymphocyte release of interleukin-2, interferon-γ and TNFα.

Adverse effects

Three CAD subjects treated with placebo, two patients receiving enalapril and one patient treated with perindopril stopped participating in the study because of the exacerbation of CAD. Three patients receiving enalapril and one treated with perindopril prematurely terminated the study due to an excessive blood pressure reduction. All of these subjects received high doses of β-blockers. Four other patients, two receiving enalapril and two receiving perindopril, dropped out due to a severe persistent cough. Two patients belonging to the control group and one CAD patient who received placebo were withdrawn because of non-compliance with the study protocol. No other serious adverse events were observed throughout the study. Baseline characteristics of the 17 subjects who were withdrawn from the study did not differ from the 157 completing the trial (data not shown).

Placebo-treated control subjects and normotensive patients with CAD

Placebo treatment of both control subjects and normotensive patients with CAD did not affect blood pressure, plasma lipids, glucose metabolism markers, hsCRP or lymphocyte-cytokine release (Tab. 2).

Effect of ACE inhibitors on blood pressure, lipid profile and glucose metabolism markers

Neither perindopril nor enalapril induced any significant changes in blood pressure or plasma lipids after 30 and 90 days of treatment. Ninety days of perindopril treatment tended to reduce the value of the HOMA index by 23.0% ($p = 0.059$). No other changes in glucose metabolism markers were induced by ACE inhibitor treatment (Tab. 2).

hsCRP

Enalapril administered for 30 and 90 days produced no effect on plasma hsCRP or decreased it by 21.1% ($p < 0.05$), respectively. Perindopril treatment reduced plasma levels of this protein by 20.8% ($p < 0.05$) and 38.7% ($p < 0.001$), respectively, after 30 days and at the end of the study (Tab. 2). The effect of 90-day perindopril treatment was stronger than that of 30 days. Post-treatment levels of hsCRP still remained higher in CAD patients than in control subjects.

Lymphocyte cytokine release

No significant changes in cytokine release were observed after 30 days of enalapril treatment (Tab. 2). At the end of the treatment period, enalapril reduced interleukin-2 and interferon-γ release by 19.3% ($p < 0.05$) and 20.7% ($p < 0.05$), respectively, and tended to reduce TNFα release by 17.5% ($p = 0.078$). After 90 days of the study, the amount of release of all cytokines remained higher in CAD patients than in control subjects.

We found a decrease in interleukin-2 release by 19.1% ($p < 0.05$) and 37.2% ($p < 0.001$), interferon-γ release by 20.4% ($p < 0.05$) and 35.4% ($p < 0.001$), and TNFα release by 20.4% ($p < 0.05$) and 36.7% ($p < 0.001$), after 30- or 90-day treatment, respectively, with perindopril. The effect of perindopril on cytokine release was stronger after 90 days than after 30 days of treatment, and post-treatment cytokine release did not differ from that observed in the control group.

Tab. 1. Baseline characteristics of participants

	Control subjects	Placebo-treated patients	Enalapril-treated patients	Perindopril-treated patients
Number of patients	40	42	47 (22, 25)	45 (20, 25)
Age (years)	51.1 ± 5.9	52.8 ± 5.6	52.5 ± 4.8 (51.9 ± 5.2, 53.1 ± 6.1)	49.0 ± 4.8 (47.9 ± 5.3; 49.8 ± 5.0)
Females *(%)*	22.5	21.4	21.3 (18.2, 24.0)	22.2 (20.0, 24.0)
BMI (kg/m^2)	26.8 ± 2.4	26.1 ± 1.9	27.3 ± 2.5 (27.1 ± 2.6, 27.5 ± 2.2)	26.6 ± 1.9 (26.1 ± 3.1, 27.0 ± 2.2)
Smokers (%)	25.0	23.8	25.5 (22.7, 28.0)	20.0 (20.0, 20.0)
Systolic blood pressure (mmHg)	123.1 ± 5.8	124.5 ± 6.4	125.2 ± 8.2 (122.3 ± 8.2, 127.9 ± 7.8)	125.6 ± 7.1 (123.8 ± 9.2, 127.0 ± 5.6)
Diastolic blood pressure (mmHg)	76.5 ± 6.8	78.2 ± 6.2	80.0 ± 6.0 (79.9 ± 7.9, 80.1 ± 7.1)	79.1 ± 5.0 (77.9 ± 6.9, 80.7 ± 6.2)
Medications				
Statins (%)	7.5	95.2***	95.7*** (95.5***, 96.0***)	93.3*** (100.0***, 88.0***)
Acetylsalicylic acid (%)	10.0	95.2***	95.7*** (90.9***, 100.0***)	93.3*** (95.0***, 92.0***)
β-*blockers* (%)	12.5	90.5***	89.4*** (90.1***, 88.0***)	88.9*** (90.0***, 88.0***)
Total cholesterol (mg/dl)	210.2 ± 15.7	213.4 ± 20.1	223.9 ± 18.2 (220.2 ± 21.1, 227.2 ± 23.2)	224.6 ± 17.0 (220.2 ± 21.2, 228.1 ± 20.2)
LDL-cholesterol (mg/dl)	139.4 ± 10.4	149.3 ± 11.1	144.1 ± 12.1 (143.9 ± 14.0, 151.2 ± 15.0)	147.0 ± 11.2 (142.7 ± 20.9, 150.3 ± 16.5)
HDL-cholesterol (mg/dl)	47.1 ± 4.0	46.8 ± 4.7	46.4 ± 5.6 (52.3 ± 6.2, 43.5 ± 6.6)	47.6 ± 5.2 (52.4 ± 5.1, 43.9 ± 5.7)
Triglycerides (mg/dl)	151.5 ± 23.2	153.2 ± 25.1	156.9 ± 30.1 (140.2 ± 31.1, 172.1 ± 43.4)	157.6 ± 32.1 (141.2 ± 33.5, 170.4 ± 40.2)
Plasma glucose (mg/dl)	89.2 ± 5.0	90.4 ± 5.9	89.3 ± 4.7 (83.1 ± 5.5, 94.9 ± 5.0)	91.3 ± 6.7 (84.3 ± 8.0, 96.8 ± 7.8)
HOMA	3.5 ± 0.9	3.7 ± 1.0	3.7 ± 0.9 (1.3 ± 0.3, 5.9 ± 0.4$^{\#\#\#}$)	4.0 ± 1.0 (1.4 ± 0.2, 6.1 ± 0.4$^{\#\#\#}$)
hsCRP (mg/l)	0.9 ± 0.3	3.7 ± 0.2 ***	3.5 ± 0.2*** (3.0 ± 0.2***, 3.8 ± 0.3***$^{\#}$)	3.8 ± 0.2*** (3.3 ± 0.5***, 4.1 ± 0.4***$^{\#}$)
Interleukin-2 release (ng/ml)	3.0 ± 0.2	4.9 ± 0.2 ***	5.1 ± 0.2*** (4.3 ± 0.3***, 5.8 ± 0.4***$^{\#}$)	5.1 ± 0.4*** (4.3 ± 0.2***, 5.7 ± 0.4***$^{\#}$)
Interferon-γ release (ng/ml)	31.2 ± 4.1	49.5 ± 3.1***	51.5 ± 4.3*** (45.2 ± 5.1***, 57.2 ± 5.8***)	51.4 ± 5.0*** (46.0 ± 5.1***, 55.7 ± 6.0***)
TNFα release (pg/ml)	199.1 ± 18.3	341.3 ± 38.1***	331.9 ± 40.2*** (274.1 ± 29.2***, 384.2 ± 39.1***$^{\#}$)	342.9 ± 39.1*** (280.1 ± 25.1***, 392.1 ± 40.1***$^{\#}$)

Each value represents the mean ± SD. The values in parentheses represent the baseline values in subgroups with normal and reduced insulin sensitivity, respectively. *** $p < 0.001$ *vs.* healthy subjects. $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ *vs.* insulin-sensitive subjects in the same treatment group

Tab. 2. Effect of angiotensin-converting enzyme inhibitors on blood pressure, lipid profile, glucose metabolism markers, plasma hsCRP and lymphocyte cytokine release in normotensive subjects with coronary artery disease

	Control subjects (n = 38)	Placebo-treated patients (n = 38)	Enalapril-treated patients (n = 40)	Perindopril-treated patients (n = 41)
Systolic blood pressure (mmHg)				
Baseline	123.4 ± 6.0	125.1 ± 7.1	125.5 ± 8.5	125.9 ± 8.2
After 30 days	122.4 ± 5.9	124.4 ± 7.5	122.1 ± 8.1	122.0 ± 7.5
After 90 days	126.0 ± 6.2	126.4 ± 8.2	120.8 ± 5.6	121.3 ± 6.2
Diastolic blood pressure (mmHg)				
Baseline	76.4 ± 6.5	78.4 ± 5.9	80.3 ± 5.3	79.2 ± 5.2
After 30 days	77.1 ± 6.2	80.0 ± 6.5	77.9 ± 5.6	77.4 ± 5.0
After 90 days	77.4 ± 5.6	79.5 ± 6.4	77.2 ± 5.8	77.1 ± 5.1
Total cholesterol (mg/dl)				
Baseline	210.0 ± 15.5	215.2 ± 22.4	224.8 ± 19.4	225.8 ± 18.7
After 30 days	208.9 ± 16.7	213.4 ± 20.1	222.1 ± 18.3	229.2 ± 15.6
After 90 days	212.4 ± 16.0	217.5 ± 21.1	228.7 ± 22.5	223.4 ± 20.3
LDL-cholesterol (mg/dl)				
Baseline	139.1 ± 10.2	149.8 ± 11.6	144.5 ± 13.8	146.8 ± 11.5
After 30 days	140.2 ± 11.0	151.2 ± 12.8	143.1 ± 12.0	143.2 ± 10.4
After 90 days	138.6 ± 10.5	152.2 ± 11.5	141.1 ± 10.5	142.1 ± 10.9
HDL-cholesterol (mg/dl)				
Baseline	47.1 ± 3.9	46.6 ± 4.9	46.8 ± 5.5	47.8 ± 5.1
After 30 days	48.0 ± 3.7	48.3 ± 4.1	48.4 ± 3.8	49.6 ± 5.0
After 90 days	48.1 ± 4.2	44.2 ± 3.9	49.2 ± 4.1	49.9 ± 4.2
Triglycerides (mg/dl)				
Baseline	151.0 ± 22.0	153.2 ± 25.8	157.2 ± 29.8	158.0 ± 30.1
After 30 days	154.2 ± 23.1	153.0 ± 20.2	143.2 ± 22.6	142.3 ± 25.6
After 90 days	152.3 ± 20.6	158.4 ± 19.2	138.1 ± 19.3	140.0 ± 22.4
Plasma glucose (mg/dl)				
Baseline	89.4 ± 5.1	90.6 ± 5.9	89.7 ± 4.6	91.3 ± 6.8
After 30 days	88.6 ± 4.9	89.3 ± 5.7	88.1 ± 4.0	89.6 ± 6.5
After 90 days	89.0 ± 5.0	90.6 ± 5.5	87.1 ± 4.2	89.0 ± 4.8
HOMA				
Baseline	3.5 ± 1.0	3.7 ± 1.0	3.7 ± 0.9	4.0 ± 0.9
After 30 days	3.5 ± 0.9	3.9 ± 0.9	3.3 ± 0.7	3.4 ± 0.8
After 90 days	3.7 ± 1.0	3.8 ± 0.9	3.1 ± 0.8	3.2 ± 0.8
hsCRP (mg/l)				
Baseline	1.0 ± 0.3	3.7 ± 0.3***	3.4 ± 0.3***	3.8 ± 0.3***
After 30 days	1.1 ± 0.3	3.8 ± 0.4***	2.9 ± 0.3***$^{\#}$	3.0 ± 0.3***$^{\#}$
After 90 days	1.0 ± 0.2	3.8 ± 0.3***	2.7 ± 0.2***$^{\#}$	2.3 ± 0.2***$^{\#\#\#\$\wedge}$
Interleukin-2 release (ng/ml)				
Baseline	3.0 ± 0.3	4.9 ± 0.3***	5.1 ± 0.3***	5.1 ± 0.5***
After 30 days	2.9 ± 0.3	5.0 ± 0.4***	4.2 ± 0.4***$^{\#}$	4.2 ± 0.4***$^{\#}$
After 90 days	2.8 ± 0.2	4.9 ± 0.3***	4.1 ± 0.3**$^{\#}$	3.2 ± 0.3$^{\#\#\#\$\wedge}$
Interferon-γ release (ng/ml)				
Baseline	31.4 ± 4.6	49.7 ± 3.5***	51.8 ± 4.7***	51.7 ± 5.3***
After 30 days	30.2 ± 5.1	48.9 ± 5.1***	42.0 ± 4.6***$^{\#}$	41.2 ± 4.8**$^{\#}$
After 90 days	29.8 ± 3.9	50.1 ± 5.3***	41.1 ± 4.7**$^{\#}$	33.4 ± 3.8$^{\#\#\#\$\wedge}$
TNFα release (pg/ml)				
Baseline	198.8 ± 19.2	341.7 ± 39.0***	332.9 ± 39.2***	344.3 ± 38.6***
After 30 days	197.0 ± 18.7	345.7 ± 32.6***	276.2 ± 28.1***$^{\#}$	273.9 ± 28.1**$^{\#}$
After 90 days	196.2 ± 19.1	347.0 ± 31.1***	274.6 ± 24.0***	217.8 ± 23.2$^{\#\#\#\$\wedge}$

Each value represents the mean ± SD. ** $p < 0.01$, *** $p < 0.001$ *vs.* control subjects. $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ *vs.* baseline values. $^{\$}$ $p < 0.05$, the effect was stronger than after 30 days of treatment. ^ $p < 0.05$, the effect of perindopril was stronger than the effect of enalapril

Tab. 3. Effect of angiotensin-converting enzyme inhibitors on plasma hsCRP and lymphocyte cytokine release in insulin-sensitive and insulin-resistant subjects with coronary artery disease and normal blood pressure

	Enalapril-treated patients		Perindopril-treated patients	
	Insulin-sensitive subjects	Insulin-resistant subjects	Insulin-sensitive subjects	Insulin-resistant subjects
HOMA				
Baseline	1.3 ± 0.3	5.9 ± 0.4***	1.4 ± 0.2	6.1 ± 0.5***
After 30 days	1.2 ± 0.2	5.1 ± 0.5***	1.2 ± 0.3	5.0 ± 0.4***
After 90 days	1.1 ± 0.3	4.9 ± 0.4***	1.2 ± 0.2	4.7 ± 0.3***#
hsCRP (mg/l)				
Baseline	2.9 ± 0.3	3.8 ± 0.3*	3.3 ± 0.4	4.1 ± 0.5*
After 30 days	2.6 ± 0.3	3.2 ± 0.3	2.8 ± 0.3	3.1 ± 0.4#
After 90 days	2.5 ± 0.2	2.8 ± 0.2#	2.3 ± 0.2##	2.3 ± 0.3###$$^
Interleukin-2 release (ng/ml)				
Baseline	4.4 ± 0.3	5.7 ± 0.4*	4.3 ± 0.2	5.8 ± 0.4*
After 30 days	3.9 ± 0.4	4.5 ± 0.4#	3.6 ± 0.3	4,6 ± 0.4#
After 90 days	3.8 ± 0.3	4.4 ± 0.3#	3.4 ± 0.3#	3.1 ± 0.3###$$$^^^
Interferon-γ release (ng/ml)				
Baseline	45.6 ± 5.3	57.5 ± 6.0	46.2 ± 5.2	56.0 ± 6.2
After 30 days	39.1 ± 4.2	44.6 ± 5.1#	37.8 ± 4.4	43.8 ± 4.8#
After 90 days	38.8 ± 4.6	43.2 ± 5.8#	35.1 ± 4.8#	32.1 ± 4.1###$$^^^
TNFα release (pg/ml)				
Baseline	275.1 ± 30.1	385.1 ± 38.4*	282.1 ± 28.1	393.0 ± 39.8*
After 30 days	243.1 ± 25.1	306.2 ± 29.8#	231.5 ± 25.2	307.1 ± 32.0#
After 90 days	242.0 ± 26.7	304.0 ± 31.2#	224.7 ± 24.7#	212.4 ± 26.2###$$^

Each value represents the mean ± SD. * p 0.05, *** p < 0.001 *vs.* insulin-sensitive patients in the same treatment group. # p < 0.05, ## p < 0.01, ### p < 0.001 *vs.* baseline values. $$ p < 0.01, $$$ p < 0.001, the effect was stronger after 90-day treatment than after 30-day treatment. ^ p < 0.05, ^^^ p < 0.001, the effect of perindopril was stronger than the effect of enalapril in the same subgroup of patients

The analysis of subgroups

Insulin-sensitive patients

No changes in blood pressure, plasma lipids and glucose metabolism markers were observed after treatment with either ACE inhibitor in insulin-sensitive subjects (data not shown).

In CAD subjects with normal sensitivity to insulin, only perindopril reduced plasma hsCRP and cytokine release (Tab. 3). Administered for 30 days, it insignificantly decreased plasma hsCRP by 15.1% (p = 0.095), interleukin-2 release by 16.3% (p = 0.080), interferon-γ release by 18.2% (p = 0.074) and TNFα release by 17.9% (p = 0.078). At the end of the study period, perindopril reduced hsCRP, interleukin-2, interferon-γ and TNFα release by 30.3% (p < 0.01), 20.9% (p < 0.05), 24.0% (p < 0.05) and 20.3% (p < 0.05), respectively.

Insulin-resistant patients

Neither enalapril nor perindopril induced any changes in blood pressure, plasma lipids and plasma glucose in insulin-resistant subjects (data not shown).

Enalapril administered for 90 days did not significantly (by 16.9%, p = 0.087) decrease the HOMA index (Tab. 3). No changes were observed after 30 days of enalapril administration. Perindopril treatment tended to reduce the HOMA index (by 18.0%, p = 0.083) after 30 days and decreased it by 23% (p < 0.05) after 90 days of treatment.

Enalapril administered for 30 days reduced plasma hsCRP by 15.7% (p = 0.074) and after 90 days decreased it by 26.3% (p < 0.05). Perindopril treatment reduced plasma levels of this protein by 24.3% (p < 0.05) after 30 days and by 43.9% (p < 0.001) at the end of the study. The effect of perindopril was more pronounced after 90 days than after 30 days of treatment.

Enalapril treatment of insulin-resistant patients reduced interleukin-2 release by 21.0% ($p < 0.05$) and 22.8% ($p < 0.05$), interferon-γ release by 22.4% ($p < 0.05$) and by 24.9% ($p < 0.05$), as well as TNFα release by 20.5% ($p < 0.05$) and by 21.1% ($p < 0.05$) after 30 and 90 days of treatment, respectively.

After 30-day treatment of insulin-resistant patients, perindopril reduced interleukin-2 release by 20.7% ($p < 0.05$), interferon-γ release by 21.8% ($p < 0.05$) and TNFα release by 21.9% ($p < 0.05$). At the end of the study, interleukin-2, interferon-γ and TNFα release, when compared to baseline, decreased by 46.6% ($p < 0.001$), 42.7% ($p < 0.001$) and by 46.0% ($p < 0.001$), respectively. The effect of perindopril was stronger after 90 days of treatment than after 30 days. At the end of the study, lymphocyte cytokine release in CAD patients did not differ from that observed in control subjects.

Comparisons between the groups

Perindopril was superior to enalapril in reducing plasma hsCRP and lymphocyte release of interleukin-2, interferon-γ and TNFα. The superiority of perindopril over enalapril was observed in the whole population of normotensive subjects with CAD and, to an even greater extent, in the subgroup of insulin-resistant patients (Tabs. 2 and 3).

Correlations

At entry, there was no correlation between plasma hsCRP and lymphocyte cytokine release and blood pressure (both systolic and diastolic), plasma lipids and glucose metabolism markers. Perindopril- and enalapril-induced changes in hsCRP correlated with their action on lymphocyte release of interleukin-2 ($r = 0.51$, $p < 0.01$ for perindopril; $r = 0.49$, $p < 0.01$ for enalapril), interferon-γ ($r = 0.53$, $p < 0.01$ for perindopril; $r = 0.48$, $p < 0.05$ for enalapril) and TNFα ($r = 0.52$, $p < 0.01$ for perindopril; $r = 0.47$, $p < 0.01$ for enalapril). The effect of ACE inhibitors on hsCRP and cytokine release correlated with their action on the HOMA index (perindopril group: $r = 0.50$, $p < 0.01$ between Δinterleukin-2 and ΔHOMA, $r = 0.52$, $p < 0.01$ between Δinterferon-γ and ΔHOMA, $r = 0.53$, $p < 0.01$ between ΔTNFα and ΔHOMA; enalapril group: $r = 0.46$, $p < 0.01$ between Δinterleukin-2 and ΔHOMA, $r = 0.43$, $p < 0.05$ between Δinterferon-γ and ΔHOMA, $r = 0.42$, $p < 0.05$ between ΔTNFα and ΔHOMA), but not with their effects on the lipid profile or arterial pressure.

Discussion

The major finding of this prospective, double-blind, placebo-controlled randomized study is that perindopril is superior to enalapril when it comes to producing a lymphocyte-suppressing effect and that this effect is especially prominent in insulin-resistant CAD subjects. This suggests that tissue-type ACE inhibitors are a better treatment option in CAD subjects with normal arterial blood pressure than plasma-type inhibitors.

Baseline lymphocyte release of interleukin-2, interferon-γ and TNFα increased and correlated with plasma hsCRP levels after treatment with tissue-type ACE inhibitors. This indicates that low-grade systemic inflammation observed in our normotensive subjects is, in part, determined by disturbed lymphocyte secretory function. Taking into account the important role of T cells in the pathogenesis of atherosclerosis and the development of its complications [7, 28] and the association between high levels of interleukin-2, interferon-γ and TNFα and the increased risk of cardiovascular events [23, 26], our results seem to indicate that CAD patients already receiving aspirin, a β-blocker and a statin often require further treatment with agents reducing lymphocyte cytokine release.

Perindopril treatment reduced interleukin-2, interferon-γ and TNFα release to the level observed in control subjects, and this effect was accompanied by a decrease in the plasma levels of hsCRP. Taking into account that CRP is directly involved in atherogenesis, and its elevated levels are strongly associated with an increased risk of cardiovascular events [8, 21], a systemic anti-inflammatory effect of simvastatin may contribute to the cardiovascular benefits of therapy with tissue ACE inhibitors in this group of patients. Previously, we found a similar association between perindopril action on hsCRP and its effect on monocyte cytokine release [10]. Their results, together with those from this study, indicate that the systemic anti-inflammatory effects of tissue ACE inhibitors results from the action of these agents on various inflammatory cells.

The lymphocyte-suppressing and previously observed monocyte-suppressing effects of perindopril and enalapril may partially contribute to the clinical benefits of ACE inhibitors. Treatment with these agents resulted in the reduction of cardiovascular death, myocardial infarction and cardiac arrest observed in perindopril-treated CAD subjects that participated in the EUROPA trial [4], in the ramipril-induced decrease in myocardial infarction, stroke or cardiovascular death in patients at high cardiovascular risk recruited to the HOPE study [29] and to an insignificant reduction in stroke incidence in subjects who underwent a previous stroke or ischemic transient attack enrolled in the PROGRESS study [20]. Our results are, however, in contrast with the results of the PEACE trial, which showed no impact of trandolapril on the incidence of non-fatal myocardial infarction, cardiovascular death and revascularization in CAD individuals with normal left ventricle function [27]. Although a higher proportion of patients included in the PEACE study used lipid-lowering agents than in the EUROPA and PROGRESS studies and lipid-lowering agents were found to reduce lymphocyte cytokine release [19], this does not seem to explain the disagreement between our results and those of the PEACE study because most of our patients received statins. In our opinion, a more likely explanation is that participants of the PEACE study had lower cardiovascular risk than our subjects. If this hypothesis is true, ACE inhibitors probably bring more benefits to CAD patients with high than rather than low cardiovascular risk.

The present study has shown the superiority of perindopril over enalapril in reducing lymphocyte-cytokine release. These results, supporting our previous studies, clearly indicate that tissue-type ACE enzyme inhibitors produce stronger anti-inflammatory effects than plasma type ones. Because the difference in the strength of the lymphocyte-suppressing effects of both these agents was particularly significant in insulin-resistant subjects, these patients should be treated with tissue-type ACE inhibitors. Interestingly, we recently observed similar dependence between perindopril and enalapril action on plasma resistin in subjects resistant to insulin [Krysiak et al., submitted], which is in line with our hypothesis.

Some previous studies showed that the presence of abnormally high blood pressure is associated with increased production of inflammatory markers [6, 16]. However, our study clearly indicates that the lymphocyte-suppressing effects of both ACE inhibitors do not result from their hypotensive properties. The effect of perindopril and enalapril on blood pressure was small and did not correlate with their effect on cytokine release. Although the presence of dyslipidemia was associated with increased lymphocyte cytokine release, and this effect was prevented by lipid-lowering agents [19], the absence of perindopril- and enalapril-induced changes in lipid profile argues against an association between ACE inhibitor action on lymphocytes and lipid metabolism. Interestingly, a subgroup analysis revealed that ACE inhibitors, particularly perindopril, inhibited the release of resistin to a greater extent in insulin-resistant than in insulin-sensitive subjects. This finding is the first that shows that the degree of tissue insulin sensitivity may determine the strength of the anti-inflammatory effect of ACE inhibitors. These results may suggest the use of these agents in CAD subjects with even mild glucose metabolism abnormalities, even if they do not meet all the criteria of the metabolic syndrome. Taking into account that we have demonstrated the superiority of perindopril over enalapril in insulin-resistant subjects, it seems that the former group of patients benefits the most from treatment with tissue-type ACE inhibitors.

Our results revealed that the lymphocyte-suppressing effects of perindopril, but not of enalapril, were more evident after 90 days of treatment than after 30. These results indicate that the strength of perindopril action on lymphocyte cytokine release increases with time, in contrast to enalapril, which produces its maximal lymphocyte-suppressing effect in the first month of treatment.

Despite treatment with statins, most patients included in our study had inappropriately high plasma levels of LDL cholesterol, which reflect the unsatisfactory effectiveness of hypolipidemic therapy in the Polish population. Therefore, the question of whether the effect of ACE inhibitors on cytokine release and systemic inflammation is similar in subjects who reach the recommended levels of plasma LDL cholesterol below 100 mg/dL (less than 16% of patients after a cardiovascular event in Poland) [25] requires further study. Two findings of our study seem to support this hypothesis. The lymphocyte-suppressing and systemic anti-inflammatory effects of both ACE inhibitors did not correlate with their action on plasma lipids. Moreover, perindopril reduced the lymphocyte cytokine release and plasma hsCRP in 8 patients with plasma LDL cholesterol levels below 100 mg/dL

(data not shown). Interestingly, taking into account our recent results, which showed that ezetimibe, despite reducing total and LDL cholesterol levels, produced only a weak anti-inflammatory effect [12, 15], combined therapy with a tissue ACE inhibitor and ezetimibe may be an interesting treatment option in CAD patients with elevated LDL cholesterol levels in whom statin therapy is either contraindicated or results in adverse effects.

Our study has also some other limitations. Firstly, the diagnosis of CAD was established only indirectly, on the basis of clinical manifestations and/or the results of the exercise test. Because no coronary angiography was performed, we cannot exclude the possibility that some participants were misdiagnosed with CAD, while the control group may have included individuals with asymptomatic atherosclerotic lesions of the coronary arteries. Secondly, the number of participants was relatively small and therefore it is possible that the effect of enalapril on lymphocyte cytokine release is stronger than that observed in our study. Finally, the definition of insulin resistance and insulin sensitivity was arbitrary, so the results obtained might be slightly different if other criteria of insulin sensitivity were used.

In summary, our study has shown that perindopril is superior to enalapril in reducing lymphocyte cytokine release. The strength of this action is determined by the level of insulin-sensitivity but is not associated with hypotensive properties of ACE inhibitors. Our results suggest that tissue-type ACE inhibitors should be preferred in the treatment of normotensive CAD patients, particularly if they are insulin resistant.

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