Plasma Levels of Nitric Oxide and Related Vasoactive Factors Following Long-Term Treatment With Angiotensin-Converting Enzyme Inhibitor in Patients With Essential Hypertension

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Several mechanisms other than the inhibition of systemic and local formation of angiotensin II (Ang II) have been proposed to play a role in mediating the hypotensive effects of angiotensin-converting enzyme (ACE) inhibitors. In the present study, we measured plasma levels of nitric oxide (NO) and the related vasoactive factors bradykinin, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) a stable metabolite of prostacyclin, and cyclic guanosine-3′,5′-monophosphate (cGMP) before and after a 4-week treatment with the ACE inhibitor lisinopril in 17 patients with essential hypertension. Plasma NO levels were measured by the Griess method after conversion of nitrate to nitrite. Long-term lisinopril treatment significantly reduced blood pressure and increased plasma NO and 6-keto $PGF_{1\alpha}$. The treatment also tended to increase plasma levels of bradykinin and cGMP, but not to a significant extent. The posttreatment NO level was inversely correlated with posttreatment systolic, diastolic, and mean blood pressure ($n = 17$, $r = -.68$, $P < .01$, $n = 17$, $r = -.54$, $P < .05$, and $n = 17$, $r = -.66$, $P < .01$, respectively). The posttreatment bradykinin level was also modestly correlated with posttreatment systolic and mean blood pressure ($n = 17$, $r = -.51$, $P < .05$ and $n = 17$, $r = -.55$, $P < .05$, respectively). In contrast, posttreatment 6-keto $PGF_{1\alpha}$ and cGMP levels were not correlated with posttreatment systolic, diastolic, or mean blood pressure. These findings raise the possibility that increased formation of NO and bradykinin, as well as inhibition of the renin-angiotensin system, contribute to the hypotensive effect of the ACE inhibitor observed in our hypertensive patients.

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ANGIOTENSIN-CONVERTING ENZYME (ACE) inhibitors are widely used in the treatment of hypertension.[1] Although the primary effect of these agents is the inhibition of systemic and local formation of angiotensin II (Ang II), a number of experimental and clinical findings have suggested that other vasodilative mechanisms play a role in mediating the hypotensive effect of ACE inhibitors.[2-12] Among them, Swartz et al[2] demonstrated that blockade of Ang II formation was not the sole factor in the depressor response of an ACE inhibitor in hypertensive patients, and that other factors, perhaps bradykinin, are also responsible for the hypotensive response to ACE inhibition. Furthermore, Shimamoto and Iimura[3] demonstrated that the hypotensive effect of the ACE inhibitors captopril and alacepril in essential hypertensive patients may be caused by an increase of plasma kinin levels in addition to a decrease in plasma Ang II. Especially in low-renin hypertensive patients, the kallikrein-kinin system is shown to contribute strongly to the hypotensive effect of an ACE inhibitor.[4]

Actually, since ACE is identical to the kininase II of the kallikrein-kinin system that inactivates bradykinin,[11] it is reasonable that a significant part of the blood pressure–lowering effect of ACE inhibitors is mediated by the accumulation of kinins.[2-6] On the other hand, kinins exert vasodilative effects through the release of different autocoids, which are mainly generated by the endothelium.[7] Activation of β_2-kinin receptors on endothelial cells leads to formation of the potent dilator nitric oxide (NO) and prostacyclin.[13-15] Actually, ACE inhibition has been shown to stimulate the formation of NO and prostacyclin in cultured human and bovine endothelial cells via enhanced accumulation of endothelium-derived bradykinin.[13-15] However, the plasma NO level after treatment with an ACE inhibitor in hypertensive patients remains to be fully investigated.

Accordingly, we measured plasma levels of NO and the related vasoactive factors bradykinin, the stable metabolite of prostacyclin, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$), and cyclic guanosine-3′,5′-monophosphate (cGMP) before and after a 4-week treatment with the ACE inhibitor lisinopril in 17 patients with essential hypertension. We also examined the relationship between blood pressure and the plasma levels of NO and other related vasoactive factors after treatment.

SUBJECTS

Entry Criteria and Study Design

Between January and December 1996, we recruited 17 patients with mild to moderate hypertension from a population of about 300 hypertensive patients in our department. All patients underwent routine laboratory studies including assays for serum electrolytes, serum creatinine, blood urea nitrogen (BUN), and fasting blood glucose, liver-function tests, urinalysis, chest roentgenography, and electrocardiography. We selected subjects with essential hypertension based on the results of laboratory tests[16] and guidelines of the World Health Organization.[17] Hypertension was defined as a systolic pressure of 160 mm Hg or greater and/or a diastolic pressure of 90 mm Hg or greater.

Secondary hypertension was excluded based on clinical history, physical examination, routine laboratory tests including measurement of plasma renin activity (PRA) and aldosterone, catecholamine, and cortisol levels, and excretory urography or renal arteriography.[16] No patients had signs or symptoms of cardiac or renal failure, pulmonary disease, or myocardial infarction. Only hypertensive patients who were not treated with antihypertensive medication were included in the study.

After the initial evaluation, patients were placed on monotherapy with lisinopril 10 mg/d. Plasma levels of NO, bradykinin, 6-keto $PGF_{1\alpha}$, and cGMP were determined before initiation of therapy and after 4

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Submitted September 12, 1998; accepted April 22, 1999.

Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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0026-0495/99/4810-0011$10.00/0

weeks of therapy. Written informed consent was obtained from each subject.

Measurements

Blood samples were obtained from the forearm in the sitting position in the morning while fasting. Serum creatinine and BUN levels were measured by a routine automatic method.[18] PRA was measured by radioimmunoassay as previously described.[16] NO levels were measured by the Griess method after conversion of nitrate to nitrite as previously described.[19] Plasma bradykinin[20] and 6-keto $PGF_{1\alpha}$[14] levels were measured by specific radioimmunoassays. cGMP levels were determined by radioimmunoassay as previously described.[21]

Arterial blood pressure was measured with a mercury sphygmomanometer after the patient rested sitting in a quiet warm room for 30 minutes. The mean of three blood pressure measurements was used.[16]

Statistical Analysis

Values are expressed as the mean ± SD. Relationships between blood pressure and various endocrine parameters were examined by linear regression analysis.[22] Pretherapeutic and posttherapeutic values were compared using a nonparametric test.

Results

Patient Characteristics

Hypertensive patients (10 men and seven women; mean age, 59 ± 14 years) with mild to moderate hypertension (161 ± 7/95 ± 11 mm Hg) formed the study group. The mean pulse rate was 73 ± 7 bpm. Renal function was normal as indicated by BUN and serum creatinine levels. The baseline PRA of most patients was within the normal range (mean PRA, 1.5 ± 1.6 ng/mL/h; range, 0.1 to 5.6).

Treatment Effects on Hemodynamic and Endocrine Parameters

Table 1 shows hemodynamic and endocrine parameters before and after treatment. After 4 weeks of lisinopril treatment, a significant reduction of systolic, diastolic, and mean blood pressure was obtained. The pulse rate at entry and BUN and serum creatinine levels at entry were not altered by the treatment.

After 4 weeks of lisinopril treatment, plasma levels of NO and 6-keto $PGF_{1\alpha}$ were significantly higher than the baseline levels. Treatment also tended to increase plasma bradykinin and cGMP, but not to a significant extent.

Table 1. Clinical Characteristics of the Patients at Baseline and After 4 Weeks of Lisinopril Treatment

Characteristic	Baseline	Posttreatment
Age (yr)	59 ± 14	
Sex (men/women)	10/7	
Systolic BP (mm Hg)	161 ± 7	141 ± 13†
Diastolic BP (mm Hg)	95 ± 11	83 ± 8*
Mean BP (mm Hg)	117 ± 8	102 ± 9†
Pulse (bpm)	73 ± 7	76 ± 7
BUN (mg/dL)	15.5 ± 3.4	15.7 ± 4.5
Serum creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.1
PRA (ng/mL/h)	1.5 ± 1.6	ND
Plasma NO (μmol/L)	2.5 ± 1.4	4.7 ± 3.5*
Plasma bradykinin (pg/mL)	63.8 ± 44.8	170.1 ± 301.3
Plasma 6-keto $PGF_{1\alpha}$ (pg/mL)	12.8 ± 6.6	18.1 ± 10.1†
Plasma cGMP (pmol/mL)	3.0 ± 1.2	3.2 ± 1.5

Abbreviations: BP, blood pressure; ND, not determined.
*$P < .01$.
†$P < .001$.

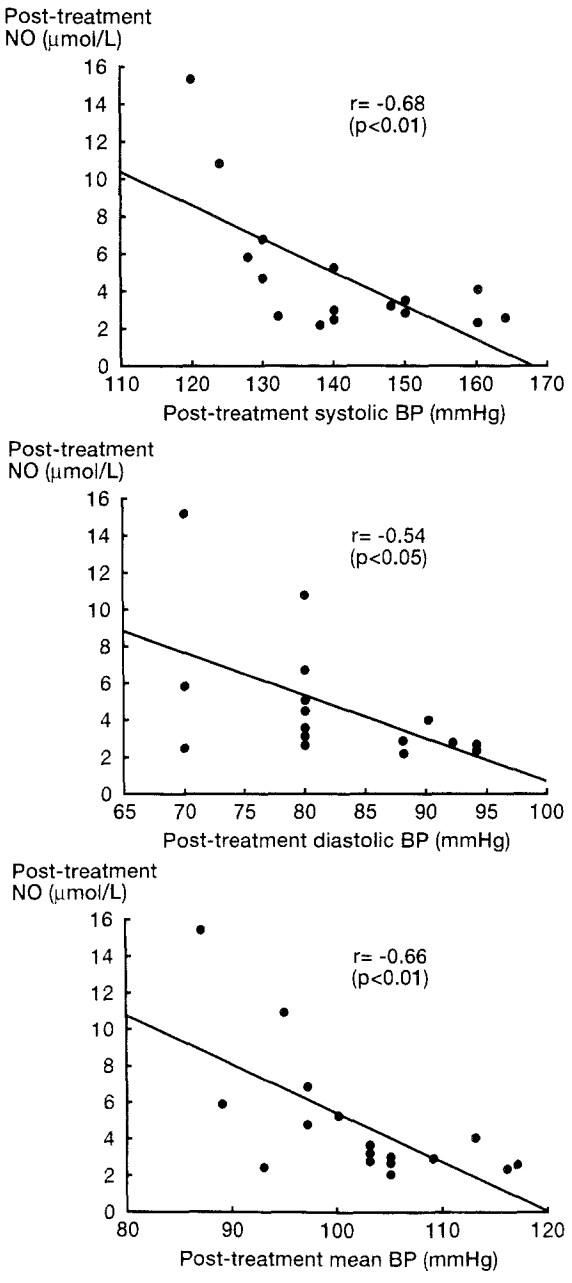

Fig 1. Correlation between the posttreatment NO level and posttreatment systolic, diastolic, and mean blood pressure.

Figure 1 shows the correlation between the posttreatment NO level and blood pressure. Posttreatment NO was inversely correlated with posttreatment systolic, diastolic, and mean blood pressure.

Figure 2 shows the correlation between the posttreatment plasma bradykinin level and blood pressure. The posttreatment bradykinin level was modestly correlated with posttreatment systolic and mean blood pressure, but was not correlated with posttreatment diastolic blood pressure.

The posttreatment plasma cGMP level was not correlated with posttreatment systolic, diastolic, or mean blood pressure

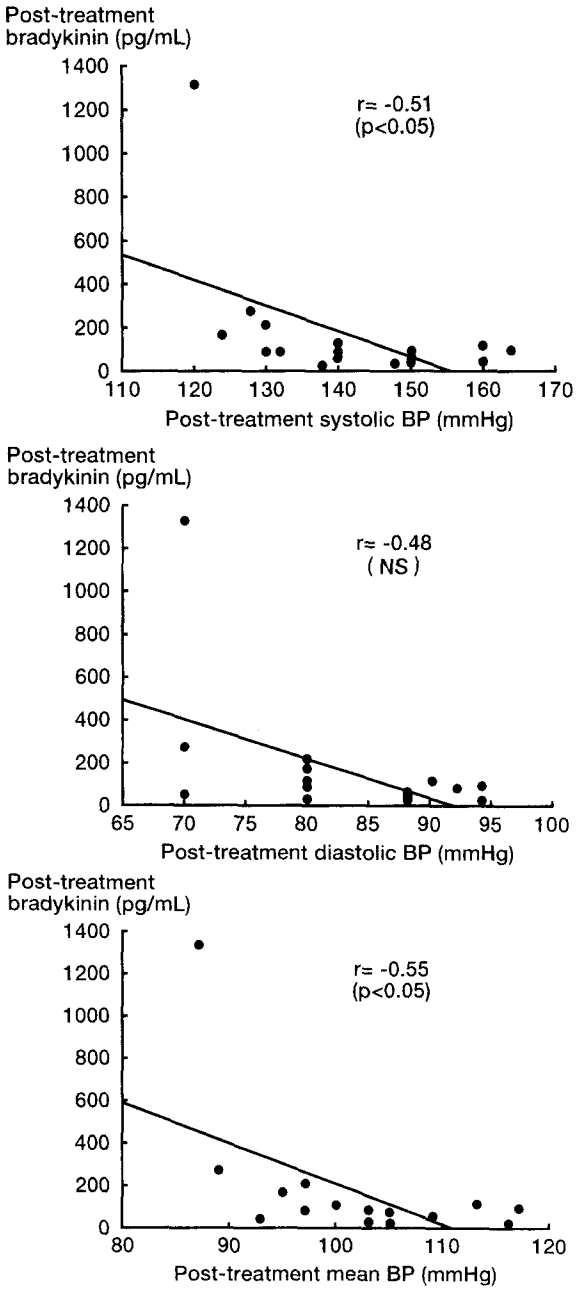

Fig 2. Correlation between the posttreatment bradykinin level and posttreatment systolic, diastolic, and mean blood pressure. NS, not significant.

(n = 17, $r = .03$, $P = .91$, n = 17, $r = -.21$, $P = .42$, and n = 17, $r = -.13$, $P = .63$, respectively). Plasma 6-keto $PGF_{1\alpha}$ also was not correlated with posttreatment systolic, diastolic, or mean blood pressure (n = 17, $r = -.24$, $P = .36$, n = 17, $r = -.01$, $P = .96$, and n = 17, $r = -.13$, $P = .61$, respectively).

DISCUSSION

The present study shows that a 4-week treatment with the ACE inhibitor lisinopril effectively decreased systolic and diastolic blood pressure and significantly increased plasma NO in our hypertensive patients. Furthermore, the posttreatment NO level was inversely correlated with posttreatment systolic, diastolic, and mean blood pressure. These findings raise the possibility that increased NO production may contribute, in part, to the hypotensive effect of lisinopril. However, cGMP levels were not significantly increased by the 4-week treatment with lisinopril. In cultured endothelial cells, the NO formed by an ACE inhibitor has been shown to increase intracellular cGMP.[10] This increase developed slowly and reached a plateau after 10 minutes and was stable for at least 30 minutes in vitro.[10] The reasons for the differences between our in vivo findings and those of in vitro studies are not entirely clear at present. One possibility is that the plasma cGMP level may be affected by mechanisms other than those related to NO. For instance, natriuretic peptides, such as atrial and brain natriuretic peptides, also may affect the plasma cGMP level.[22] Another possibility is that the elevated cGMP might return to near-baseline after a 4-week treatment with lisinopril, although we have no direct evidence.

The present study also shows that a 4-week treatment with lisinopril tended to increase the plasma bradykinin level. Previously, plasma kinins were found to be increased after ACE inhibition in normotensive healthy subjects and hypertensive patients.[3,4] We also showed that 6-keto $PGF_{1\alpha}$ is increased after 4-week lisinopril treatment. Since ACE is identical to the kinase II of the kallikrein-kinin system that inactivates bradykinin,[11] our findings suggest that increased bradykinin formation resulting from ACE inhibition may lead to a sustained stimulation of prostacyclin in our hypertensive patients. However, this study did not clearly establish the origin of the elevated plasma levels of bradykinin and prostacyclin or of NO.

A modest and inverse correlation was observed between the posttreatment bradykinin level and posttreatment systolic and mean blood pressure. Previous reports[23,24] indicated that endogenous bradykinin plays an important role in blood pressure control by attenuating the pressor effect of various vasoconstrictors such as Ang II in rats. Furthermore, Bao et al[25] demonstrated that a potentiation of endogenous bradykinin contributes to the chronic antihypertensive action of ACE inhibitors in renovascular hypertension. Together with these findings, our results raise the possibility that endogenous bradykinin also contributes, in part, to the hypotensive effect of ACE inhibition. However, since the correlation was modest, this possibility should be reexamined in a larger trial.

On the other hand, the posttreatment 6-keto $PGF_{1\alpha}$ level was not correlated with blood pressure levels. This suggests that prostacyclin formed by an ACE inhibitor may not play an important role in the hypotensive effect of ACE inhibition in our hypertensive patients.

In conclusion, treatment of hypertensive patients with lisinopril for 4 weeks was accompanied by significant increases in plasma NO. Furthermore, posttreatment NO values were inversely correlated with posttreatment systolic, diastolic, and mean blood pressure. These findings suggest that the elevated plasma NO levels induced by ACE inhibition contributed, in part, to the hypotensive effect of the ACE inhibitor in our hypertensive patients.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Atsumi Ohnishi, Yuka Inoshita, and Kazuko Shinagawa (Division of Hypertension and Atherosclerosis, First Department of Internal Medicine, Osaka City University Medical School).

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