Clinical Investigations

Effects of Imidapril Therapy on Endogenous Fibrinolysis in Patients with Recent Myocardial Infarction

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Summary

Background: Treatment with an angiotensin-converting enzyme (ACE) inhibitor in patients with myocardial infarction has been shown to modify endogenous fibrinolysis.

Hypothesis: We investigated the effects of the ACE inhibitor imidapril on endogenous fibrinolysis in association with the serum ACE activity.

Methods: In a randomized, double-blind, placebo-controlled study beginning 4 weeks after uncomplicated myocardial infarction, 15 patients received imidapril (5 mg daily) (imidapril group) and another 15 received placebo therapy (placebo group) for 4 weeks. Blood sampling was performed before the start of administration and on Days 3, 7, and 28 after the start of administration. Serum ACE activity and plasma fibrinolytic variables [plasminogen activator inhibitor (PAI) activity, plasminogen activator inhibitor type 1 (PAI-1) antigen level, and tissue type plasminogen activator (TPA) antigen level] were measured.

Results: There was no difference between the imidapril and placebo groups in serum ACE activity or plasma fibrinolytic variables before administration. Serum ACE activity decreased significantly on Days 3, 7, and 28 in the imidapril

group. The decrease of PAI activity and PAI-1 antigen levels was significantly less on Days 7 and 28, but not on Day 3. The TPA antigen level in the imidapril group was unchanged. None of the parameters in the placebo group was changed.

Conclusion: The ACE inhibitor imidapril modified fibrinolysis, but the effects occurred after the inhibition of serum ACE activity.

Key words: angiotensin-converting enzyme inhibitor, angiotensin-converting enzyme activity, plasminogen activator inhibitor activity and antigen, tissue type plasminogen activator antigen

Introduction

To elucidate the effects of angiotensin-converting enzyme (ACE) inhibitors on endogenous fibrinolysis, we investigated the effects in association with serum ACE activity.

The central component of the fibrinolytic system is plasmin, which degrades fibrin or thrombus and which is converted from plasminogen by tissue type plasminogen activator (TPA).¹ However, TPA in plasma is rapidly inhibited by plasminogen activator inhibitor (PAI).² Thus, the net fibrinolytic activity in plasma reflects the balance between PAI and TPA. TPA promotes fibrinolysis,¹ whereas its specific inhibitor, PAI, rapidly inhibits it by forming a complex with TPA. PAI activity, mainly as PAI-1 activity, is a major determinant of overall fibrinolytic activity.^{1,3} Large-scale, placebo-controlled studies have shown that the administration of ACE inhibitors in patients after myocardial infarction (MI) reduces the incidence of recurrent coronary thrombosis.^{4,5} We and others have reported that an impaired endogenous fibrinolytic system is associated with an increased risk of intravascular thrombosis.^{6–8} Decreased level of plasminogen activator inhibitor has been shown to be associated with a reduced incidence of reinfarction.^{9,10}

On the other hand, angiotensin II is known to increase PAI-1.¹¹ Furthermore, treatment with an ACE inhibitor has been shown to decrease elevated plasma PAI-1 activity.¹² However, the effects of ACE inhibitors have been studied only at

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chronic phase and the relationship with ACE activity is not clear. Therefore, we compared the changes in serum ACE activity, plasma PAI activity, PAI-1 antigen levels, and TPA antigen levels on the day before the start of administration and on Days 3, 7, and 28 after the start of administration between the patients who received imidapril (Tanatril®, Tanabe Seiyaku Co. Ltd., Osaka, Japan) and those who received placebo. Imidapril is a prodrug type of ACE inhibitor whose pharmacologic effects are derived from its active metabolite (imidaprilat). Imidaprilat is almost as effective as enalaprilat and about twice as effective as captopril in inhibiting ACE activity.¹³

Methods

Study Population

Thirty patients who had experienced first MI were recruited 4 weeks after the onset of MI. In addition to a typical history, the patients showed pathologic Q waves on the electrocardiogram (ECG) or had a peak creatine kinase level at least twice the upper limit of the normal range. All patients took calcium antagonists, long-acting nitrates, and 81 mg of aspirin daily from the time of admission to hospital, and this drug protocol remained unchanged during the study period. None of the patients had previously taken an ACE inhibitor. At the time of recruitment, a full clinical assessment was made and a chest radiograph and symptom-limited treadmill exercise test were performed. No patients had clinical or radiological signs of heart failure or evidence of ischemia or arrhythmia requiring additional therapy. Fifteen patients were randomized to imidapril treatment (imidapril group) and the other 15 to placebo treatment (placebo group).

Study Protocol

This study was designed as a randomized, double-blind, placebo-controlled trial. Entry into the study was at 4 weeks after the onset of MI to avoid any acute-phase response. Patients received 5 mg of imidapril or a tablet of placebo each morning for 4 weeks. No patients had significant hypotensive response or abnormal electrolytes during this study. Patients fasted from 10:00 P.M. the previous night, took their usual drugs at 7:00 A.M., and attended the outpatient department at 8:00 A.M. on the day before the start of administration, and on Days 3, 7, and 28 after the start of imidapril or placebo administration at 7:00 A.M.¹⁴ All patients lay recumbent, and a 21-gauge needle was inserted into an antecubital vein. Then, an initial 3 ml of blood was used for measuring lipid concentrations, and a further 4.5 ml of blood for assay of fibrinolytic variables was drawn into sodium citrate (0.13 mol/l, pH 7.5). Then another 5 ml of blood for measurement of ACE activity was drawn into a plain glass tube. The samples for assay of fibrinolytic variables were immediately centrifuged at 3,000 rpm for 10 min at 4°C. The samples for lipid concentrations and ACE activity assay were left in room air for 30 min and centrifuged in the same way. After centrifugation, the samples

were immediately stored at -80°C until analysis. The study protocol followed the guidelines of the ethics committee at our institution. Informed consent was obtained from each patient and control subject.

Assays

Serum ACE activity assay: Serum ACE activity was measured by colorimetry with an ACE color (Fujirebio Inc., Tokyo) using the substrate (p-hydroxybenzoyl-glycyl-L-histidyl-L-leucine).¹⁵ The results are expressed in IU/l. The intra- and interassay coefficients of variation in this kit were 5.8 and 14.3%, respectively. The normal mean $\pm$ standard error of mean (SEM) value for ACE activity measured in our laboratory ($n = 19$) was 14.5 ± 0.9 IU/l.

Plasma PAI activity assay: Plasma PAI activity was determined by a chromogenic substrate assay with a Spectrolyse (PL) reagent kit (Biopool, Umeå, Sweden).¹⁶ The results are expressed in IU/ml. The intra- and interassay coefficients of variation in this kit were 9.4% and 11.4%, respectively. The normal mean $\pm$ SEM value for PAI activity measured in our laboratory ($n = 33$) was 5.2 ± 0.5 IU/ml.

Plasma PAI-1 antigen level assay: Plasma PAI-1 antigen level was determined by a two-site enzyme-linked immunosorbent assay with an TintElize PAI-1 reagent kit (Biopool, Umeå, Sweden).¹⁷ The results are expressed in ng/ml. The intra- and interassay coefficients of variation in this kit were 5.8 and 8.5%, respectively. The normal mean $\pm$ SEM value for PAI-1 antigen level measured in our laboratory ($n = 25$) was 15.3 ± 0.9 ng/ml.

Plasma TPA antigen level assay: Plasma TPA antigen level was measured by an enzyme-linked immunosorbent assay with an ASSERACHROM TPA reagent kit (Diagnostica Stago, Inca, Franconville, France).¹⁸ The results are expressed in ng/ml. The intra- and interassay coefficients of variation in this kit were 2.4 and 4.7%, respectively. The normal mean $\pm$ SEM value for TPA antigen level measured in our laboratory ($n = 33$) was 5.6 ± 0.3 ng/ml.

Lipids: Total plasma cholesterol and triglyceride levels were determined enzymatically and high-density lipoprotein cholesterol levels were determined using magnesium dextran.

Statistical Analyses

All data are shown as mean $\pm$ SEM. The clinical characteristics were compared between imidapril and placebo groups using the unpaired *t*-test for continuous data and χ^2 test for frequency data. The levels of serum ACE activity, plasma PAI activity, PAI-1 antigen, and TPA antigen between the imidapril and placebo groups at baseline were compared using the Mann-Whitney U test. Levels over the time course in the two patient groups were compared by two-way ANOVA with repeated measures. When the difference was significant, the significance level for correlations was given after application of the Bonferroni method for comparison of within group changes, and the Mann-Whitney U test was used for comparison of the group data. Probability levels <0.05 were considered significant.

TABLE I Baseline characteristics in the placebo and imidapril groups (mean $\pm$ SEM)

	Placebo group (n = 15)	Imidapril group (n = 15)
Age (years)	68 $\pm$ 2	67 $\pm$ 2
Men/women (n)	11/4	12/3
Systemic hypertension (n)	6	8
Serum total-cholesterol (mg/dl)	180 $\pm$ 7	186 $\pm$ 8
Serum triglyceride (mg/dl)	139 $\pm$ 20	131 $\pm$ 14
Serum HDL cholesterol (mg/dl)	38 $\pm$ 2	39 $\pm$ 2
Cigarette smoking (n)	6	5
Diabetes mellitus (n)	4	3
Obesity (n)	4	4
Drugs		
Calcium antagonists (n)	9	8
Long-acting nitrates (n)	10	11
Aspirin (n)	15	15

Abbreviations: HDL = high-density lipoprotein, SEM = standard error of mean.

Results

Baseline Characteristics

As shown in Table I, there was no significant difference in baseline characteristics between the imidapril and placebo groups as shown in Table 1. There were no significant differences in age, gender, hypertension, obesity, diabetes mellitus, history of smoking, or in the levels of total cholesterol, triglyceride, or high-density lipoprotein cholesterol between the two groups, neither was there a significant difference in medications.

Baseline ACE Activity and Fibrinolytic Variables

As shown in Table II, there were no differences between the imidapril and placebo groups in the mean values of serum ACE activity, plasma PAI activity, plasma PAI-1 antigen, or plasma TPA antigen on the day before the start of administration.

Serum ACE Activity After Administration

There was a significant difference in serum ACE activity (mean $\pm$ SEM IU/l) after administration of imidapril: it changed from 14.9 $\pm$ 1.4 at baseline to 5.1 $\pm$ 0.9 on Day 3, to 6.1 $\pm$ 1.2 on Day 7, and to 6.2 $\pm$ 1.2 on Day 28 of administration ($p < 0.01$; Fig. 1). Serum ACE activity in the placebo group was unchanged. The levels on Days 3, 7, and 28 in the imidapril group were significantly lower than those in the placebo group ($p < 0.01$).

Plasma PAI Activity after Administration

Plasma PAI activity (mean $\pm$ SEM IU/ml) after administration of imidapril changed from 9.5 $\pm$ 1.0 at baseline to 7.4 $\pm$ 1.1 on Day 3, to 6.8 $\pm$ 0.9 on Day 7, and to 5.3 $\pm$ 1.0 on Day 28 of administration, with a significant difference on Day 28

TABLE II Fibrinolytic variables and angiotensin-converting enzyme activity at baseline in the placebo and imidapril groups (mean $\pm$ SEM)

	Placebo group (n = 15)	Imidapril group (n = 15)
ACE activity (IU/l)	15.3 $\pm$ 1.1	15.3 $\pm$ 1.1
PAI activity (IU/ml)	11.7 $\pm$ 1.0	12.0 $\pm$ 0.9
PAI-1 antigen level (ng/ml)	25.0 $\pm$ 1.4	24.2 $\pm$ 1.5
TPA antigen level (ng/ml)	10.7 $\pm$ 1.0	10.4 $\pm$ 1.0

Abbreviations: ACE = angiotensin-converting enzyme, PAI = plasminogen activator inhibitor, PAI-1 = plasminogen activator inhibitor type 1, TPA = tissue type plasminogen activator, SEM = standard error of mean.

($p < 0.01$; Fig. 2). Plasma PAI activity in the placebo group was unchanged. The levels in the imidapril group were significantly lower than those in the placebo group on Day 7 ($p < 0.05$) and Day 28 ($p < 0.01$).

Plasma PAI-1 Antigen Level after Administration

The plasma PAI-1 antigen level (mean $\pm$ SEM ng/ml) after administration of imidapril changed from 24.2 $\pm$ 1.5 at baseline to 21.4 $\pm$ 1.8 on Day 3, to 19.8 $\pm$ 1.5 on Day 7, and to 17.3 $\pm$ 1.5 on Day 28 of administration, with a significant difference on Day 28 ($p < 0.01$; Fig. 3). The plasma PAI-1 antigen level in the placebo group was unchanged. The levels in the imidapril group were significantly lower than those in the placebo group on Day 7 ($p < 0.05$) and on Day 28 ($p < 0.01$).

Plasma TPA Antigen Level after Administration

The plasma TPA antigen level (mean $\pm$ SEM ng/ml) did not change significantly after administration of imidapril (Fig. 4). The plasma TPA antigen level in the placebo group was unchanged.

FIG. 1 Serial changes of the serum angiotensin-converting enzyme (ACE) activity in the imidapril and placebo groups. —●— = Imidapril, —○— = placebo. ** $p < 0.01$ vs. before administration, †† = $p < 0.01$ vs. placebo.

FIG. 2 Serial changes of the plasma plasminogen activator inhibitor (PAI) activity in the imidapril and placebo groups. ● = Imidapril, ○ = placebo. ** $p < 0.01$ vs. before administration, † $p < 0.05$ vs. placebo, †† $p < 0.01$ vs. placebo.

FIG. 3 Serial changes of the plasma plasminogen activator inhibitor type 1 (PAI-1) antigen levels in the imidapril and placebo groups. ● = Imidapril, ○ = placebo. ** $p < 0.01$ vs. before administration, † $p < 0.05$ vs. placebo, †† $p < 0.01$ vs. placebo.

Discussion

Both the Studies of Left Ventricular Dysfunction (SOLVD) Prevention Trial and the Survival and Ventricular Enlargement (SAVE) Trial have revealed that ACE inhibition reduced the risk of acute coronary syndrome in patients after myocardial infarction.^{4,5} The decrease of PAI was associated with a reduced incidence of reinfarction.^{9,10} The plasma PAI-1 activity, after 4 weeks treatment with an ACE inhibitor, has been also shown to be lower than that before the treatment.¹² In the present study, the fibrinolytic variables (plasma PAI activity, plasma PAI-1 antigen level, plasma TPA antigen level), and serum ACE activity were examined at four sampling points to compare the effects of imidapril on endogenous fibrinolysis with

FIG. 4 Serial changes of the plasma tissue type plasminogen activator (TPA) antigen levels in the imidapril and placebo groups. ● = Imidapril, ○ = placebo.

those on serum ACE activity. The serum ACE activity decreased significantly within 3 days after the start of imidapril administration, and the levels on Days 7 and 28 were also significantly lower. However, the plasma PAI activity and PAI-1 antigen level were significantly lower on Days 7 and 28, but not on Day 3 after the start of administration, indicating that the decrease in plasma PAI after imidapril therapy occurs later than that in serum ACE activity. Recently, the expression of PAI-1 has been suggested to be mediated by angiotensin IV, which is a metabolite of angiotensin II.¹⁹ ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II in the renin-angiotensin system, which results in a decrease of angiotensin II level. Furthermore, the decrease in angiotensin II leads to the reduction of angiotensin IV, which stimulates the expression of PAI-1. The residual angiotensin IV in the vascular tissue after administration of imidapril may be a cause of this time difference in the reduction in plasma PAI and serum ACE activity. At 5 mg/kg/day, imidapril is almost as potent as enalapril and more potent than captopril in inhibiting ACE activity in aorta, lungs, and brain tissue of spontaneously hypertensive rats after 10 weeks of oral treatment.¹³ The ACE activity in the aorta tissue 9 days after imidapril treatment was still at the reduced level, whereas that after treatment with enalapril and captopril almost returned to the control level. Therefore, the time difference may also be caused by the delay in inhibition of vascular tissue ACE caused by the slow tissue penetration and tissue washout of imidapril.

Recently, ACE has been shown to play a dual role. It is responsible for converting angiotensin I to angiotensin II in the renin-angiotensin system and for the degradation of bradykinin to inactive by-products in the kinin pathway.²⁰ By activation of ACE, the angiotensin II level is increased and the bradykinin level is decreased. Conversely, by ACE inhibition, the angiotensin II level is decreased and bradykinin concentration is increased. Bradykinin has been suggested to stimulate

the release of the plasminogen activator.²¹ However, in our study, the TPA antigen level did not change after imidapril administration, probably because imidapril has weaker activity to inhibit bradykinin hydrolysis by ACE than enalapril.²²

In this randomized, placebo-controlled study, the fibrinolytic variables and ACE activity were examined at four sampling points. Serum ACE activity was decreased more rapidly than the plasma levels of PAI activity and PAI-1 antigen in patients receiving imidapril.

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