Pharmacological Reports
2008, 60, 514–523
ISSN 1734-1140

Pleiotropic effects of angiotensin-converting enzyme inhibitors in normotensive patients with coronary artery disease

Robert Krysiak, Bogusław Okopień

Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Medyków 18, PL 40-752 Katowice, Poland

Correspondence: Robert Krysiak, e-mail: r.krysiak@poczta.onet.pl

Abstract:
Angiotensin-converting enzyme inhibitors proved to be effective in the primary and secondary prevention of cardiovascular diseases. Clinical effectiveness of this group of agents may largely depend on their pleiotropic effects. The purpose of this study was to compare the effects of plasma- and tissue-type angiotensin-converting enzyme inhibitors on blood pressure and on systemic inflammation, hemostasis and oxidative functions in normotensive patients with stable coronary artery disease.
Ninety patients with stable coronary artery disease enrolled into the study were randomly divided into three different groups, simultaneously treated with enalapril (20 mg/d, n = 30), perindopril (4 mg/d, n = 30) or placebo (n = 30). Plasma lipid profile and the levels of oxidized low density lipoproteins (LDLs), monocyte chemoattractant protein (MCP)-1, interleukin-10, C-reactive protein (CRP), fibrinogen and plasminogen activator inhibitor (PAI)-1 were determined at the beginning of the study and after 30 and 90 days of treatment. Seventy-six patients completed the trial.
Neither enalapril nor perindopril affected blood pressure or plasma lipids. Perindopril significantly reduced plasma levels of oxidized LDLs, CRP, MCP-1, fibrinogen and PAI-1, and increased interleukin-10. The effect of enalapril on these markers of systemic inflammation, hemostasis and oxidative functions was much less pronounced.
The results showed that enalapril and perindopril were devoid of a blood pressure-lowering effect in normotensive patients with stable coronary artery disease. Perindopril was superior to enalapril in exhibiting antioxidant, antithrombotic and profibrinolytic activities. The treatment-induced changes in the balance between pro- and antiinflammatory cytokines and in hemostasis may contribute to the clinical effectiveness of tissue angiotensin-converting enzyme inhibitors in the therapy of atherosclerosis-related disorders.

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

Abbreviations: ACE – angiotensin-converting enzyme, CRP – C-reactive protein, ELISA –enzyme-linked immunosorbent assay, HDL – high density lipoproteins, hsCRP – high sensitivity C-reactive protein, ICAM-1 – intercellular adhesion molecule-1, LDL – low density lipoproteins, MCP-1 – monocyte chemoattractant protein-1, MMP-9 – matrix metalloproteinase 9, PAI-1 – plasminogen activator inhibitor-1, TNF-α – tumor necrosis factor-α VCAM-1 – vascular cell adhesion molecule-1

Introduction

Recently conducted three large clinical trials have shown that angiotensin-converting enzyme (ACE) inhibitors: perindopril (EUROPA) [8], ramipril (HOPE) [40] and, although to a less extent, trandolapril (PEACE)

[1] reduce mortality and fatal and non-fatal cardiovascular events in atherosclerotic patients without heart failure and left ventricular systolic dysfunction. A combined analysis of these trials [4] provides similar results and indicates that the use of ACE inhibitors should be considered in all patients with atherosclerosis. Perindopril [2, 8] reduced cardiovascular events more efficiently than expected for the small reduction in blood pressure. This may result from the fact that these drugs produce many other beneficial effects, including regulation of smooth muscle cell proliferation and migration, cytoprotection of vascular endothelium, anti-inflammatory and antioxidant effects, beneficial action on endogenous fibrinolysis and antiplatelet effects [13, 19, 21, 34].

Although ACE inhibitors were found to reduce the production of interleukin-1β [25], interleukin-6 [37], interleukin-8 [30], C-reactive protein (CRP) [6, 17], interleukin-12 [3], tumor necrosis factor-α (TNF-α) [25], interferon-γ [3], E-selectin [12], intercellular adhesion molecule (ICAM)-1 [12], vascular cell adhesion molecule-1 (VCAM-1) [12], monocyte chemoattractant protein (MCP)-1 [12, 30], matrix metalloprotease 9 (MMP-9) [28], and to increase the production of interleukin-10 [28], in some studies they were devoid of these effects [31, 36]. It should be stated that most of the studies in which ACE inhibitors revealed their pleiotropic anti-inflammatory effects included patients with arterial hypertension or congestive heart failure often not suffering from coronary artery disease, or inflammatory cells were obtained from healthy subjects. Therefore, it remains unresolved whether similar benefits are observed also in subjects suffering only from coronary artery disease.

ACE inhibitors differ in their solubility, oral bioavailability, hepatic extraction, elimination half-life of active compounds and protein binding capacity [26]. Depending on their relative affinity to tissue, ACE are categorized into two groups: lower-affinity or plasma inhibitors (perindopril, quinapril, ramipril and banazepril) and higher-affinity or tissue inhibitors (captopril, enalapril and lisinopril) [7, 39]. However, it still remains unclear whether the beneficial action of ACE inhibitors on inflammation, hemostasis and oxidative functions results from their "class effect" or differs from drug to drug.

In this prospective, double-blind, placebo-controlled randomized trial, we assessed the effect of a 90-day treatment with ACE inhibitors on the plasma levels or activities of some inflammatory and hemostatic risk factors for coronary artery disease, namely MCP-1, interleukin-10, CRP, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) in normotensive subjects with coronary artery disease. The second aim of our trial was to investigate which of two drugs, a tissue-specific (perindopril) or plasma-type (enalapril) ACE inhibitor [7, 39] is more efficient in normalizing stable coronary disease-related changes in the established plasma markers of cardiovascular risk. 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 40–69 years) were enrolled in the study if they met the following criteria: (1) established stable coronary artery disease; (2) the presence of clinical symptoms despite treatment with aspirin, a β-blocker and a statin; (3) for women, at least 24 months since the last menstruation, hysterectomy, ovariectomy, or barrier contraception.

The diagnosis of coronary artery disease was established on the basis of clinical symptoms and/or the exercise test performed using a bicycle ergometer with electric brakes. A positive result on exercise stress testing was defined as horizontal or downsloping ST-segment depression of at least 1 mm at 80 ms after the J point.

The exclusion criteria were as follows: (1) any form of acute coronary syndrome or a previous history of acute coronary syndromes; (2) chronic coronary artery disease being 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 WHO/ISH 1999); (8) any acute and chronic inflammatory processes; (9) impaired renal or hepatic function; (10) malabsorption syndromes; (11) hemorrhagic diathesis; (12) previous treatment with ACE inhibitors; (13) any contraindication to ACE inhibitor therapy; (14) ongoing hormonal replacement therapy or oral contraception and (15) poor patient compliance.

Study design

Ninety patients met the entry criteria. All patients gave their written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Bioethical Committee of the Medical University of Silesia.

The participants were randomized in a double-blind fashion to enalapril (20 mg daily; n = 30), perindopril (4 mg daily; n = 30) or placebo (n = 30) according to a computer-generated randomization procedure. Because enalapril was administered twice daily while perindopril only once in the morning, in place of the evening dose of perindopril, the patients received placebo. In the placebo-treated group, placebo was administered both in the morning and evening. The treatment lasted 90 days and no changes in the therapy were made during the study.

Taking history, clinical examination, measurement of blood pressure and venous blood sampling, for evaluating safety laboratory parameters, were performed before and 15, 30, 60 and 90 days after the start of therapy. 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. All measurements were made on the left arm. 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. During each visit patients were interviewed for adverse effects such as cough, hypotonia, allergic reactions as well as for the symptoms of renal or liver insufficiency, potassium excess, neutropenia and trombocytopenia. Laboratory tests included total and differential blood cell count, blood sedimentation rate, electrolytes, creatinine, bilirubin, alanine and aspartate aminotransferases, γ-glutamyltransferase, alkaline phosphatase, total proteins, creatine kinase, urine examination and 12-lead ECG. Symptomatic hypotonia, acute renal insufficiency, angioneurotic edema, elevation of plasma creatinine > 1.5 mg/dl or potassium > 5.3 mmol/l, neutropenia or thrombocytopenia were considered indications for withdrawal from the study.

Laboratory assays

Lipid profile, and plasma levels of glucose, oxidized low density lipoproteins (LDLs), MCP-1, interleukin-10, high sensitivity CRP (hsCRP), fibrinogen and PAI-1 were determined before and after 30 and 90 days of therapy. Blood samples were taken after an overnight fast in a quiet, temperature-controlled room (24–25°C) between 8.00 and 9.00 a.m. (to avoid circadian fluctuations of the parameters studied) and collected into tubes containing sodium citrate. The samples were immediately coded so that the person performing laboratory assay was blinded to subject identity and study sequence. All the tests were performed according to manufacturers' instructions within 48 h after collection. To minimize analytical errors, all assays were carried out in duplicate.

Plasma glucose concentrations were measured by a glucose oxidase method (Beckman, Palo Alto, CA). The serum levels of total cholesterol, LDL-cholesterol, high density lipoprotein (HDL)-cholesterol and triglycerides were assessed colorimetrically using commercial kits (bioMerieux, Marcy l'Etoile, France). Oxidized LDL-cholesterol levels were determined by an enzyme-linked immunosorbent assay (ELISA) method (Mercodia, Uppsala, Sweden). Plasma interleukin-10 and MCP-1 levels were estimated using commercial ELISA kits (R&D Systems, McKinley Place N.E. Minneapolis, MN) according to the manufacturer's instructions. Plasma levels of CRP were measured using a high-sensitivity monoclonal antibody assay (MP Biomedicals, Orangeburg, NY). Fibrinogen levels were determined by the Clauss method using a semi-automated blood coagulation analyzer OPTION 2 Plus using reagents obtained from bioMerieux (Marcy l'Etoile, France). PAI-1 antigen levels were assessed by a commercially available ELISA method (Asserachrom, Diagnostica Stago, Asnieres, France). The minimum detectable levels for the assessed parameters were: 5.0 pg/ml, 0.8 pg/ml, 0.1 mg/l, 0.1 g/l and 1.0 ng/ml, respectively, for MCP-1, interelukin-10, hsCRP, fibrinogen and PAI-1. The intra- and interassay coefficients of variation in our laboratory were as follows: hsCRP – 4.3% and 5.9%, MCP-1 – 4.0% and 4.8%, interleukin-10 – 3.2% and 3.8%, fibrinogen – 3.6% and 2.3%, PAI-1 – 8.7% and 5.0%, and oxidized LDLs – 4.0% and 7.4%, respectively.

Statistical analysis

Results are presented as the mean ± SD. Comparisons between the groups were performed using one-way ANOVA followed by the *post-hoc* Newman-Keuls test (blood pressure, lipid profile and plasma glucose) or using the Kruskall-Wallis test followed by the Mann-Whitney U test (MCP-1, interleukin-10, hsCRP,

fibrinogen and PAI-1). To compare pre-, inter- and post-therapy data within the same treatment group paired Student's *t*-test (blood pressure, lipid profile and plasma glucose) or the Wilcoxon test (MCP-1, interleukin-10, hsCRP, fibrinogen and PAI-1) were applied. Correlations were calculated with the Kendall Tau test; p values less than 0.05 were regarded as statistically significant. Statistical analysis was performed using the GraphPad Prism 2.01 software (GPA-26576-117).

Results

Baseline characteristics

At study entry, there was no difference between the treatment groups in terms of sex, weight, age, medical background, clinical characteristics and safety parameters. Demographic data and baseline blood test values in patients who completed the trial are presented in Table 1.

Adverse effects

Five patients, two subjects who were given placebo, two other subjects treated with enalapril and one patient who received perindopril, dropped out because of the exacerbation of coronary artery disease. Three other patients belonging to the enalapril-treated group, who had been already receiving high doses of β-blockers, were withdrawn from the trial due to excessive blood pressure lowering. After reducing the doses of β-blockers in all three cases blood pressure normalized despite continuation of treatment with the same dose of enalapril. One patient who received enalapril and two patients treated with perindopril prematurely terminated the trial because of severe persistent cough. Two patients belonging to the control group and one patient who received enalapril dropped out because of non-compliance with the study regimen. In the remaining patients, neither significant adverse

Tab. 1. Baseline characteristics of patients who completed the trial

	Placebo-treated group	Enalapril-treated group	Perindopril-treated group
Number of patients	26	23	27
Age (years)	56.0 ± 11.0	53.4 ± 6.6	48.8 ± 5.8
Female/Male	5/21	6/17	4/23
BMI (kg/m^2)	28.3 ± 3.3	27.5 ± 2.7	28.3 ± 2.3
Smokers (%)	19.2	17.4	14.8
Systolic blood pressure (mmHg)	124.2 ± 7.5	125.5 ± 9.0	125.2 ± 7.4
Diastolic blood pressure (mmHg)	78.3 ± 6.7	79.8 ± 7.5	80.2 ± 6.1
Mean blood pressure (mmHg)	93.6 ± 6.5	95.0 ± 7.7	95.2 ± 6.0
Total cholesterol (mg/dl)	210.4 ± 39.0	232.0 ± 42.7	221.0 ± 26.0
LDL-cholesterol (mg/dl)	139.9 ± 34.1	156.4 ± 31.5	141.4 ± 15.0
HDL-cholesterol (mg/dl)	46.5 ± 11.6	46.2 ± 9.9	49.6 ± 8.7
Triglycerides (mg/dl)	121.3 ± 48.8	179.8 ± 99.3	136.0 ± 64.4
Glycemia (mg/dl)	90.7 ± 10.4	89.5 ± 9.1	90.5 ± 8.2
Oxidized LDL (IU/l)	38.4 ± 4.1	41.9 ± 4.6	43.0 ± 4.3
MCP-1 levels (pg/ml)	119.5 ± 8.6	126.7 ± 12.2	121.3 ± 8.2
Interleukin-10 (pg/ml)	13.10 ± 1.21	12.22 ± 0.96	12.53 ± 1.15
hsCRP (mg/l)	3.08 ± 0.43	3.14 ± 0.23	3.14 ± 0.35
Fibrinogen (g/l)	3.91 ± 0.58	4.03 ± 0.65	4. 09 ± 0.61
PAI-1 antigen (ng/ml)	88.3 ± 11.2	81.7 ± 8.9	84.6 ± 9.1

Each value represents the mean ± SD

effects nor any other complications were reported throughout the study and all laboratory safety parameters remained within normal limits. Baseline characteristics of the 14 subjects who left the study did not differ from the 76 completing the trial (data not shown).

Placebo-treated group (Tab. 2, Fig. 1, 2)

In placebo-treated patients with coronary artery disease, blood pressure, lipid/lipoprotein profile, glucose level, plasma levels/activity of hsCRP, MCP-1, interleukin-10, fibrinogen, PAI-1 and oxidized LDLs remained at the similar level throughout the study.

Effect of ACE inhibitors on blood pressure and lipid profile (Tab. 2)

Neither of the tested drugs produced any significant effect on blood pressure and lipid profile after 30 and 90 days of treatment.

Effect of ACE inhibitors on measured parameters of inflammation, hemostasis and oxidative processes

MCP-1

Only perindopril decreased (by 7.9%) plasma MCP-1 levels after 30 days ($p < 0.05$) while no effect was produced by enalapril. Both these drugs decreased plasma MCP levels at the end of the study. For enalapril this reduction was by 13.3% ($p < 0.05$) while for perindopril by 18.1% ($p < 0.01$) (Fig. 1).

Interleukin-10

Both enalapril and perindopril increased plasma interleukin-10 levels. Enalapril increased interleukin-10 insignificantly by 16.9% ($p = 0.069$) and by 21.8% ($p < 0.01$) after 30 and 90 days of treatment, respectively. Perindopril increased plasma levels of this cy-

Tab. 2. The effect of enalapril and perindopril on blood pressure, lipid profile and plasma glucose levels in normotensive patients with coronary artery disease

	Placebo-treated group			Enalapril-treated group			Perindopril-treated group		
	Baseline	After 30 days	After 90 days	Baseline	After 30 days	After 90 days	Baseline	After 30 days	After 90 days
Systolic blood pressure [mmHg]	124.2 ± 7.5	122.6 ± 5.4	121.1 ± 4.9	125.5 ± 9.0	123.4 ± 7.6	121.1 ± 6.8	125.2 ± 7.4	122.5 ± 7.1	120.2 ± 6.5
Diastolic blood pressure [mmHg]	78.3 ± 6.7	77.0 ± 5.1	75.9 ± 5.3	79.8 ± 7.5	77.9 ± 6.7	76.2 ± 7.5	80.2 ± 6.1	78.2 ± 6.9	75.6 ± 6.9
Mean blood pressure [mmHg]	93.6 ± 6.5	92.2 ± 5.2	91.0 ± 5.1	95.0 ± 7.7	93.1 ± 6.6	91.2 ± 6.9	95.2 ± 6.0	93.0 ± 6.7	90.5 ± 6.2
Total cholesterol (mg/dl)	210.4 ± 39.0	205.2 ± 42.1	204.0 ± 32.0	232.0 ± 42.7	227.9 ± 42.4	223.2 ± 36.9	221.0 ± 26.0	211.3 ± 33.1	213.1 ± 28.0
LDL-cholesterol (mg/dl)	139.9 ± 34.1	126.9 ± 28.8	127.5 ± 33.0	156.4 ± 31.5	151.0 ± 24.1	147.2 ± 24.9	141.4 ± 15.0	133.8 ± 27.1	137.3 ± 22.3
HDL-cholesterol (mg/dl)	46.5 ± 11.6	49.7 ± 12.5	47.4 ± 11.1	46.2 ± 9.9	43.3 ± 5.3	49.2 ± 7.1	49.6 ± 8.7	47.5 ± 9.2	45.1 ± 6.0
Triglycerides (mg/dl)	121.3 ± 48.8	145.1 ± 52.0	153.4 ± 49.1	179.8 ± 99.3	141.3 ± 66.6	140.0 ± 48.0	136.0 ± 64.4	150.2 ± 52.9	135.6 ± 49.6
Glycemia (mg/dl)	90.7 ± 10.4	86.3 ± 10.8	86.7 ± 11.2	89.5 ± 9.1	85.6 ± 9.8	84.2 ± 10.5	90.5 ± 8.2	86.1 ± 7.8	86.1 ± 10.5

Each value represents the mean ± SD

tokine by 20.2% ($p < 0.05$) after 30 days and by 31.0% ($p < 0.001$) after 90 days of treatment (Fig. 1).

hsCRP

Both ACE inhibitors administered for 30 days did not produce any significant effect on plasma hsCRP. After 90 days, perindopril reduced plasma levels of this protein by 11.8% ($p < 0.05$) while the effect of enalapril was still insignificant ($p = 0.094$) (Fig. 1).

Fibrinogen

Enalapril produced no significant effect on plasma fibrinogen. Perindopril decreased plasma fibrinogen levels by 13.8% ($p < 0.01$) and by 19.6% ($p < 0.01$) after 30 and 90 days, respectively (Fig. 2).

PAI-1

Plasma PAI-1 levels tended to decrease by 5.6% ($p = 0.098$) and 8.2% ($p = 0.064$) and decreased significantly by 10.4% ($p < 0.05$) and 17.4% ($p < 0.01$) after 30 and 90 days of treatment with enalapril and perindopril, respectively (Fig. 2).

Oxidized LDLs

Oxidized LDLs were not affected by 30-day enalapril treatment but they showed an insignificant decrease

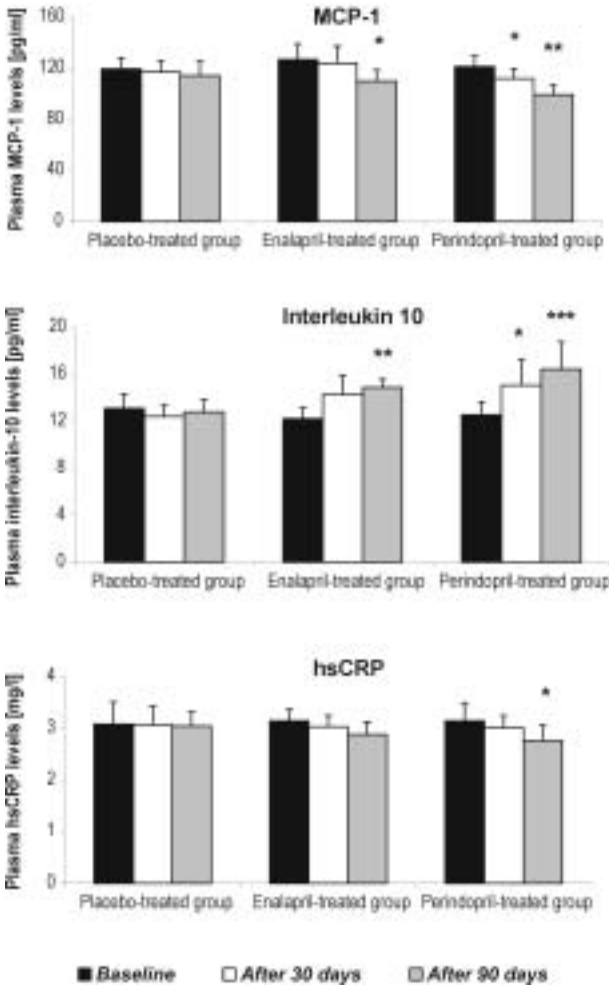

Fig. 1. Effect of angiotensin-converting enzyme (ACE) inhibitors on plasma levels of cytokines and high sensitivity C-reactive protein (hsCRP). Data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* pretreatment values

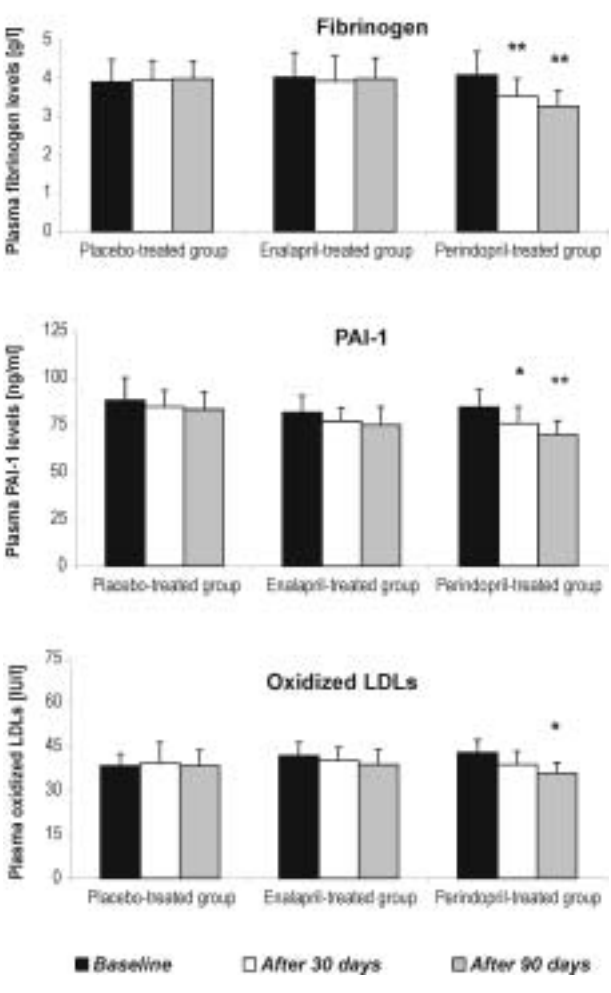

Fig. 2. Effect of angiotensin-converting enzyme (ACE) inhibitors on plasma levels of fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and oxidized low density lipoproteins (LDLs). Data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$ *vs.* pretreatment values

(by 7.2%; p = 0.087) after 90 days. After 30 days of treatment with perindopril, oxidized LDLs tended to decrease (by 10.2%; p = 0.061), while at the end of the study they showed a significant decrease by 16.5% ($p < 0.05$) (Fig. 2).

Correlations

At baseline, none of the groups showed any correlations between plasma levels of the studied factors and blood pressure, lipid profile and glucose level. The effect of enalapril and perindopril on hsCRP correlated positively with their action on MCP-1 and negatively with their action on interleukin-10. However, these correlations were weak (r values between 0.48 and 0.56, $p < 0.05$ for MCP-1, and between –0.45 and –0.51, $p < 0.05$ for interleukin-10). There were no correlations between the effects of enalapril and perindopril on blood pressure, lipid profile and glucose level and their effects on the studied markers.

Discussion

The major finding of our prospective trial is that perindopril exhibits stronger anti-infammatory, anticoagulant and antioxidant effects compared with enalapril. Although the decrease in MCP-1 was induced by both studied drugs, the effect of perindopril appeared earlier and was more prominent than that of enalapril. Perindopril was also superior to enalapril in increasing plasma levels of interleukin-10. Furthermore, perindopril but not enalapril reduced significantly plasma levels of hsCRP, fibrinogen and PAI-1. So far, only a few studies were conducted to compare the effects of various ACE inhibitors on cytokine production and hemostasis, and their results are inconclusive. Schindler et al. [29] observed that captopril, enalapril and cilazapril, but not ramipril, lisinopril, perindopril and spirapril, reduced the synthesis of interleukin-1 and TNF-α by stimulated human peripheral blood mononuclear cells. In another study [3], both captopril and lisinopril significantly inhibited interleukin-12 production by these cells and suppressed inferferon-γ production by activated human T cells. TNF-α production by both human blood mononuclear cells and *in vivo* in mice was significantly inhibited by a single supraphysiological dose of captopril, delapril and cilazapril [9]. Captopril, idrapril or fosinopril inhibited synthesis of tissue factor in human monocytes from healthy subjects [20] but different ACE inhibitors had distinct effects on platelet thromboxane B_2 formation in patients with essential hypertension [18]. Although assessment of only one drug in each group does not allow us to generalize, our results may suggest that tissue-type ACE inhibitors are more effective drugs in the treatment of chronic coronary artery disease in normotensive patients than the plasma-type subgroup of these agents.

Our study has revealed that ACE inhibitors altered plasma cytokine levels, providing data that the modulation of cytokine production may contribute to the positive effect of these agents on the incidence and severity of acute vascular events, reported previously in some large clinical trials [8, 27, 40]. By reducing MCP-1 and increasing interleukin-10 plasma levels ACE inhibitors, mainly perindopril, shifted the balance between pro- and anti-inflammatory cytokines toward the latter ones. Moreover, peridopril (and much less also enalapril) reduced hsCRP plasma levels, an important marker of systemic inflammation, the elevated levels of which are strongly associated with an increased risk of cardiovascular events and which, as recent results show, is directly involved in atherogenesis [32]. Taking into account that CRP stimulates MCP-1 [24] and inhibits interleukin-10 [33] production, the treatment-induced changes in plasma content of both studied cytokines may partially result from CRP-lowering action of ACE inhibitors. However, the fact that the changes in hsCRP were less prominent, reached the level of significance only in the case of perindopril, and correlated weakly with the treatment-induced changes in plasma levels of both these cytokines suggests that cytokine production by inflammatory cells is regulated also by other factors. Therefore, plasma hsCRP content does not reflect precisely the effect of ACE inhibitors on the balance between pro- and anti-inflammatory cytokines.

Taking into consideration that both fibrinogen and PAI-1 belong to cardiovascular risk factors [35, 38] and that even small differences in fibrinogen level among the population change the risk of coronary artery disease and its complications [14], the action of perindopril on fibrinogen and PAI-1 may be clinically relevant. Moreover, it is likely the beneficial effect of perindopril on hemostasis is partially indirect because this agent decreased oxidized LDLs which enhance the expression of tissue factor in macrophages, inter-

fere with endothelial thrombomodulin expression and produce an inhibitory effect on fibrinolysis [11].

There are some evidence both from our [15] and other [10, 16] laboratories that arterial hypertension is associated with the increased levels of the markers of inflammation and hemostasis. However, the beneficial effects of the ACE inhibitors on coagulation, inflammation and oxidative processes in our study were not secondary to the reduction in arterial blood pressure. The fact that treatment-induced changes in the studied markers were accompanied by only small and insignificant changes in arterial blood pressure and there was no correlation between them, speaks against the hypothesis that the blood-lowering action is responsible for the pleiotropic effects of ACE inhibitors in the studied group of patients. This finding supports the rationale of using these drugs, especially perindopril, also in normotensive patients with coronary artery disease. Furthermore, the lack of the effect on lipid/lipoprotein profile and blood glucose indicates that the observed changes do not also result from the action of ACE inhibitors on lipid/carbohydrate metabolism, although both dyslipidemia [22] and even small abnormalities in carbohydrate metabolism [23] unfavorably affect inflammation and hemostasis.

Little is known about the minimal period of therapy which is required to reveal pleiotropic effect of ACE inhibitors [5]. The fact that some beneficial effects on inflammation and hemostasis were observed after only 30 days of therapy indicates that this action appears already in the first month of treatment. Interestingly enough, our study has shown that 90-day treatment was superior to 30-day therapy in decreasing MCP-1, hsCRP, fibrinogen, PAI-1 and oxidized LDLs, and in increasing interleukin-10. This suggests that a treatment longer than that used in the present study might produce a stronger normalizing effect on inflammation, hemostasis and oxidation. If this hypothesis is true, perindopril and enalapril should be administered for a certain minimal period of time to produce its full efficacy in the primary and secondary prevention of acute coronary events.

Discussing the benefits of ACE inhibitors, it should be clearly stated that pleiotropic effects of these agents were observed despite the fact that patients had been already using aspirin, a β-blocker and a statin. This strongly indicates that perindopril and to a less extent also enalapril exhibit their pleiotropic action even in patients in whom coronary artery disease has been already, according to the older recommendations, optimally treated. Our findings are in favor of implementation of treatment with tissue-specific ACE inhibitors and its continuation even in patients who do not experience any reduction in blood pressure.

Although it was not the primary goal of our study, we have found that both enalapril and perindopril administered at the daily dose of 20 and 4 mg, respectively, were well-tolerated and in most patients devoid of any significant effect on arterial blood pressure. This indicates that if administered to normotensive patients, these drugs are safe and, if not used together with high doses of antihypertensives, do not lead to the increased risk of hypotonia.

Our study has had two major limitations. Coronary artery disease was diagnosed indirectly (suggestive clinical symptoms and the results of the exercise test) and the diagnosis was not verified by coronary angiography. The perindopril group was on the average seven years younger and the percentage of smokers in this group was smaller. Although these differences did not reach significance level, they might have slightly influenced the baseline values of the examined parameters.

In conclusion, our study has revealed that perindopril produces much more prominent pleiotropic effects than enalapril. These effects, which were non-blood pressure-lowering- and non-lipid-lowering-related, may contribute to the clinical efficacy of ACE inhibitors in the treatment of arteriosclerosis-associated disorders. Our study indicates that tissue-specific ACE inhibitors appear to be a better treatment option than plasma-type ACE-inhibitors in patients with coronary artery disease and normal blood pressure.

Acknowledgments:
We are indebted to Mrs. Jarosława Sprada for her excellent technical support. This work was supported by the statutory grant NN-1/284/05 of the Medical University of Silesia.
None of the authors has any conflict of interest. The experiments comply with the current laws of Poland.

References:

1. Braunwald E, Domanski MJ, Fowler SE, Geller NL, Gersh BJ, Hsia J, Pfeffer MA et al.: PEACE Trial Investigators: Angiotensin-converting-enzyme inhibition in stable coronary artery disease. N Engl J Med, 2004, 351, 2058–2068.

2. Campbell DJ: A review of perindopril in the reduction of cardiovascular events. Vasc Health Risk Manag, 2006, 2, 117–124.
3. Constantinescu CS, Goodman DB, Ventura ES: Captopril and lisinopril suppress production of interleukin-12 by human peripheral blood mononuclear cells. Immunol Lett, 1998, 62, 25–31.
4. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S: Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. Lancet, 2006, 368, 581–558.
5. Dendorfer A, Dominiak P, Schunkert H: ACE inhibitors and angiotensin II receptor antagonists. Handb Exp Pharmacol, 2005, 170, 407–442.
6. Di Napoli M, Papa F: Angiotensin-converting enzyme inhibitor use is associated with reduced plasma concentration of C-reactive protein in patients with first-ever ischemic stroke. Stroke, 2003, 34, 2922–2929.
7. Dzau VJ, Bernstein K, Celermajer D, Cohen J, Dahlöf B, Deanfield J, Diez J et al.: Pathophysiologic and therapeutic importance of tissue ACE: a consensus report. Cardiovasc Drugs Ther, 2002, 16, 149–160.
8. Fox KM; EURopean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease Investigators: efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). Lancet, 2003, 362, 782–788.
9. Fukuzawa M, Satoh J, Sagara M, Muto G, Muto Y, Nishimura S, Miyaguchi S et al.: Angiotensin converting enzyme inhibitors suppress production of tumor necrosis factor-alpha in vitro and in vivo. Immunopharmacology, 1997, 36, 49–55.
10. Garanty-Bogacka B, Syrenicz M, Syrenicz A, Gębala A, Lulka D, Walczak M: Serum markers of inflammation and endothelial activation in children with obesity-related hypertension. Neuro Endocrinol Lett, 2005, 26, 242–246.
11. Holvoet P: Oxidized LDL and coronary heart disease. Acta Cardiol, 2004, 59, 479–484.
12. Jilma B, Li-Saw-Hee FL, Wagner OF, Beevers DG, Lip GY: Effects of enalapril and losartan on circulating adhesion molecules and monocyte chemotactic protein-1. Clin Sci, 2002, 103, 131–136.
13. Kothari SA, Le MK, Gandhi PJ: Effects of angiotensin converting enzyme inhibitors on thrombotic mediators: potential clinical implications. J Thromb Thrombolysis, 2003, 15, 217–225.
14. Krysiak R, Okopień B, Herman ZS: Effects of HMG-CoA reductase inhibitors on coagulation and fibrinolysis processes. Drugs, 2003, 63, 1821–1854.
15. Madej A, Okopień B, Kowalski J, Haberka M, Herman ZS: Plasma concentrations of adhesion molecules and chemokines in patients with essential hypertension. Pharmacol Rep, 2005, 57, 878–881.
16. Makris TK, Tsoukala C, Krespi P, Hatzizacharias A, Gialeraki A, Papargyriou J, Votteas V et al.: Haemostasis balance disorders in patients with essential hypertension. Thromb Res, 1997, 88, 99–107.
17. Mitrovic V, Klein HH, Krekel N, Kreuzer J, Fichtlscherer S, Schirmer A, Paar WD et al.: Influence of the angiotensin converting enzyme inhibitor ramipril on high-sensitivity C-reactive protein (hs-CRP) in patients with documented atherosclerosis. Z Kardiol, 2005, 94, 336–342.
18. Moser L, Callahan KS, Cheung AK, Stoddard GJ, Munger MA: ACE inhibitor effects on platelet function in stages I–II hypertension. J Cardiovasc Pharmacol, 1997, 30, 461–467.
19. Naftilan AJ: Role of the tissue renin-angiotensin system in vascular remodeling and smooth muscle cell growth. Curr Opin Nephrol Hypertens, 1994, 3, 218–227.
20. Napoleone E, Di Santo A, Camera M, Tremoli E, Lorenzet R: Angiotensin-converting enzyme inhibitors downregulate tissue factor synthesis in monocytes. Circ Res, 2000, 86, 139–143.
21. Neultel JM: Effect of the renin-angiotensin system on the vessel wall: using ACE inhibition to improve endothelial function. J Hum Hypertens, 2004, 18, 599–606.
22. Okopień B, Kowalski J, Krysiak R, Łabuzek K, Stachura-Kułach A, Kułach A, Zieliński et al.: Monocyte suppressing action of fenofibrate. Pharmacol Rep, 2005, 57, 367–372.
23. Okopień B, Krysiak R, Herman ZS: Effects of short-term fenofibrate treatment on circulating markers of inflammation and hemostasis in patients with impaired glucose tolerance. J Clin Endocrinol Metab, 2006, 91, 1770–1778.
24. Paffen E, Vos HL, Bertina RM: C-reactive protein does not directly induce tissue factor in human monocytes. Arterioscler Thromb Vasc Biol, 2004, 24, 975–981.
25. Peeters AC, Netea MG, Kullberg BJ, Thien T, van der Meer JW: The effect of renin-angiotensin system inhibitors on pro- and anti-inflammatory cytokine production. Immunology, 1998, 94, 376–379.
26. Piepho RW: Overview of the angiotensin-converting-enzyme inhibitors. Am J Health Syst Pharm, 2000, 57, Suppl 1, S3–S7.
27. PROGRESS Collaborative Group: Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. Lancet, 2001, 358, 1033–1041.
28. Schieffer B, Bunte C, Witte J, Hoeper K, Boger RH, Schwedhelm E, Drexler H: Comparative effects of AT1-antagonism and angiotensin-converting enzyme inhibition on markers of inflammation and platelet aggregation in patients with coronary artery disease. J Am Coll Cardiol, 2004, 44, 362–368.
29. Schindler R, Dinarello CA, Koch KM: Angiotensin-converting-enzyme inhibitors suppress synthesis of tumour necrosis factor and interleukin 1 by human peripheral blood mononuclear cells. Cytokine, 1995, 7, 526–533.
30. Schmeisser A, Soehnlein O, Illmer T, Lorenz HM, Eskafi S, Roerick O, Gabler C et al.: ACE inhibition lowers angiotensin II-induced chemokine expression by reduction of NF-κB activity and AT1 receptor expression. Biochem Biophys Res Commun, 2004, 325, 532–540.

31. Sheth T, Parker T, Block A, Hall C, Adam A, Pfeffer MA, Stewart DJ et al.; IMPRESS Investigators: Comparison of the effects of omapatrilat and lisinopril on circulating neurohormones and cytokines in patients with chronic heart failure. Am J Cardiol, 2002, 90, 496–500.
32. Singh SK, Suresh MV, Voleti B, Agrawal A: The connection between C-reactive protein and atherosclerosis. Ann Med, 2008, 40, 110–120.
33. Singh U, Devaraj S, Dasu MR, Ciobanu D, Reusch J, Jialal I: C-reactive protein decreases interleukin-10 secretion in activated human monocyte-derived macrophages via inhibition of cyclic AMP production. Arterioscler Thromb Vasc Biol, 2006, 26, 2469–2475.
34. Sironi L, Nobili E, Gianella A, Gelosa P, Tremoli E: Anti-inflammatory properties of drugs acting on the renin-angiotensin system. Drugs Today, 2005, 41, 609–622.
35. Song JC, White CM: Do HMG-CoA reductase inhibitors affect fibrinogen? Ann Pharmacother, 2001, 35, 236–241.
36. Tikiz C, Utuk O, Pirildar T, Bayturan O, Bayindir P, Taneli F, Tikiz H et al.: Effects of angiotensin-converting enzyme inhibition and statin treatment on inflammatory markers and endothelial functions in patients with long-term rheumatoid arthritis. J Rheumatol, 2005, 32, 2095–2101.
37. Trevelyan J, Brull DJ, Needham EW, Montgomery HE, Morris A, Mattu RK: Effect of enalapril and losartan on cytokines in patients with stable angina pectoris awaiting coronary artery bypass grafting and their interaction with polymorphisms in the interleukin-6 gene. Am J Cardiol, 2004, 94, 564–569.
38. Tsikouris JP, Suarez JA, Meyerrose GE: Plasminogen activator inhibitor-1: physiological role, regulation, and the influence of common pharmacologic agents. J Clin Pharmacol, 2002 42, 1187–1192.
39. Wojewódzka-Żeleźniakowicz M, Chabielska E, Mogielnicki A, Kramkowski K, Karp A, Opadczuk A, Domaniewski T et al.: Antithrombotic effect of tissue and plasma type angiotensin converting enzyme inhibitors in experimental thrombosis in rats. J Physiol Pharmacol, 2006, 57, 231–245.
40. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G: Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med, 2000, 342, 145–153.

Received:
September 13, 2007; in revised form: April 15, 2008.