Bradykinin Stimulates the Release of Tissue Plasminogen Activator in Human Coronary Circulation: Effects of Angiotensin-Converting Enzyme Inhibitors

Kazu Minai, MD, Tetsuya Matsumoto, MD, Hajime Horie, MD, Naoto Ohira, MD, Hiroyuki Takashima, MD, Hiroshi Yokohama, MD, Masahiko Kinoshita, MD

Otsu, Japan

OBJECTIVES
The goal of this study was to determine: 1) whether bradykinin (BK) directly stimulates tissue plasminogen activator (tPA) secretion in human coronary circulation, and 2) whether angiotensin-converting enzyme (ACE) inhibition favorably alters the fibrinolytic balance regulated by BK.

BACKGROUND
Bradykinin is a potent stimulator of tPA secretion in endothelial cells; however, the effect of BK on tPA release in the human coronary circulation has not been studied.

METHODS
Fifty-six patients with atypical chest pain were randomly assigned to two groups: 25 patients were treated with the ACE inhibitor enalapril (ACE inhibitor group), and 31 were not treated with ACE inhibitors (non-ACE inhibitor group). Graded doses of BK (0.2, 0.6, 2.0 μg/min), acetylcholine (ACh) (30 μg/min) and papaverine (PA) (12 mg) were administered into the left coronary artery. Coronary blood flow (CBF) was evaluated by Doppler flow velocity measurement. Blood samples were taken from the aorta (Ao) and the coronary sinus (CS).

RESULTS
Bradykinin induced similar increases in CBF in both groups. The net tPA release induced by BK was dose-dependently increased in both groups, and the extent of that increase in the ACE inhibitor group was greater than that in the non-ACE inhibitor group. Bradykinin did not alter plasminogen activator inhibitor-1 (PAI-1) levels in the Ao or CS in either group. Neither ACh nor PA altered tPA levels or PAI-1 levels in either group.

CONCLUSIONS
Intracoronary infusion of BK stimulates tPA release without causing any change in PAI-1 levels in the human coronary circulation. In addition, this effect of BK is augmented by an ACE inhibitor. (J Am Coll Cardiol 2001;37:1565–70) © 2001 by the American College of Cardiology

It is widely accepted that vascular endothelial cells, through the release of nitric oxide (NO), prostacyclin, tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1), play an important role in the regulation of thrombosis and fibrinolysis. Reduced fibrinolytic activity is associated with an increased risk of myocardial infarction and cardiovascular mortality (1,2).

Bradykinin (BK) is a vasoactive polypeptide that has cardioprotective effects (3). There is a growing body of evidence that BK causes endothelium-dependent vasodilation through the production of NO, prostacyclin and endothelium-derived hyperpolarizing factor through the B2 receptor in human coronary arteries (4,5). On the other hand, it has been shown that BK is a potent stimulus for tPA secretion in cultured endothelial cells (6,7). Brown et al. (8) demonstrated that infusion of BK results in an increase in tPA levels throughout the entire body. Recently, it has been shown that intra-arterial administration of BK stimulated tPA release in human brachial arteries (9,10). Nevertheless, the effect of BK on tPA release has not been examined in the human coronary circulation.

Angiotensin-converting enzyme (ACE) inhibition has been shown to improve coronary endothelial function (11,12) and decrease the incidence of ischemic cardiovascular events in patients with left ventricular dysfunction (13,14). In patients treated with ACE inhibitors, the mechanisms that underlie the reduction in coronary thrombotic events remain unknown. Thus, it is unclear whether ACE inhibitors favorably alter the fibrinolytic balance by increasing BK-induced tPA release, decreasing angiotensin II-mediated PAI-1 release, or both (8,15). Furthermore, the effects of ACE inhibition on BK-mediated fibrinolytic and vasomotor responses have not been assessed concurrently in coronary circulation in the same patients.

In this study, we examined whether BK directly stimulates tPA secretion in human coronary circulation and whether ACE inhibition favorably alters the fibrinolytic balance regulated by BK.
Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ACh = acetylcholine
ANOVA = analysis of variance
Ao = aorta
BK = bradykinin
CBF = coronary blood flow
CS = coronary sinus
LAD = left anterior descending
MAP = mean arterial pressure
NO = nitric oxide
PAI-1 = plasminogen activator inhibitor-1
PARC = plasma active renin concentration
tPA = tissue plasminogen activator

METHODS

Study patients. The eligible subjects for our study were randomly assigned to two groups: 25 patients were treated with enalapril (10 mg once daily) (ACE inhibitor group) for a duration of seven days, and 31 patients were not treated with an ACE inhibitor (non-ACE inhibitor group). 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. Secondary hypertension was excluded based on history, physical examination and appropriate laboratory testing (including renal arteriogram, if indicated). Patients with diabetes mellitus, myocardial infarction, congestive heart failure, cardiomyopathy or valvular heart disease were excluded from the study. All cardiac medications except for ACE inhibitors were discontinued for at least 72 h before the study. 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 AM and 11 AM in the fasting state. A 0.014-inch Doppler-tipped guidewire (FloWire, Cardiometrics Inc., Mountain View, California) was advanced to the proximal segment of the left anterior descending (LAD) coronary artery to measure blood flow velocity. 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 inserted via the right femoral vein into the coronary sinus (CS) for blood sampling. Baseline coronary blood flow (CBF) velocity, blood sampling and coronary angiography were performed and confirmed to be unchanged by a 2-min infusion of saline at 1 ml/min. 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, CBF velocity reached a peak at about 60 s and maintained a plateau by 60 s.
2) After completing the protocol with the intracoronary injection of BK, we waited at least 10 min before beginning the infusion of acetylcholine (ACh) (muscarinic receptor-operated endothelium-dependent vasodilator), by which time the coronary diameter and coronary blood flow velocity had returned to their baseline values.
3) Acetylcholine was given at 30 µg/min for 2 min.
4) Finally, papaverine (endothelium-independent vasodilator) was given into the left coronary artery at 12 mg for 20 s.

Coronary angiography was performed after each infusion.

Quantitative coronary angiography and measurements of coronary blood flow. Coronary cineangiograms were recorded using a Philips cineangiographic system (Philips Medical Systems, Tokyo, Japan). 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 angiography was performed using the Judkins technique with contrast material (Omnipaque, Dai-ichi Pharmaceutical Co., Tokyo, Japan). Coronary angiograms were analyzed by QCA using the Cardiovascular Measurement System (CMS-MEDICS Medical Imaging Systems, Leiden, the Netherlands). Peak CBF velocity was continuously monitored using a fast Fourier transform-based spectral analyzer (FloMap, Cardiometrics Inc.). Coronary blood flow was derived from the CBF velocity and diameter measurements by the formula: \( \pi \times \text{averaged peak CBF velocity} \times 0.125 \times (\text{arterial diameter})^2 \) (16).

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

Blood sampling and biochemical assays. Paired blood samples in the aorta (Ao) and CS were taken simultaneously before the infusion of BK and after the infusion of BK, ACh and papaverine for the measurement of plasma tPA antigen and PAI-1 antigen. Blood samples in the Ao and CS were also obtained at 2 and 4 min after cessation of the infusion of BK at 2.0 µg/min.

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 t-PA and PAI-1 was collected in tubes containing 0.105 mol/L sodium citrate. Antigen levels were determined using a two-site ELISA (Biopool, AB, Umeå, Sweden) (8).

Arteriovenous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected CS venous and arterial blood. Thus, net release or uptake rates at each time point were calculated as Net Release = \((C_{CS} - C_A) \times (\text{CBF} \times [(100 - \text{Hematocrit})/100])\).

Statistics. Data are expressed as means ± SEM. Discrete variables were expressed as counts or percentages and compared with the chi-square test. Continuous variables
were compared using the unpaired Student t test or a one-way analysis of variance (ANOVA). When serial changes in systemic and coronary hemodynamic variables and fibrinolytic parameters in response to the graded doses of BK were compared between the two groups, a two-way ANOVA for repeated measures followed by Bonferroni’s multiple-comparison test was used. A p value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics. There were no significant differences in baseline characteristics between the ACE inhibitor group and the non-ACE inhibitor group, except for mean arterial pressure (MAP), plasma active renin concentration (PARC) and ACE. Mean arterial pressure and ACE were lower in the ACE inhibitor group than in the non-ACE inhibitor group, while PARC was higher in the ACE inhibitor group than in the non-ACE inhibitor group (Table 1).

Systemic and coronary hemodynamics. As illustrated in Figure 1, intracoronary infusion of BK resulted in a significant decrease in MAP in the ACE inhibitor group, and this effect was dose-dependent. However, BK did not alter MAP in the non-ACE inhibitor group. After the infusion of each of three graded doses of BK, MAP was significantly lower in the ACE inhibitor group than it was in the non-ACE inhibitor group (p < 0.0001). With increasing doses of BK, heart rate did not change in either group (Fig. 1). The addition of either ACh at 30 μg/min or papaverine at 12 mg did not cause changes in MAP or heart rate in either group. Intracoronary infusion of BK increased CBF in a dose-dependent manner in the two groups, and no significant differences were observed in the response to BK between the two groups (Fig. 1). Intracoronary infusion of papaverine caused an increase in CBF, which was equipotent to that of BK (2.0 μg/min) in both groups. There were no significant differences in the CBF response to either ACh or papaverine between the two groups.

Fibrinolytic parameters. Baseline tPA antigen levels at either the Ao or CS in the ACE inhibitor group (Ao: 6.07 ± 0.50, CS: 6.19 ± 0.56 ng/ml) were similar to those in the non-ACE inhibitor group (Ao: 5.93 ± 0.56, CS: 5.87 ± 0.53 ng/ml).

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ACE (n = 25)</th>
<th>non-ACE Inhibitor (n = 31)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>61.8 ± 2.25</td>
<td>60.3 ± 2.0</td>
<td>0.61</td>
</tr>
<tr>
<td>Gender, male:female</td>
<td>17:8</td>
<td>23:8</td>
<td>0.83</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>13 (52%)</td>
<td>10 (32%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4 ± 0.5</td>
<td>23.0 ± 0.6</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>106.4 ± 4.2</td>
<td>119.3 ± 3.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65.4 ± 2.1</td>
<td>65.3 ± 1.9</td>
<td>0.99</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>191.9 ± 5.9</td>
<td>193.9 ± 5.6</td>
<td>0.80</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>46.1 ± 4.6</td>
<td>44.9 ± 2.1</td>
<td>0.78</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>126.6 ± 11.0</td>
<td>125.3 ± 10.8</td>
<td>0.93</td>
</tr>
<tr>
<td>PARC, pg/ml</td>
<td>73.5 ± 28.3</td>
<td>15.2 ± 6.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Aldosterone, pg/ml</td>
<td>65.0 ± 5.7</td>
<td>70.3 ± 6.8</td>
<td>0.56</td>
</tr>
<tr>
<td>ACE, IU/l</td>
<td>2.04 ± 0.33</td>
<td>9.88 ± 0.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>69.4 ± 9.4</td>
<td>94.9 ± 9.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>239.5 ± 18.9</td>
<td>299.9 ± 36.9</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Mean ± SE. ACE = angiotensin-converting enzyme; HDL = high density lipoproteins; PARC = plasma active renin concentration.

Figure 1. The effects of bradykinin (0.2, 0.6, 2.0 μg/min), acetylcholine (ACh) (30 μg/min) and papaverine (PA) (12 mg) on the mean arterial pressure (upper panel), heart rate (middle panel) and percent change in coronary blood flow (lower panel) in the ACE inhibitor group (open box) and non-ACE inhibitor group (closed circle). *p < 0.05 versus baseline.
In both the non-ACE inhibitor group and the ACE inhibitor group, the level of tPA in the Ao was not changed by the infusion of BK. This level in the ACE inhibitor group was increased at 2 min after the cessation of BK (Fig. 2, left). In the non-ACE inhibitor group, the level of tPA in the CS was significantly increased in a dose-dependent manner by the infusion of graded doses of BK and gradually declined at 2 min and 4 min after cessation of the infusion of BK (Fig. 2, right). In the ACE inhibitor group, the level of tPA in the CS immediately reached a plateau with the infusion of BK at 0.2 μg/min and remained constant throughout and for 2 min after cessation of the infusion of BK (Fig. 2, right). The level of tPA in response to BK in the CS in the ACE inhibitor group was significantly higher than that in the non-ACE inhibitor group (p < 0.0001) (Fig. 2, right), whereas the level of tPA in response to BK in the Ao was not significantly different between the two groups (Fig. 2, left). The net tPA release induced by the intracoronary infusion of BK was increased in both groups, and the extent of this increase in the ACE inhibitor group was markedly greater than that in the non-ACE inhibitor group (p = 0.0002) (Fig. 3).

Infusion of ACh at a dose of 30 μg/min did not alter the level of tPA in either the Ao or the CS in either group nor did infusion of papaverine alter the level of tPA in either the Ao or the CS in either group. Neither ACh nor papaverine caused a significant change in net tPA release in either group.

The basal level of PAI-1 in either the Ao or the CS did not differ between the two groups. With an increase in BK dosage, the level of PAI-1 in the CS tended to decrease compared with that in the Ao in both groups; however, there were no significant differences in either group. Neither ACh nor papaverine had any significant effects on the level of PAI-1 in either the Ao or CS in either group (Fig. 4).

**DISCUSSION**

This is the first report that demonstrates that intracoronary infusion of BK caused a significant increase in tPA antigen levels, and ACE inhibitors augmented this stimulatory effect of BK in the human coronary circulation.

**Systemic and coronary hemodynamic changes.** In the ACE inhibitor group, MAP was significantly decreased in a dose-dependent fashion, whereas this effect was not observed in the non-ACE inhibitor group. These results were consistent with previous studies in which BK was given at doses of up to 10 μg/kg/min (8). Bradykinin relaxes human coronary arteries through the stimulation of endothelial B2 receptors, leading to the production of NO, prostacyclin and endothelium-derived hyperpolarizing factor (4,5). Angiotensin-converting enzyme inhibitors may act on kininase II and increase the tissue concentrations of BK, which would augment the vasodilation induced by BK. Previous studies have shown that intracoronary infusion of BK increased coronary artery diameter and CBF, and these effects were augmented by acute intravenous administration of enalaprilat in human subjects (17). In this study, the increases in CBF induced by BK at 2.0 μg/min in the ACE inhibitor group were comparable with those in the non-ACE inhibitor group. Therefore, our results suggest that the effects of ACE inhibitors on fibrinolytic changes in response to BK were not associated with coronary hemodynamic changes.
tPA. Tissue plasminogen activator, the key factor in the initiation of fibrinolysis, is synthesized in endothelial cells, stored in small, dense vesicles and secreted by endothelial cells in response to several vasoactive agents (18).

In both groups, the net tPA antigen release induced by BK was increased, suggesting that tPA antigen is released during transcardiac passage. The extent of that increase in the ACE inhibitor group was markedly greater than that in the non-ACE inhibitor group. Therefore, the present data may provide insight into the mechanisms through which ACE inhibitors reduce the risk of ischemic cardiovascular events in clinical trials (13,14). On the basis of our data, ACE inhibitors may not only improve coronary endothelial vasomotor function (11,12), but may also have favorable effects on the coronary fibrinolytic balance through potentiation of the effects of BK in humans, resulting in a decrease in ischemic cardiovascular events.

Brown et al. (8) reported that systemic administration of BK caused an increase in venous tPA antigen concentrations in captopril- or quinapril-pretreated subjects. Subsequently, they demonstrated that BK stimulates tPA release in human brachial arteries. However, few previous studies have examined the mechanisms underlying the stimulation of tPA release induced by BK in humans.

Intracoronary infusion of ACh did not change the plasma level of tPA antigen in either the Ao or CS. The effects of tPA release induced by ACh in the human vasculature are controversial (9,19). One possible explanation is that the stimulation of endothelial muscarinic receptors induced by methacholine may cause local tPA production in human forearm circulation since ACh and methacholine differ with regard to their potency for stimulating tPA release. In this study, the increase in CBF induced by ACh at a concentration of 30 µg/min was significantly less than that induced by BK, but a further increase in the dose of ACh (up to 100 µg/min) directly contracts human coronary vascular smooth muscle cells apart from endothelium (data not shown). Therefore, as far as human coronary arteries are concerned, it is likely that the dose of ACh administered or the number of human coronary endothelial muscarinic receptors may have been insufficient to stimulate tPA release. Further studies are needed to determine whether B2 receptor-specific stimulation and subsequent intracellular signal transduction cause the release of tPA in human coronary circulation.

Increased shear stress stimulates tPA release in cultured human umbilical vein endothelial cells (7). In this study, intracoronary infusion of papaverine, an endothelium-independent vasodilator, did not increase tPA release in human coronary circulation, even though it has a vasodilatory effect equipotent to that of BK. Therefore, flow-induced coronary vasodilation may not be responsible for the observed effect of BK on tPA release. We assumed that BK stimulates tPA release through a direct action (possibly by stimulating the B2-specific receptor) in human coronary circulation.

In this study, ACE inhibitors had no effect on baseline levels of tPA antigen or PAI-1 antigen in human coronary circulation. Treatment with either captopril for four weeks (20) or ramipril for two weeks (21) caused a significant decrease in the level of tPA or PAI-1 antigen in patients with myocardial infarction. The chronic effects of ACE inhibitors on tPA and PAI-1 release should be examined further.

PAI-1. Elevated levels of PAI-1 have been implicated in the pathogenesis of myocardial infarction (22) and may contribute to the risk of reinfarction in patients who have suffered a previous myocardial infarction (23). It has been shown that angiotensin II increased PAI-1 antigen levels in humans (15). Many factors influence the production of PAI-1 (e.g., glucose, insulin, estrogen and angiotensin II) (24–26). Regulation of plasma levels of PAI-1 cannot be explained solely by the renin-angiotensin system.

Intracoronary infusion of BK had no effects on PAI-1 levels at the Ao or CS in either group. Previous studies demonstrated that infusion of BK or substance P, an endothelium-dependent vasodilator, had no effects on circulating PAI-1 levels but increased circulating tPA levels in human forearm circulation (9,27). In this study, PAI-1 levels at the CS tended to decrease with increasing doses of BK in both groups. On the other hand, infusion of papaverine did not change PAI-1 levels in either the Ao or CS. These results raise the possibility that the production of PAI-1 is regulated by other factors.
and secretion of PAI-1 are modulated by the increased tPA release in response to BK.

Study limitations. We did not examine whether the presence of coronary risk factors affect tPA release in response to BK in the human coronary circulation. Further studies are needed to determine whether there is a relationship between the human coronary fibrinolytic response to BK and disorders such as hypercholesterolemia, hypertension and insulin resistance.

Blood flow in the CS is derived not only from LAD coronary arteries but also from the left circumflex and right coronary arteries. The amount of blood flow in LAD coronary arteries is greater than that in the left circumflex or right coronary arteries and is related to that in the left circumflex and right coronary arteries (28). Therefore, we defined net tPA release as the product of the CBF of LAD coronary arteries and the difference in concentration between the Ao and the CS, although we did not have an accurate net CBF.

Summary and clinical implications. We obtained two novel observations in this study. First, intracoronary infusion of BK stimulated tPA release in human coronary circulation. Second, this effect of BK was augmented by the administration of an ACE inhibitor. Therefore, ACE inhibitors may have favorable effects on the coronary fibrinolytic balance in humans through the potentiation of BK, resulting in a decrease in ischemic cardiovascular events.

Reprint requests and correspondence: Dr. Tetsuya Matsumoto, First Department of Internal Medicine, Shiga University of Medical Science, Seta Tsukinowa, Otsu, Shiga 520-2192, Japan. E-mail: tetsuyam@belle.shiga-med.ac.jp.

REFERENCES