Long-Term Treatment With Imidapril but Not With Nifedipine Enhances Plasma NOx Concentration in Patients With Essential Hypertension

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Abstract. We investigated whether long-term treatment with the angiotensin-converting enzyme (ACE) inhibitor imidapril or the calcium channel antagonist nifedipine increases systemic nitric oxide (NO) production in patients with essential hypertension. Twenty-nine patients with essential hypertension were randomly divided into two groups, and they were treated with either imidapril or nifedipine once daily p.o. for 4 weeks. Long-term treatment with imidapril significantly decreased blood pressure and increased plasma NOx concentration. Long-term treatment with nifedipine also caused a comparable extent of significant decrease in blood pressure, but failed to alter plasma NOx levels. The imidapril treatment significantly inhibited serum ACE activity and increased plasma bradykinin concentration. Furthermore, the extent of inhibition of serum ACE activity and the extent of increase in plasma bradykinin concentration in response to the imidapril treatment were both significantly correlated with the extent of increase in plasma NOx concentration. In contrast, no such changes were noted after the nifedipine treatment. These results provide the first evidence that long-term treatment with imidapril enhances plasma NOx concentration in patients with essential hypertension. This effect does not seem to be due to the decrease in blood pressure. The increase in bradykinin concentration may be involved in the enhancing effect of the ACE inhibitor on NOx production in vivo.

Keywords: angiotensin-converting enzyme inhibitor, calcium channel antagonist, human, hypertension, nitric oxide

Introduction

Nitric oxide (NO) has multiple important actions that contribute to the maintenance of body homeostasis (1–3). Endothelium-derived NO is constitutively synthesized from L-arginine by endothelial NO synthase (eNOS). It has been revealed that transgenic mice overexpressing the eNOS gene manifest hypotension (4), and mice deficient in the eNOS gene exhibit hypertension (5). Furthermore, it has been shown that both endothelium-dependent NO-mediated vascular responses (6) and whole-body NO production (7) are impaired in patients with essential hypertension. Thus, reduced NO production is closely associated with essential hypertension (8, 9).

Hypertension is a critical risk factor for arteriosclerotic cardiovascular diseases such as coronary artery disease, stroke, and heart failure. Since NO plays not only an anti-hypertensive role, but also an important protective role against hypertensive vascular compli-
cations (i.e., arteriosclerosis) (10), the restoration of reduced NO production in hypertensive subjects by pharmacological agents is of particular interest. Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension. Blockade of the renin-angiotensin system with ACE inhibitors has also been proved to be effective in reducing the risk of major arteriosclerotic cardiovascular events (11). Although the precise mechanisms of such beneficial actions of ACE inhibitors remain to be completely understood, their enhancing effect on NO production may be involved. Indeed, three previous studies suggested a potential ACE inhibitor-induced improvement in plasma nitrite and nitrate (NOx) concentration, a marker of systemic NO production, in patients with essential hypertension. However, one of the three studies examined only a small number of patients (n = 7) (12), and the remaining two studies did not assign a comparative group (13, 14). Thus, it still remains to be fully elucidated whether or not long-term treatment with ACE inhibitors increases systemic NO production in hypertensive individuals.

To address this point, we investigated the effect of a long-term blockade of ACE with imidapril (a long-acting ACE inhibitor with the lowest incidence of an adverse effect of dry cough) (15) on plasma NOx concentration in patients with essential hypertension, and that effect was compared with the effect of long-term treatment with nifedipine (a long-acting calcium channel inhibitor).

Materials and Methods

Study subjects

Twenty-nine patients with mild to moderate essential hypertension were studied. Blood pressure was measured by the Korotkov method at 10 min after quiet sitting before the blood sampling. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. None of the patients had histories of cerebrovascular, cardiovascular, renal, or liver diseases or diabetes mellitus. Patients with secondary forms of hypertension were excluded on the basis of clinical history and physical examination. Before the study, no patient received nitrates, ACE inhibitors, or angiotensin II type-1 receptor blockers that might affect NO metabolism. The present study was reviewed and approved by the Human Research Committee at the University of Occupational and Environmental Health, School of Medicine, Japan, and it was performed according to the Institutional Guidelines. Informed consent for participation was obtained from all of the subjects.

Study protocol

The patients were randomly assigned into 2 groups and were treated with either imidapril hydrochloride (5 mg/day; Tanabe Pharmaceutical Co., Ltd., Tokyo) or nifedipine (20 mg/day; Bayer Yakuhin, Ltd., Osaka) once daily p.o. for 4 weeks. The blood pressure measurement and the blood sampling were carried out before and 4 weeks after the treatment.

Measurement

In order to minimize the influence of foods and beverages on plasma NOx concentration, we instructed our patients to fast overnight, and we collected the blood in the morning under fasting conditions in all the patients studied. Vacuum tubes with and without sodium EDTA were used for obtaining the plasma and the serum, respectively. The blood samples were immediately centrifuged at 3,000 rpm, 4°C for 15 min, and the supernatants were stored at −80°C. Plasma NOx concentration were assessed by the Griess method, as we previously reported (16, 17). Serum ACE activity was measured by the Kasahara method (18). Plasma bradykinin concentration was determined by radio-immunoassay (19). Plasma concentration of asymmetric dimethylarginine (ADMA) was assayed by high performance liquid chromatography (20). Plasma biochemical data were analyzed with a SMAC autoanalyzer (Technicon Instruments Corporation, New York, NY, USA).

NOx might exist in the air or water. To avoid contamination of such NOx in our NOx measurement, we minimized the time of exposure of blood samples to the air and used ultrapure water purified with the Milli-Q system (Millipore Corporation, Billerica, MA, USA) when all solutions were prepared (21). NOx from laboratory ware is also a major source of NOx contamination (22). Indeed, when we substituted the plasma samples by freshly prepared ultrapure water purified with the Milli-Q system throughout whole procedure from blood sampling to NOx quantification, we detected a low level of NOx (6.8 ± 0.7 µmol/L, n = 8). Importantly, variation (S.E.M.) of NOx level in the ultrapure water was significantly smaller than the difference in the NOx data in Fig. 2A (P < 0.01, by an unpaired t-test), indicating the reliability of our results. On the other hand, there was no significant difference in NOx levels among the freshly prepared ultrapure water and ultrapure water stored for 1 week (6.7 ± 0.7 µmol/L), 2 weeks (8.5 ± 0.8 µmol/L), and 3 weeks (6.6 ± 0.8 µmol/L) (n = 8 each), suggesting little influence of different storage periods or different occasion on our NOx assay. We measured NOx levels of all the samples at one time during the same day.
Statistical analyses

Results are expressed as the mean value ± S.E.M. Throughout the text, n means the number of patients. Statistical analysis was performed by a paired t-test, an unpaired t-test, a chi-square test, regression analysis (least squares linear estimation), or repeated measure ANOVA. When data within a group were compared, a paired t-test was used; and when data between two groups before and after different treatments were analyzed, repeated measure ANOVA followed by Scheffe’s post-hoc test was employed. The values were considered to be statistically significant when P was less than 0.05.

Results

Patient characteristics

Baseline patient characteristics, including age, gender, blood pressure, heart rate, and blood biochemical data were comparable between the imidapril and the nifedipine groups (Table 1). Plasma concentration of NOx, bradykinin, and ADMA (an endogenous NO synthase inhibitor) and serum ACE activity were also comparable between the two groups before the study (Table 1).

Effect of treatment with imidapril or nifedipine on blood pressure

Long-term treatment with imidapril significantly decreased systolic, diastolic, and mean blood pressure in the patients with essential hypertension (all P<0.05, n = 15) (Fig. 1). Long-term treatment with nifedipine also caused a significant decrease in systolic, diastolic, and mean blood pressure in the patients with essential hypertension (all P<0.05, n = 14) (Fig. 1). The antihypertensive effect of imidapril was similar to that of nifedipine (Fig. 1).

Effect of treatment with imidapril or nifedipine on plasma NOx level

Long-term treatment with imidapril significantly increased plasma NOx concentration in the patients with essential hypertension (P<0.05) (Fig. 2A). In contrast, long-term treatment with nifedipine did not significantly affect plasma NOx levels in the patients (Fig. 2A). The plasma NOx level in the imidapril-treated group after 4 weeks was significantly higher than that in the nifedipine-treated group (P<0.05) (Fig. 2A).

Effect of treatment with imidapril or nifedipine on plasma ADMA level

Long-term treatment with imidapril (0.52 ± 0.03 to 0.51 ± 0.02 µmol/L) or nifedipine (0.47 ± 0.02 to 0.47 ± 0.03 µmol/L) did not significantly change plasma concentration of ADMA.

Effect of treatment with imidapril or nifedipine on serum ACE activity and plasma bradykinin level

Long-term treatment with imidapril significantly inhibited serum ACE activity (P<0.01) (Fig. 2B) and increased plasma bradykinin concentration (P<0.01) (Fig. 2C). Both levels were significantly different.

Table 1. Clinical characteristics of the study patients

<table>
<thead>
<tr>
<th></th>
<th>Imidapril (n = 15)</th>
<th>Nifedipine (n = 14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 ± 3</td>
<td>60 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Male/Female</td>
<td>10/5</td>
<td>8/6</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>163 ± 5</td>
<td>163 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>89 ± 3</td>
<td>92 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>114 ± 3</td>
<td>116 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/minutes)</td>
<td>71 ± 3</td>
<td>73 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192 ± 29</td>
<td>215 ± 34</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>35 ± 10</td>
<td>48 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>146 ± 52</td>
<td>114 ± 56</td>
<td>NS</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>16 ± 1</td>
<td>15 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma NOx (µmol/L)</td>
<td>59 ± 8</td>
<td>43 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ACE activity (IU/L)</td>
<td>12 ± 1</td>
<td>14 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma bradykinin (ng/ml)</td>
<td>13 ± 5</td>
<td>13 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma ADMA (µmol/L)</td>
<td>0.52 ± 0.03</td>
<td>0.47 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M. HDL indicates high-density lipoprotein; ACE, angiotensin-converting enzyme; ADMA, asymmetric dimethylarginine; NOx, nitrite plus nitrate; n, number of patients; NS, not significant.
between imidapril- and nifedipine-treated groups at 4 weeks of the treatment. After long-term treatment with imidapril, the extent of inhibition of serum ACE activity ($P<0.05$) (Fig. 3A) and the extent of increase in plasma bradykinin concentration ($P<0.05$) (Fig. 3B) were both significantly correlated with the extent of increase in plasma NOx concentration. In contrast, no such significant changes were noted after long-term treatment with nifedipine (Fig. 2: B and C).

Discussion

The novel findings of the present study are that long-term treatment with the ACE inhibitor imidapril, but not with the calcium channel antagonist nifedipine, enhanced plasma NOx concentration in patients with essential hypertension and that this action was associated with an inhibition of serum ACE activity and an increase in plasma bradykinin levels. To the best of our knowledge, this is the first clinical demonstration of the stimulatory effect of imidapril on systemic NOx production in patients with essential hypertension.

NOx as a marker of NO formation in vivo

Because NOx was widely used as a marker of systemic NO production in the human body, we used it...
in this study (23). Besides the present study, we also previously demonstrated that long-term treatment with simvastatin, a HMG-CoA reductase inhibitor, significantly enhances plasma NOx concentration in patients with hypercholesterolemia (16) and that plasma NOx level is inversely correlated with plasma level of low-density lipoprotein cholesterol in humans (24). However, whether NOx is a reliable marker of NO formation in vivo is still controversial, and indeed there are conflicting facts regarding plasma NOx level in hypertensive individuals: two studies reported that plasma NOx concentrations were significantly reduced in patients with essential hypertension compared to those in normotensive healthy subjects (12, 25) whereas one study showed that plasma NOx levels were similar in refractory hypertensive patients and normotensive healthy subjects (26). In addition, it has been suggested that NO$_2^-$, but not NOx, is a useful marker for assessing NO production (22, 27). However, we have recently demonstrated that plasma NOx concentration as well as urinary NOx excretion are significantly lower in accordance with the number of disrupted NOS isoforms in the order of singly, doubly, and triply NOS$^{-/-}$ mice (28). In agreement with our findings, previous studies also revealed that plasma NOx concentration is significantly decreased after long-term pharmacological inhibition of NOS activity in rats (29). Furthermore, significantly higher levels of plasma NOx concentration in rats after long-term treatment with L-arginine, a precursor of NO synthesis (30), and in mice overexpressing eNOS gene (4) have been reported. These results suggest that NOx can be of use as a marker of systemic NO production to some extent. At least, when long-term response, but not short-term response, to therapy is monitored in individual patients, NOx is thought to be reliable as a marker of NO formation in vivo (22, 23).

No causal role of blood pressure on imidapril-induced NOx production

Treatment with imidapril decreased arterial blood pressure and increased plasma NOx concentration in the patients with essential hypertension. Treatment with nifedipine also elicited a comparable extent of an antihypertensive effect, but had no effect on plasma NOx concentration. These results suggest that an increase in plasma NOx concentration induced by the imidapril treatment was not caused by changes in arterial blood pressure. Consistent with our findings, it has been shown that long-term treatment with an ACE inhibitor, but not with a calcium channel antagonist, improves impaired endothelium-dependent NO-mediated renal vascular relaxation in patients with essential hypertension, independent of its effect in lowering blood pressure (31).

No involvement of ADMA in imidapril-induced NOx production

Since ADMA is thought to be an important endo-
genous inhibitor of NO synthase in humans (32, 33), we examined plasma ADMA concentration in our hypertensive patients. However, plasma ADMA levels were unchanged after long-term treatment with imidapril, suggesting that ADMA is not involved in the enhancing effect of imidapril on plasma NOx concentration.

Involvement of ACE and bradykinin in imidapril-induced NOx production

ACE, which is identical to the kininase II of the kallikrein-kinin system, not only converts angiotensin I to angiotensin II, but also degrades bradykinin into inactive peptide (34). Inhibition of ACE by its inhibitors, therefore, leads to an increase in local and circulating levels of bradykinin (35). Indeed, in this study, after long-term treatment with imidapril, a significant increase in plasma bradykinin concentration was observed, concomitant with suppression of serum ACE activity. Bradykinin can stimulate the vascular endothelium to release NO via the endothelial bradykinin B2 receptor (36), and endothelium-dependent vasodilatation by ACE inhibitors is shown to be related to an increase in vascular bradykinin levels in humans (37, 38). In this study, following the imidapril treatment, changes in serum ACE activity and plasma bradykinin concentration were significantly linked to those in plasma NOx concentration. Thus, it is conceivable that the enhancing effect of imidapril on plasma NOx concentration was mediated by increased bradykinin concentration in our hypertensive subjects.

Angiotensin II stimulates NADH/NADPH oxidase in vascular smooth muscle cells via the AT1 receptor and causes vascular superoxide generation (39). Superoxide, in turn, inactivates endothelium-derived NO (40). Thus, the mechanism for the protection against superoxide-induced inactivation of endothelium-derived NO by imidapril may also be involved in the enhancing effect of imidapril on plasma NOx concentration.

Beneficial effects of ACE inhibitors through NO production

ACE inhibitors are effective in the treatment of hypertension and heart failure (41). Recent large clinical trials have revealed that ACE inhibitors are also effective for reducing coronary artery disease, stroke, renal disease, and overall mortality (41). NO is synthesized in almost all tissues and organs; and it exerts multiple beneficial actions, including a vascular relaxant action, an anti-atherogenic action, an anti-thrombotic action, and an anti-oxidant action, under both physiological and pathological conditions (1–3). We have recently indicated that NO does indeed elicit such important actions in vivo, using mice with complete deletion of the NO synthase system that we have recently developed (28, 42). From these points of view, the improvement of plasma NOx concentration by imidapril observed in this study must have important clinical significance. Thus, the present findings could explain, at least in part, why ACE inhibitors are beneficial for the prevention and treatment of a variety of cardiovascular disorders.

Conclusion

In summary, we were able to demonstrate that a long-term blockade of ACE with imidapril augments systemic NO synthesis in patients with essential hypertension. Inhibition of ACE and increased bradykinin appear to be involved in the enhancing effect of imidapril on NO production. Our findings should contribute to a better understanding of the beneficial cardiovascular effects of the ACE inhibitor.

Acknowledgments

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