Effects of Cyclooxygenase Inhibition on Endothelial Function in Hypertensive Patients Treated with Angiotensin-Converting Enzyme Inhibitors

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Summary

Background: Cyclooxygenase inhibition restores endothelium-dependent vasodilatation in hypertension, but it is unknown whether it restores endothelial function in hypertensive patients treated with angiotensin-converting enzyme (ACE) inhibitors.

Hypothesis: The purpose of the present study was to evaluate the effects of cyclooxygenase inhibition on endothelial function in hypertensive patients treated with ACE inhibitors.

Methods: Endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent glyceryl trinitrate-induced dilatation were investigated in 10 patients treated with enalapril (ACE group), 11 patients treated with manidipine and metoprolol (non-ACE group), and 12 normotensive control subjects. After administration of 1000 mg of aspirin, FMD was investigated once again. Plasma cyclic guanosine monophosphate (cGMP) and eicosanoids were also measured during reactive hyperemia before and after aspirin administration.

Results: Flow-mediated dilatation was more impaired in the non-ACE group than in the ACE group ($8.3 \pm 3.8\%$, $5.7 \pm 1.7\%$, respectively, $p < 0.04$). Glyceryl trinitrate-induced dilatation was similar in the ACE group, the non-ACE group, and in the control subjects. In the ACE group, FMD was reduced after administration of aspirin ($5.3 \pm 4.2\%$, $p < 0.05$). The percent change in FMD after administration of aspirin correlated significantly with percent change in cGMP ($r = 0.77$, $p < 0.03$; y-intercept, -62.1% , $p < 0.01$). After aspirin

administration, levels of thromboxane B₂ and 6-keto-prostaglandin_{1α} were significantly decreased compared with those before aspirin administration in all groups.

Conclusions: Cyclooxygenase inhibition may reduce the beneficial effect on endothelium-dependent vasodilatation induced by ACE inhibitors. The results suggested that prostacyclin in addition to nitric oxide plays a significant role in the restoration of endothelial function in hypertensive patients treated with ACE inhibitors.

Key words: endothelial function, hypertension, angiotensin-converting enzyme inhibitor, cyclooxygenase, prostacyclin

Introduction

Angiotensin-converting enzyme (ACE) inhibitors are generally used for the treatment of hypertensive patients. It has been shown that ACE inhibitors improve endothelial function in various animal models of cardiovascular disease and in patients with hypertension and cardiovascular disease.^{1–6} Bradykinin, which is produced locally against the breakdown, appears to be the mediator of the improvement of endothelial function that is induced by ACE inhibitors in diseased arteries. Bradykinin is a very potent vasodilator that exerts its vasodilatory actions by causing endothelial release of nitric oxide (NO) and/or prostacyclin. In contrast, bradykinin also produces cyclooxygenase-dependent contracting factors, such as thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂), via phospholipase A₂. It was shown that a cyclooxygenase inhibitor in hypertensive patients without medication restored endothelial function to reduce TXA₂, PGH₂, or superoxide anion-impaired NO production.⁷ However, it is unknown whether cyclooxygenase inhibition restores endothelial function in hypertensive patients treated with an ACE inhibitor despite reduction of prostacyclin.

The purpose of the present study was to evaluate the effects of cyclooxygenase inhibition on endothelial function in hypertensive patients treated with ACE inhibitors. We also investigated whether NO and prostacyclin participated in the changes in endothelial function.

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Methods

Subjects

The subjects in this study included 21 Japanese patients with essential hypertension. Of these, 10 patients (Group 1) were treated with the ACE inhibitor enalapril (10 mg/day) for at least 6 months. The remaining 11 patients (Group 2) were treated with manidipine (20 mg/day) and metoprolol (60 mg/day) for at least 6 months. Group 1 consisted of five men and five women (average age, 65 ± 10 years; age range, 52–79 years), while Group 2 consisted of seven men and four women (average age, 66 ± 9 years; age range, 52–81 years). The duration of hypertension in Group 1 ranged from 2 to 20 years (average, 9 ± 6 years) and that in Group 2 from 3 to 17 years (average, 8 ± 5 years). No patient in either group had cardiovascular disease or diabetes mellitus. Patients in both groups had normal renal function as evidenced by normal serum creatinine and negative results of urinalysis. Five patients in Group 1 and six patients in Group 2 had hyperlipidemia, and they had been treated with simvastatin or pravastatin for more than 6 months. Patients smoking more than five cigarettes per day were excluded from the study; two patients in each of the two groups smoked less than five cigarettes per day. The patients took their antihypertensive and antihyperlipidemic medications as usual in the morning of each day during the study period. The 12 age- and gender-matched healthy control subjects (Group 3) who took part in the study were normotensive (6 men, 6 women; average age, 61 ± 78 years; age range, 51–75 years). Normalcy was determined by careful examination of history, physical examination, and laboratory analysis. This study was performed after informed consent had been obtained from each subject according to the Declaration of Helsinki.

Study Design

The ultrasound method for measuring endothelium-dependent and endothelium-independent arterial dilatation has been described in detail by ourselves⁸ and by Celermajer *et al.*⁹ Initially, scans in all three groups were taken only at rest and during reactive hyperemia. Subsequently, at 60 min after oral administration of 1,000 mg of aspirin (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), scans were taken at second rest, during reactive hyperemia, and after administration of 300 µg sublingual glyceryl trinitrate (GTN) by one puff of a spray device (Myocor Spray, Toa Eiyo Co., Ltd., Tokyo, Japan).

Blood was drawn from the right median cubital vein for measurement of low-density (LDL-C) and high-density lipoprotein cholesterol (HDL-C) before the first resting scan. After both the first and the second hyperemic scans, blood was drawn again from 8 of the 10 patients in Group 1, 10 of the 11 patients in Group 2, and 6 of the 12 control subjects (Group 3) for plasma cyclic guanosine monophosphate (cGMP), plasma thromboxane B₂ (TXB₂), a stable metabolite of TXA₂ and plasma 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}), a stable metabolite of prostacyclin. The percent change in cGMP was calculated to compare cGMP level before and after administration of aspirin.

Data Analysis

The vessel diameter was automatically measured in the method described by our group.⁹ Endothelium-dependent flow-mediated vasodilatation (FMD) with endothelium-independent vasodilatation caused by sublingual GTN (GTN-induced dilatation) was used to assess endothelial function. The vessel diameters in FMD- and GTN-induced dilatation were expressed as a percentage of the diameter of the artery in the first resting scan. The percent change in FMD was calculated to compare FMD before and after aspirin administration. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by the heart rate and the cross-sectional area of the vessel. Reactive hyperemia was calculated as the maximal flow recorded in the first 15 s after cuff deflation divided by the flow during the resting scan.

Interobserver variability was determined by calculating the mean and standard deviation of the difference in the two observers' results from 20 arterial studies. The interobserver variability for measurement of FMD was $0.2 \pm 2.3\%$.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation. Comparison among groups was performed by analysis of variance (ANOVA) and Scheffe's test. A paired Student's *t*-test was used to analyze differences before and after administration of aspirin. The relationship between percent change of FMD and percent change of cGMP was determined by simple linear regression analysis. A *p* value < 0.05 was considered to be statistically significant.

Results

Basal-Line Characteristics

Mean blood pressures, heart rates, vessel sizes during the resting scan, as well as LDL-C and HDL-C values were similar in all three groups (Table I).

Comparison of Vascular Responses before Aspirin Administration

The degrees of reactive hyperemia were similar in all three groups. Flow-mediated dilatation was more reduced in Group 2 than in Groups 1 and 3 (Group 1, $8.3 \pm 3.8\%$, $p < 0.04$ compared with Group 2; Group 3, $10.3 \pm 1.7\%$, $p < 0.0003$ compared with Group 2; Group 2, $5.8 \pm 2.2\%$). Glyceryl trinitrate-induced dilatation and vessel size at the first resting scan were similar in all three groups ($12.8 \pm 4.9\%$, $12.8 \pm 2.5\%$, and $15.6 \pm 3.1\%$, respectively) (Fig. 1).

Comparison of Vascular Response after Aspirin Administration

Vessel size at the resting scan and the degree of reactive hyperemia were similar before and after aspirin administration in the three groups (Table I), and there was no difference in mean

TABLE I Characteristics of the patients included in the study

	Group 1	Group 2	Group 3	p Value
Number of patients	10	11	12	
Age (years)	65 ± 10	66 ± 9	62 ± 8	0.47
Sex (male/female)	5/5	7/4	6/6	0.76
LDL-C (mg/dl)	119 ± 27	113 ± 21	123 ± 14	0.57
HDL-C (mg/dl)	62 ± 23	55 ± 14	63 ± 12	0.49
Before aspirin				
Mean BP (mmHg)	89 ± 9	94 ± 8	92 ± 7	0.23
Heart rate (/min)	62 ± 11	62 ± 7	67 ± 12	0.52
Vessel size (mm)	4.22 ± 0.72	4.09 ± 0.40	4.25 ± 0.52	0.77
Reactive hyperemia (%)	345 ± 64	328 ± 71	382 ± 116	0.33
After aspirin				
Mean BP (mmHg)	89 ± 11	95 ± 7	89 ± 6	0.18
Heart rate (/min)	62 ± 7	60 ± 7	62 ± 11	0.87
Vessel size (mm)	4.18 ± 0.74	4.11 ± 0.40	4.22 ± 0.51	0.89
Reactive hyperemia (%)	357 ± 108	320 ± 92	385 ± 150	0.45

Abbreviations: BP = blood pressure, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol.

FIG. 1 (A) Flow-mediated dilatation (FMD) before and after aspirin administration. Before aspirin administration in the non-ACE group (Group 2), FMD was significantly impaired compared with that in the angiotensin-converting enzyme (ACE) group (Group 1) and control subjects (Group 3). Moreover, FMD after aspirin administration was reduced to a greater extent after aspirin administration than before aspirin administration in Group 1. (B) Glyceryl trinitrate (GTN)-induced dilatation in the three groups. There was no difference among GTN-induced dilatations in the three groups.

blood pressure and heart rate between resting scans before and after aspirin administration (Table I). Moreover, aspirin administration did not change mean blood pressure and reactive hyperemia in any group (Table I). Flow-mediated dilatation after aspirin administration was more reduced than that before aspirin administration in Group 1 ($8.3 \pm 3.8\%$, $5.3 \pm 4.2\%$, $p < 0.05$) (Fig. 1). However, FMD was similar before and after aspirin administration in Groups 2 and 3. There was no difference in TXB_2 , 6-keto-PGF $_{1\alpha}$, and cGMP among the three groups before aspirin administration. After aspirin administration, levels of TXB_2 and 6-keto-PGF $_{1\alpha}$ were significantly decreased in all three groups compared with those before aspirin administration (Table II). Percent change in FMD correlated significantly with percent change in cGMP in Group 1 ($r = 0.77$, $p < 0.03$; y-intercept, -62.1% , $p < 0.01$) (Fig. 2).

Discussion

The major findings of the present study were (1) FMD was more impaired in Group 2 than in Groups 1 and 3; (2) FMD in Group 1 was reduced after administration of aspirin; and (3) percent change in FMD correlated significantly with percent change in cGMP in Group 1. These data suggest that prostacyclin in addition to NO participates in the improvement of endothelial function induced by ACE inhibitors.

Experimental studies^{10, 11} have raised the possibility that bradykinin plays an important role in the regulation of vascular tone at rest and during flow-stimulated conditions. Bradykinin, through activation of endothelial B₂ kinin receptors, induces NO synthesis. In patients with hypertension, enhanced NO synthesis induced by ACE inhibitors improved endothe-

TABLE II Prostanoids and cGMP before and after aspirin administration

	Group 1		Group 2		Group 3	
	Before	After	Before	After	Before	After
6-keto-PGF _{1α} (pg/ml)	17.0 ± 8.9	12.0 ± 9.2 ^a	22.4 ± 3.4	18.4 ± 5.5 ^a	20.4 ± 8.1	17.4 ± 6.9 ^a
TXB ₂ (pg/ml)	13.0 ± 4.2	4.8 ± 2.0 ^b	12.0 ± 8.3	5.1 ± 4.1 ^b	14.4 ± 12.2	6.7 ± 6.6 ^a
cGMP (pmol/ml)	7.2 ± 7.1	12.3 ± 8.0	3.1 ± 3.1	3.9 ± 5.6 ^c	7.4 ± 14.4	5.6 ± 6.8

^a $p < 0.05$ vs. before aspirin.^b $p < 0.01$ vs. before aspirin.^c $p < 0.05$ vs. Group 1.Abbreviations: TXB₂ = thromboxane B₂, Before = before aspirin, After = after aspirin, cGMP = cyclic guanosine monophosphate.

lial-dependent vascular function.^{12, 13} On the other hand, B₂ kinin receptors stimulate phospholipase A₂, leading to the mobilization of arachidonic acid, the precursor of eicosanoid. Therefore, bradykinin produces not only cyclooxygenase-dependent vasodilators, such as prostacyclin, but also cyclooxygenase-dependent contracting factors, such as TXA₂ and PGH₂. The larger release of PGH₂ in spontaneous hypertensive rats is associated with a higher expression of cyclooxygenase in these blood vessels,¹⁴ and therefore a higher expression of cyclooxygenase may arise similarly in human hypertension. It has been reported that endothelial vasodilatation due to acetylcholine in hypertensive patients without medication was improved by inhibition of cyclooxygenase and that cyclooxygenase-dependent contracting factors and superoxide anion impaired NO production.⁷ However, it was unknown whether inhibition of cyclooxygenase in patients treated with ACE inhibitors affects endothelial vasodilatation during a flow-stimulated condition. In the present study, aspirin administration reduced FMD in hypertensive patients

treated with ACE inhibitors. Obviously, we do not know the mechanisms of reduced FMD after aspirin administration. However, based on animal experiments,^{10, 15, 16} we propose two possible mechanisms. First, reduction of prostacyclin, which occurred as a consequence of the inhibition of cyclooxygenase, caused a decrease in FMD. The increased production of bradykinin due to an ACE inhibitor causes an increase in the production of prostacyclin.¹⁷ As a result of the increased production of prostacyclin, FMD was restored. Reduced production of prostacyclin by aspirin, therefore, might cause reduced FMD. In the present study, aspirin administration significantly reduced 6-keto-PGF_{1α}. However, 6-keto-PGF_{1α} before aspirin administration was similar among the subjects in the three groups. These findings may be caused by the difference between blood concentration and tissue level concentration of prostacyclin; therefore, the prostacyclin level in the vessel wall should be measured in a future study. Second, there is a possibility that the production of endothelium-dependent contracting factors increases after aspirin administration. In human brain endothelial cells, inhibition of cyclooxygenase increased constitutive and angiotensin II-stimulated secretion of endothelin-1.¹⁸ However, the concentration of endothelium-dependent contracting factors, including endothelin-1, was not measured in the present study. Therefore, the effect of endothelin-1 on endothelial vasodilatation in hypertensive patients treated with ACE inhibitors should be evaluated in a future study.

Bradykinin causes endothelial release of NO as well as prostacyclin in a flow-stimulating condition. In the present study, we measured the level of cGMP, which was the second messenger of NO, to evaluate the effect of NO on FMD.¹⁹ A significant linear correlation was found between percent changes in FMD and cGMP before and after aspirin administration. This linear correlation suggests that NO partially participates in FMD in hypertensive patients. Moreover, the y-intercept of the regression line also revealed statistical significance; it shifted from the origin of the coordinate axes downward. This finding may suggest that vasodilatory action decreased because of reduction of prostacyclin after aspirin administration. Therefore, prostacyclin plays an important role in the regulation of vasomotor tone in hypertensive patients treated with ACE inhibitors.

FIG. 2 Relationship between percent change in flow-mediated dilatation (FMD) and percent change in cyclic guanosine monophosphate (cGMP) in Group 1. Percent change in FMD correlated significantly with percent change in cGMP. The y-intercept of the regression line shifted significantly downward from the origin of the coordinate.

Study Limitations

The number of patients investigated was relatively small. Our results showed that endothelial vasodilatation was restored to a greater extent in Group 1 than in Group 2. However, improvement in endothelial function by use of an ACE inhibitor in hypertension is controversial.^{20, 21} Thus, care must be taken in the extrapolation of these findings to other populations. The FMD in both Groups 1 and 2 was measured during medication. As we did not examine differences in the FMD before and after medication in each group, we could not exactly evaluate the effect of medication on FMD. However, factors that affect the FMD, such as age, LDL-C, HDL-C, smoking, and duration of hypertension, were similar in the two groups. Therefore, our results suggest that the ACE inhibitor caused the difference in FMD between Groups 1 and 2.

Conclusion

Inhibition of cyclooxygenase may reduce the beneficial effect on endothelial vasodilatory function induced by ACE inhibitors. These results suggest that prostacyclin in addition to NO plays a significant role in the restoration of endothelial function in hypertensive patients treated with ACE inhibitors.

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