Original Article

Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism Modulates Coronary Release of Tissue Plasminogen Activator in Response to Bradykinin

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The aim of this study was to assess the relationship between the angiotensin converting enzyme gene (ACE) genotype and endothelium-dependent coronary vasomotor and fibrinolytic activity. The ACE DD genotype has been reported to be a risk factor for myocardial infarction. However, the mechanism is unknown. The fibrinolytic and renin-angiotensin systems are linked via ACE at the vascular beds. We studied 73 patients (II: n = 24; ID: n = 37; DD: n = 12) who underwent diagnostic cardiac catheterization. Graded doses of bradykinin (BK) (0.2, 0.6, 2.0 $\mu$g/min) and acetylcholine (30,100 $\mu$g/min) were administered into the left coronary artery. Coronary blood flow (CBF) was evaluated by measuring Doppler flow velocity. Blood samples were taken from the aorta (Ao) and the coronary sinus (CS). Coronary release of tPA antigen was determined as a CS-Ao gradient $\Delta$ CBF $\Delta$[(100 · hematocrit) / 100]. ACE genotypes were determined using polymerase chain reaction. The ACE genotype did not appear to affect coronary macro- and microvascular responses induced by BK or acetylcholine. Coronary tissue plasminogen activator (tPA) release induced by BK was depressed in subjects with the ACE DD genotype. ACE levels in the DD genotype were significantly higher than those in the ID or II genotype. In all of the subjects, there was a significant negative correlation between the serum level of ACE activity and net coronary tPA release in response to BK at 0.6 $\mu$g/min. In conclusions, the DD genotype of the ACE gene impairs the coronary release of tPA induced by BK.

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Key Words: tissue plasminogen activator, bradykinin, angiotensin-converting enzyme, DD genotype

Introduction

Angiotensin-converting enzyme (ACE) is a key part of the renin-angiotensin system. It converts angiotensin I to angiotensin II and inactivates bradykinin (BK). The ACE gene has been mapped to chromosome 17q23, and an insertion/deletion (I/D) polymorphism, involving a 287-base pair alu repeat sequence, has been located in intron 16 (1). Cambien et al. showed that the DD genotype was a risk factor for myocardial infarction (2), although a later study found no correlation between myocardial infarction and this genotype (3).

Few studies have assessed the possible mechanisms by which the ACE D allele causes these detrimental effects.

The level of plasma ACE is partly under genetic control, and the DD genotype is associated with higher circulating and tissue ACE concentrations (4). An increase in ACE activity may impair endothelial function by increasing angiotensin II and accelerating bradykinin degradation. Therefore, the D allele has been shown to be associated with impaired endothelium-dependent vasodilatation (5, 6).

Bradykinin is an endothelium-dependent vasodilator that stimulates the release of nitric oxide (NO), prostacyclin, and endothelium-dependent hyperpolarizing factors. Bradykinin...
is also a potent stimulator for tissue plasminogen activator (tPA) in endothelial cells (7). We recently reported that BK stimulated tPA release in the human coronary circulation, and this effect was augmented by the inhibition of ACE (8). In some large-scale studies, ACE inhibitors have been shown to reduce the incidence of myocardial infarction (9, 10). However, it is unclear whether the inhibition of ACE reduces coronary thrombotic events via a favorable effect on coronary endothelial function. We hypothesized that in subjects with the ACE DD genotype, higher levels of ACE may cause the inactivation of BK and decrease tPA stimulation, and contribute and thereby contribute to coronary thrombogenesis. No previous studies have analyzed the relationship between ACE I/D polymorphism and coronary fibrinolytic activity. Therefore, we here examined whether the ACE I/D polymorphism and plasma ACE activity influence the coronary vasomotor and fibrinolytic function as estimated by responses to BK.

Methods

Study Patients

Our study population consisted of 55 men and 18 women. All of the study patients underwent diagnostic cardiac catheterization for the evaluation of atypical chest pain or myocardial ischemia on electrocardiogram and had angiographically normal coronary arteries. Patients with myocardial infarction, coronary vasospastic angina, congestive heart failure, cardiomyopathy, or valvular heart disease were excluded from the study. All cardiac medications were discontinued for at least 72 h before the study. For at least seven days before the study, the subjects received a low-sodium diet (NaCl 5 g/day). The Ethical Committee on Human Research of our institution approved the study protocols, and written informed consent was obtained from all patients.

Protocol

Cardiac catheterization was performed between 9 and 11 AM in the fasting state. A 0.014-inch Doppler-tipped guide wire (JoMetrics Flowire; JoMed, Rancho Cordova, USA) was advanced to the area between the proximal and middle segments of the left anterior descending (LAD) coronary artery to measure blood flow velocity, as previously reported (8, 11). All drugs were infused directly into the left main coronary artery via the guide catheter at infusion rates ranging between 0.5 and 1 ml/min. A 6F multipurpose catheter (GCS6; Goodtec, Gifu, Japan) was advanced via the right femoral vein into the coronary sinus (CS) for blood sampling.

The following studies were then performed.

1) Bradykinin was started at 0.2 µg/min and then increased to 0.6 and 2.0 µg/min for 2 min. During BK infusion, the coronary blood flow (CBF) velocity reached a peak at about 60 s and remained constant at this peak level thereafter.

2) After completing the protocol with the intracoronary injection of BK, we waited at least 10 min before beginning the infusion of acetylcholine (ACh), by which time the coronary diameter and coronary blood flow velocity had returned to their baseline values.

3) Acetylcholine was administered at 30 µg/min and then increased to 100 µg/min for 2 min.

4) Finally, papaverine was infused into the left coronary artery at 12 mg for 20 s.

Coronary angiography was performed after each infusion.

Quantitative Coronary Angiography and Measurements of CBF

The change in diameter of the LAD coronary artery was measured in a vessel segment 2.5 mm beyond the tip of the Doppler wire. Coronary angiograms were analyzed by QCA, using the Cardiovascular Measurement System (CMS-MEDIS Medical Imaging Systems, Leiden, The Netherlands). Peak CBF velocity was continuously monitored using a fast-Fourier transform-based spectral analyzer (FloMap; Cardiometrics Inc., Mountain View, USA). Coronary blood flow was derived from the CBF velocity and measurements of the diameter by the formula: \[ \pi \cdot \text{average peak CBF velocity} \cdot 0.125 \cdot \text{(arterial diameter)}^2. \]

Phasic and mean arterial blood pressures, heart rate, and 12-lead electrocardiograms were continuously monitored using a polygraph system (Nihon Kohden, Tokyo, Japan) and recorded on a multichannel recorder.

Blood Sampling and Biochemical Assays

Paired blood samples in the aorta (Ao) and CS were taken simultaneously before and after the infusion of BK for the measurement of plasma tPA antigen and plasminogen activator inhibitor-1 (PAI-1) antigen. Since neither ACh nor papaverin increased net tPA release (8), no blood was drawn during the infusion of ACh or papaverin. Blood samples were collected on ice and centrifuged immediately, and plasma was stored at -70°C until the time of assay. Blood for the measurement of tPA and PAI-1 was collected in tubes containing 0.105 mol/l sodium citrate. Antigen levels were determined using a two-site ELISA (Biopool, Umeå, Sweden).

Arteriovenous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected CS venous and arterial blood. Thus, the net release of tPA at each time point was calculated as (concentration CS - concentration Ao) \( \cdot \) (CBF \( \cdot \) (100 - hematocrit)/100).

Determination of ACE Genotype

DNA was extracted from peripheral blood using a DNA ex-
tractor WB kit (Wako, Osaka, Japan). Angiotensin-converting enzyme genotypes were determined using the polymerase chain reaction (PCR) method, as previously reported (12, 13). In brief, the sense oligonucleotide primer was 5' GTG AGACCATCCCCATCTTTCT-3' and the antisense primer was 5' GATGTGGCCATCACATTCGTCAGAT-3'. DNA was amplified for 35 cycles of denaturation at 94 ºC for 60 s, annealing at 58 ºC for 60 s, and extension at 74 ºC for 120 s. The PCR products were resolved on 1.5% agarose gels and visualized with ethidium bromide staining.

Statistics

Data were expressed as the mean ± SEM. Discrete variables were expressed as counts or percentages and compared using the χ² test. Continuous variables were compared using the unpaired Student’s t-test or a one-way analysis of variance (ANOVA). When serial and fibrinolytic parameters in response to graded doses of BK were compared between the two groups, a two-way ANOVA for repeated measures followed by Fisher’s PLSD multiple comparison test was used. Values of p < 0.05 were considered statistically significant.

Results

Baseline Characteristics

The frequencies of the DD, ID, and II genotypes (0.16, 0.51, and 0.33, respectively) did not deviate significantly from those predicted by the Hardy-Weinberg equilibrium (0.17, 0.49, 0.34). There were no significant differences in baseline clinical characteristics between the ACE genotypes, except for ACE activity (Tables 1, 2). The distribution of hypertension, diabetes mellitus, and hyperlipidemia did not differ among the three groups. ACE levels were significantly higher in the DD group than in the II group (p < 0.01).

Systemic and Coronary Hemodynamics

Baseline systemic and coronary hemodynamics were similar in all three groups (Tables 1, 2). Intracoronary infusion of BK did not alter systemic blood pressure or heart rate in each group (Fig. 1). Intracoronary infusion of BK increased CBF in a dose-dependent manner in all three groups, and no significant differences were observed in the CBF responses to

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<th>Table 1. Baseline Clinical Characteristics</th>
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<td>Age (years old)</td>
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<td>Sex (male:female)</td>
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Data are presented as the mean ± SEM or number (%) of subjects. BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein.

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<th>Table 2. Baseline Neurohormonal and Fibrinolytic Parameters</th>
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<td>PARC (pg/ml)</td>
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<td>ACE (IU/l)</td>
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<td>Aldosterone (pg/ml)</td>
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<td>tPA antigen (CS) (ng/ml)</td>
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<td>tPA antigen (Ao) (ng/ml)</td>
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<td>PAI-1 antigen (CS) (ng/ml)</td>
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<td>PAI-1 antigen (Ao) (ng/ml)</td>
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Data are presented as the mean ± SEM. *p < 0.05 vs. II group. PARC, plasma-active renin concentration; ACE, angiotensin converting enzyme; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; CS, coronary sinus; Ao, aorta.
BK among the three groups (Fig. 2). The increases in CBF in response to ACh were comparable among the three groups (30 \(\mu\)g/min: II group 69 \(\pm\) 19%, ID group 74 \(\pm\) 15%, DD group 57 \(\pm\) 38%; 100 \(\mu\)g/min: II group 18 \(\pm\) 26%, ID group 30 \(\pm\) 20%, DD group 14 \(\pm\) 22%). Intracoronary infusion of 12 mg papaverine increased CBF by similar percentages in the three groups (II group 356 \(\pm\) 37%, ID group 334 \(\pm\) 28%, DD group 332 \(\pm\) 40%).

**Fibrinolytic Parameters**

Baseline levels of tPA antigen at either the Ao or CS did not differ among the three groups. In all groups, the level of tPA in the Ao was not changed by the infusion of BK, except at a dose of 2.0 \(\mu\)g/min (Fig. 3). The level of tPA in the CS was significantly increased in a dose-dependent manner by the infusion of a graded dose of BK in all three groups (Fig. 3). The CS-Ao gradient of tPA antigen in response to BK in the DD genotype was lower than that in the II genotype (Fig. 4). The net tPA release induced by the intracoronary infusion of BK was increased in all groups (Fig. 4), and there was a progressive decrease in net tPA release in the presence of the D allele, with the lowest response in patients homozygous for the D allele, an intermediate response in those with the ID genotype, and the greatest response in those homozygous for the I allele (Fig. 4). At a dose of 0.6 \(\mu\)g/min BK, there was a significant negative correlation between net tPA release and the ACE level (Fig. 5).

The baseline levels of PAI-1 in either the Ao or CS did not differ among the three groups (Table 2). With an increase in the dosage of BK, the level of PAI-1 did not change in any of the three groups (Fig. 6).

**Discussion**

The major finding of the present study was that the D allele of the ACE gene was associated with the impairment of tPA release in response to BK in the human coronary circulation. The D allele has been associated with various cardiovascular diseases (\(1, 14, 15\)). Some, but not all, studies have reported that the D allele was associated with an increased risk of myocardial infarction (\(2, 3\)). The mechanisms by which ACE polymorphism may influence coronary artery disease remain to be elucidated. The present study suggested that the
D allele may cause an impairment of coronary fibrinolytic activity associated with future cardiovascular events in patients with angiographically normal coronary arteries. Ludwig et al. reported that, in their cohort, the distribution of ACE genotypes was not associated with the degree of coronary atherosclerosis (16). Iwai et al. previously reported that the DD genotype of the ACE gene was associated with a shorter period of time between the first anginal pain and the onset of myocardial infarction compared to the ID and II genotypes, suggesting increased thrombogenicity in patients with the DD genotype (17). Therefore, it is conceivable that ACE polymorphism may be associated with an increased risk of myocardial infarction via the impairment of coronary fibrinolytic activity rather than the development of coronary atherosclerosis. In addition, our results suggest that thrombolytic therapy for myocardial infarction may be less effective in patients with the DD genotype.

Fibrinolytic activity is primarily determined by the balance between the levels of tPA and PAI-1 (10). The fibrinolytic system and the renin-angiotensin system are linked via ACE at the level of the vascular endothelium (1, 8, 18). Inhibition of ACE favorably alters the fibrinolytic balance by increasing BK-induced tPA release, decreasing angiotensin II-mediated PAI-1 release, or both. BK causes endothelium-dependent vasodilation through the production of NO, prostacyclin, and endothelium-derived hyperpolarizing factor through the B2 receptor in human coronary arteries (19). Recently, we reported that ACE inhibitors augmented the release of tPA induced by BK in the human coronary circula-

**Fig. 3.** The effects of bradykinin (0.2, 0.6, 2.0 µg/min) on tPA antigen in the aorta (Ao) and coronary sinus (CS) in the II (closed circle), ID (open circle) and DD (closed triangle) genotypes. *p < 0.05 vs. baseline.

**Fig. 4.** The effects of bradykinin (0.2, 0.6, 2.0 µg/min) on the CS-Ao gradient of tPA antigen (left side) and the effects of bradykinin (0.2, 0.6, 2.0 µg/min) on the net tPA release (right side) in the II (closed circle), ID (open circle) and DD (closed triangle) genotypes. *p < 0.05 vs. baseline.

**Fig. 5.** The relationship between ACE activity and net tPA release in response to BK at a dose of 0.6 µg/min.

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tion. In the present study, the ACE level was highest in ACE D homozygotes, lowest in ACE I homozygotes, and intermediate in heterozygotes, and the net coronary tPA release induced by BK showed a significant negative correlation with ACE levels. Systemic hemodynamics and neurohumoral parameters other than the ACE level did not differ among the three groups. As shown in the computer-simulation study of Takahashi et al., mild changes in ACE activity associated with an ACE gene polymorphism do not alter the renin-angiotensin system sufficiently to affect hemodynamics (20).

It has been reported that the venous kinin concentration is significantly correlated with net tPA release (21). Therefore, the D allele may suppress endogenous coronary fibrinolytic function and promote coronary thrombosis via the promotion of BK degradation by an increase in circulating and tissue ACE levels. On the other hand, the D allele could impair BK-induced coronary fibrinolytic responses through other mechanisms. An increase in plasma ACE may enhance the conversion of angiotensin I to angiotensin II, and increase the generation of superoxide anions that degrade NO release in response to BK. Therefore, the D allele may impair the release of tPA through increased reactive oxygen stress, while an NO-generating pathway contributes to tPA release (22). Brown et al. reported that BK stimulates tPA release from the forearm vascular endothelium through a B2 receptor-dependent, NO-synthase-independent, and cyclooxygenase-independent pathway (23), suggesting that EDHF may contribute to BK-stimulated tPA release. Further studies are needed to examine the mechanisms by which BK stimulates coronary tPA release. In the present study, we did not observe differences in the increase in CBF induced by BK between the different ACE genotypes. Prasad et al. also reported that neither the ACE I/D genotype nor serum ACE levels correlated with coronary microvascular responses to BK in patients with atherosclerosis, which is consistent with our findings (24). These results suggest that ACE I/D polymorphism may play a role in coronary fibrinolytic function independent of coronary vasomotion in response to BK. In the present study, the ACE genotype did not affect the coronary flow response to ACh. We previously reported that intracoronary infusion of ACh did not affect plasma levels of tPA antigen in either the Ao or CS (8). There are conflicting reports regarding the relation between the ACE genotype and vasomotor responses induced by ACh (25, 26). Butler, et al. demonstrated that the D allele is associated with an impairment of the forearm blood flow responses induced by acetylcholine, while there is no difference in the response to nitroprusside in normal humans (25). Prasad et al. found no difference in ACh-mediated coronary vasomotion between the ACE genotypes in patients with atherosclerosis (26). The effects of the ACE genotype on endothelial vasomotor function cannot be extensively evaluated using ACh as a pharmacological tool, since ACh has not only an endothelium-dependent vasodilating effect but also a constricting effect on vascular smooth muscle.

In the present study, ACE polymorphism had no influence on circulating PAI-1 antigen levels, although several studies have reported that elevated PAI-1 levels were observed in subjects with the ACE DD genotype (27, 28) and were related to an increased risk of cardiovascular thrombotic events (29).

However, because the present study employed relatively few subjects, and particularly subjects with the DD genotype, further trials will be needed to confirm these findings.

In conclusion, the D allele of the ACE gene was associated with impaired tPA release in response to BK in the human coronary circulation. Our results suggest that thrombolytic therapy for acute coronary syndromes may be less effective in patients with the DD genotype. Furthermore, the inhibition of ACE would be expected to cause a greater improvement in coronary fibrinolytic function to reduce future coronary thrombotic events in patients with the II or ID genotype than in patients with the DD genotype.

References

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