Hypertension

Effect of the Angiotensin-Converting Enzyme Inhibitor Imidapril on Reactive Hyperemia in Patients With Essential Hypertension: Relationship Between Treatment Periods and Resistance Artery Endothelial Function

Yukihito Higashi, MD, PhD,* Shota Sasaki, MD,* Keigo Nakagawa, MD,* Hideo Matsuura, MD, PhD,*
Goro Kajiyama, MD, PhD,* Tetsuya Oshima, PhD†

Hiroshima, Japan

OBJECTIVES   The purpose of this study was to evaluate the effects of the angiotensin-converting enzyme (ACE) inhibitor imidapril and the calcium antagonist amlodipine on endothelial function before and after 2, 4, 8, 12, 24 and 48 weeks of treatment.

BACKGROUND   There are limited data on whether and how long endothelial function is improved after initiation of ACE inhibitor treatment and how the grade of endothelial function further progresses after improvement of endothelial dysfunction in patients with essential hypertension.

METHODS   The forearm blood flow (FBF) was measured in 25 patients with essential hypertension and in 25 normotensive subjects by using strain-gauge plethysmography during reactive hyperemia (RH) (280 mm Hg for 5 min) and after sublingual administration of nitroglycerin (NTG, 0.3 mg).

RESULTS   The FBF of patients with essential hypertension during RH was significantly less than that of normotensive subjects. The increase in FBF after sublingual NTG was similar in both groups. Both imidapril (n = 13) and amlodipine (n = 12) significantly reduced systolic blood pressure and diastolic after eight weeks of treatment from the pretreatment values. Forearm vascular resistance was significantly decreased after two weeks of treatment. Imidapril significantly augmented RH after 12 weeks of treatment from the pretreatment values (31.6 ± 5.7 to 38.2 ± 6.0 ml/min per 100 ml tissue, p < 0.05), whereas amlodipine did not alter RH for each treatment period. The ability of imidapril to improve RH was maintained throughout the 48-week treatment period. There was no significant difference in RH at 12, 24 and 48 weeks. The increase in FBF after sublingual administration of NTG was similar in all treatment periods for the two groups. The infusion of N^G-monomethyl-L-arginine, a nitric oxide (NO) synthase inhibitor, abolished the enhancement of RH in hypertensive patients treated with imidapril.

CONCLUSIONS   These findings suggest that the ACE inhibitor imidapril augments RH after 12 weeks of treatment in patients with essential hypertension and that this ACE inhibitor-induced augmentation of RH may be due to an increase in NO. (J Am Coll Cardiol 2001;37:863–70) © 2001 by the American College of Cardiology

Endothelium plays an important role in the regulation of vascular tone by producing various vasoactive factors, including vasodilating substances such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) and vasoconstricting substances such as endothelin, angiotensin II, thromboxane A_2 and prostaglandin H_2 (1–4). The endothelium is both a target of and a contributor to hypertension. Hypertension is associated with alterations in the resistance vessels' endothelial function. We and other investigators have reported that endothelial function is impaired in patients with essential hypertension (5–10).

Several lines of evidence have shown that the impaired endothelial function of brachial (11), renal (12) and small arteries (13) of patients with essential hypertension can be restored by long-term treatment with angiotensin-converting enzyme (ACE) inhibitors. Recently, we also have shown that a 12-week treatment period with the ACE inhibitor imidapril improves endothelium-dependent renovascular relaxation in patients with essential hypertension (14,15). These findings are supported by the results of animal studies using ACE inhibitors (16–18). These ben-

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Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
BP	=	blood pressure
EDHF	=	endothelium-dependent hyperpolarizing factor
FBF	=	forearm blood flow
FVR	=	forearm vascular resistance
L-NMMA	=	N^G-monomethyl-L-arginine
NO	=	nitric oxide
NTG	=	nitroglycerin
RH	=	reactive hyperemia

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eficial effects of ACE inhibitors on endothelial function are thought to increase NO activity through the inhibition of angiotensin II–mediated production of superoxide, which inactivates NO (19), along with inhibition of bradykinin degradation, which mediates NO production (20). Creager et al. (21), however, reported that antihypertensive treatment for up seven to eight weeks with ACE inhibitors, such as captopril and enalapril, did not augment endothelium-dependent vasodilation in the forearm circulation of hypertensive patients. There is little information on whether and how long endothelial function is improved after initiation of ACE inhibitor treatment and how the grade of endothelial function further progresses after improvement of endothelial dysfunction in patients with essential hypertension.

Thus, we evaluated the effects of the ACE inhibitor imidapril and the calcium antagonist amlodipine on endothelial function before and after 2, 4, 8, 12, 24 and 48 weeks of treatment in patients with essential hypertension. For this purpose, we measured the responses of forearm blood flow (FBF) to reactive hyperemia (RH), an index of endothelium-dependent vasodilation, and sublingually administered nitroglycerin (NTG), an index of endothelium-independent vasodilation.

METHODS

Subjects. We studied 25 Japanese patients with essential hypertension (15 men and 10 women; mean age 53 ± 10 years) and 25 normotensive subjects (14 men and 11 women; mean age 48 ± 12 years). Hypertension was defined as systolic blood pressure (BP) >160 mm Hg and/or diastolic BP >95 mm Hg, in a sitting position, on at least three different occasions. Patients with secondary forms of hypertension were excluded on the basis of a complete history, physical examination, radiologic and ultrasound examinations, urinalysis, plasma renin activity, concentrations of plasma aldosterone, norepinephrine, serum creatinine, potassium, calcium and free thyroxine and 24-h urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids and vanillylmandelic acid. None of the patients had a history of cardiovascular or cerebrovascular disease, diabetes mellitus, liver disease or renal disease. Normotension was defined as systolic BP <130 mm Hg and

diastolic BP <80 mm Hg. The normotensive control subjects had no history of serious disease and took no medications for at least four weeks before the study. The study protocol was approved by the Ethics Committee of the First Department of Internal Medicine of Hiroshima University. Written, informed consent for participation was obtained from all subjects.

Measurement of FBF. Forearm blood flow was measured using a mercury-filled, strain-gauge Silastic plethysmograph (EC-5R, D. E. Hokanson, Inc., Issaquah, Washington), as previously described (5,8,9). Briefly, a strain gauge was attached to the upper part of the left arm and connected to a plethysmograhy device and was supported above the right atrium. A wrist cuff was inflated to 50 mm Hg above the systolic BP to exclude the hand circulation from the measurements taken 1 min before measurement of FBF. The upper arm–congesting cuff was inflated to 40 mm Hg for 7 s in each a 15-s cycle to occlude venous outflow from the arm by using a rapid cuff inflator (EC-20, D. E. Hokanson, Inc., Issaquah, Washington). The FBF output signal was transmitted to a recorder (U-228, Advance Co., Nagoya, Japan). Forearm blood flow was expressed as ml/min per 100 ml of tissue of forearm volume. Forearm vascular resistance (FVR) was calculated as the mean BP divided by FBF and was expressed as a mm Hg/ml/min per 100 ml of tissue of forearm volume.

Study protocol. None of the patients had a history of antihypertensive treatment before the study. After a four-week run-in period, first, the pretreatment values of patients with essential hypertension were compared to those of normotensive control subjects. Active treatment was then followed for 48 weeks, and the time courses of the effects of imidapril and amlodipine were evaluated. Patients were treated with single daily doses of the ACE inhibitor imidapril (Tanabe Pharmaceutical Co., Osaka, Japan), 5 mg, or the calcium antagonist amlodipine (Pfizer Pharmaceutical Co., Tokyo, Japan), 5 mg, in the morning during the 48-week active treatment period. The patients were randomly assigned to the imidapril group (n = 13 [7 men and 6 women]; mean age 53 ± 9 years) or the amlodipine group (n = 12 [8 men and 4 women]; mean age 54 ± 11 years).

Measurements of FBF were performed at the beginning of treatment (0 weeks) and after 2, 4, 8, 12, 24 and 48 weeks of treatment. The study began at 8:30 AM. Subjects fasted the previous night for at least 12 h. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature 22°C to 25°C) throughout the study. Thirty minutes after maintaining the supine position, basal FBF was measured. Then, the effects of RH and sublingual NTG on FBF were measured. To obtain RH, FBF was occluded by inflating the cuff on the left upper arm at a pressure of 280 mm Hg for 5 min. After the release of ischemic cuff occlusion, FBF was measured for 3 min. Nitroglycerin was sublingually administered at the dose of 0.3 mg by one tablet (Nihonkayaku Co., Tokyo, Japan).

Table 1. Clinical Characteristics in Normotensive Subjects and Patients With Essential Hypertension Treated With the Angiotensin-Converting Enzyme Inhibitor Imidapril and the Calcium Antagonist Amlodipine Before (0 Weeks) and After 2, 4, 8, 12, 24 and 48 Weeks

Variable	Normotensive Subjects (n = 25)	Before Treatment (0 Weeks) Imidapril (n = 13)	Amlodipine (n = 12)	After 2 Weeks Imidapril (n = 8)	Amlodipine (n = 10)	After 4 Weeks Imidapril (n = 13)	Amlodipine (n = 12)
Body mass index (kg/m^2)	23.8 ± 2.8	24.1 ± 2.7	24.3 ± 2.6	25.0 ± 3.1	24.2 ± 2.9	24.1 ± 2.7	24.2 ± 2.5
Systolic blood pressure (mm Hg)	116.4 ± 10.2	168.1 ± 11.5*	170.3 ± 12.6*	150.8 ± 10.2*†	144.6 ± 10.5*†	146.8 ± 9.7*†	143.5 ± 9.5*†
Diastolic blood pressure (mm Hg)	69.1 ± 8.8	98.8 ± 8.9*	96.6 ± 10.8*	96.6 ± 8.7*	87.4 ± 8.1*†‡	94.4 ± 8.6*	86.1 ± 8.5*†‡
Heart rate (beats/min)	68.4 ± 8.1	69.3 ± 7.3	66.7 ± 8.6	70.2 ± 7.8	71.2 ± 8.9	71.0 ± 8.0	72.4 ± 8.8
Total cholesterol (mmol/liter)	4.83 ± 0.71	5.11 ± 0.80	5.04 ± 0.78	5.08 ± 0.78	5.01 ± 0.85	5.01 ± 0.74	4.98 ± 0.86
Triglycerides (mmol/liter)	1.13 ± 0.41	1.20 ± 0.48	1.17 ± 0.50	1.22 ± 0.47	1.14 ± 0.39	1.18 ± 0.42	1.14 ± 0.46
HDL cholesterol (mmol/liter)	1.47 ± 0.33	1.45 ± 0.43	1.44 ± 0.39	1.46 ± 0.44	1.45 ± 0.36	1.46 ± 0.43	1.44 ± 0.42
LDL cholesterol (mmol/liter)	3.12 ± 0.58	3.42 ± 0.71	3.35 ± 0.68	3.37 ± 0.69	3.33 ± 0.64	3.31 ± 0.65	3.30 ± 0.69
Serum glucose (mmol/liter)	4.8 ± 0.5	4.9 ± 0.4	4.8 ± 0.4	4.8 ± 0.3	4.8 ± 0.5	4.9 ± 0.5	4.8 ± 0.4
FBF (ml/min per 100 ml tissue)	4.5 ± 1.2	4.4 ± 1.3	4.6 ± 1.4	4.5 ± 1.4	4.5 ± 1.3	4.4 ± 1.4	4.5 ± 1.4
FVR (mm Hg/ml/min per 100 ml tissue)	18.8 ± 3.7	27.7 ± 4.2*	26.4 ± 4.1*	25.4 ± 3.8*†	23.7 ± 3.6*†	25.3 ± 3.9*†	23.4 ± 3.5*†
Smoker (n)	3	3	2	1	2	3	2

Variable	8 Weeks Imidapril (n = 13)	Amlodipine (n = 11)	After 12 Weeks Imidapril (n = 13)	Amlodipine (n = 12)	After 24 Weeks Imidapril (n = 10)	Amlodipine (n = 12)	After 48 Weeks Imidapril (n = 8)	Amlodipine (n = 10)
Body mass index (kg/m^2)	24.0 ± 2.7	24.4 ± 2.6	24.0 ± 2.7	24.1 ± 2.5	24.5 ± 3.0	24.2 ± 2.6	24.4 ± 2.9	24.6 ± 2.7
Systolic blood pressure (mm Hg)	144.3 ± 9.8*†	142.6 ± 10.2*†	146.1 ± 10.1*†	143.3 ± 10.3*†	146.3 ± 10.5*†	143.1 ± 10.2*†	148.8 ± 11.2*†	142.9 ± 11.1*†
Diastolic blood pressure (mm Hg)	88.2 ± 8.4*†	85.2 ± 9.4*†	89.0 ± 8.8*†	86.1 ± 8.9*†	88.0 ± 8.9*†	82.8 ± 9.0*†	90.2 ± 8.7*†	84.2 ± 9.5*†
Heart rate (beats/min)	68.1 ± 7.9	70.5 ± 8.9	70.3 ± 7.8	69.8 ± 7.9	71.1 ± 8.2	68.7 ± 8.0	69.8 ± 7.9	67.9 ± 8.4
Total cholesterol (mmol/liter)	5.06 ± 0.78	5.03 ± 0.84	5.10 ± 0.82	4.99 ± 0.87	5.06 ± 0.76	4.96 ± 0.83	5.09 ± 0.81	5.02 ± 0.90
Triglycerides (mmol/liter)	1.19 ± 0.44	1.21 ± 0.51	1.10 ± 0.40	1.20 ± 0.52	1.17 ± 0.48	1.22 ± 0.53	1.18 ± 0.48	1.21 ± 0.54
HDL cholesterol (mmol/liter)	1.44 ± 0.39	1.42 ± 0.53	1.42 ± 0.38	1.40 ± 0.48	1.44 ± 0.40	1.41 ± 0.46	1.43 ± 0.41	1.42 ± 0.49
LDL cholesterol (mmol/liter)	3.40 ± 0.70	3.39 ± 0.72	3.46 ± 0.74	3.35 ± 0.76	3.39 ± 0.70	3.31 ± 0.74	3.42 ± 0.72	3.36 ± 0.78
Serum glucose (mmol/liter)	4.7 ± 0.3	4.9 ± 0.5	4.6 ± 0.4	4.8 ± 0.4	4.8 ± 0.3	4.7 ± 0.5	5.0 ± 0.5	4.9 ± 0.4
FBF (ml/min per 100 ml tissue)	4.3 ± 1.3	4.4 ± 1.3	4.4 ± 1.3	4.5 ± 1.4	4.4 ± 1.2	4.4 ± 1.3	4.5 ± 1.4	4.6 ± 1.3
FVR (mm Hg/ml/min per 100 ml tissue)	24.8 ± 4.1*†	24.2 ± 4.4*	24.5 ± 3.8*	23.4 ± 3.9*†	24.4 ± 4.0*†	23.9 ± 3.8*†	24.3 ± 4.1*†	22.8 ± 4.2*†
Smoker (n)	3	2	3	2	1	2	1	2

*$p < 0.05$ versus normotensive subjects. †$p < 0.5$ versus hyperpertensive patients before treatment (0 weeks). ‡$p < 0.05$ versus imidapril (same weeks). All data are presented as the mean value ± SD.

FBF = forearm blood flow; FVR = forearm vascular resistance; HDL = high density lipoprotein; LDL = low density lipoprotein.

After administration of NTG, FBF was measured for 5 min. These studies were carried out in a randomized fashion. Each study proceeded after FBF had returned to baseline. In the preliminary study, after the release of cuff occlusion or sublingual NTG, FBF returned to baseline within 10 min. Thus, the end of the response to RH or sublingual NTG was followed by a 15-min recovery period. Baseline fasting serum concentrations of total cholesterol, high density lipoprotein cholesterol, triglycerides, creatinine, insulin, glucose and electrolytes, as well as plasma renin activity, were obtained after a 30-min rest period before the study.

To evaluate the effects of imidapril and amlodipine on NO release, we measured the FBF response to RH in the presence of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) (CLINALFA Co., Läufelfingen, Switzerland) in 6 of 13 hypertensive patients treated with imidapril and in 6 of 12 patients treated with amlodipine after 12 weeks of treatment. A 23-gauge polyethylene catheter (Hakkow Co., Yokayama, Japan) was inserted under local anesthesia (1% lidocaine) into the left brachial artery for infusion of L-NMMA. After maintaining the supine position for 30 min, we measured basal FBF. Then, the effect of RH on forearm hemodynamic data was measured. After a 15-min recovery period, L-NMMA was infused intra-arterially at a dose of 8 μmol/min for 5 min

before FBF measurement. We performed RH after initiation of a 5-min infusion of L-NMMA.

In the preliminary study, we evaluated the effects of oral administration of imidapril (5 mg/day for 12 weeks) on the circulating levels of ACE, angiotensin II and bradykinin in eight patients with essential hypertension (6 men and 2 women; mean age 49 ± 10 years). After 12 weeks of treatment, imidapril significantly decreased serum ACE activity from 13.1 ± 3.1 to 3.9 ± 1.1 IU/liter (p < 0.01) and the plasma angiotensin II level from 17.2 ± 8.3 to 4.1 ± 1.3 pg/ml (p < 0.01) and increased the plasma bradykinin level from 15.6 ± 4.8 to 24.9 ± 13.2 pg/ml (p < 0.05). We confirmed the reproducibility of RH and sublingual NTG-induced vasodilation twice each month in seven healthy male subjects (mean age 25 ± 4 years). The coefficients of variation were 4.1% and 2.5%, respectively.

Analytical methods. Samples of venous blood were placed in tubes containing EDTA-Na (1 mg/ml) and in polystyrene tubes. The EDTA-containing tubes were promptly chilled in an ice bath. Plasma was immediately separated by centrifugation at 3,100 rpm at 4°C for 10 min, and serum at 1,000 rpm (at room temperature) for 10 min. The samples were stored at −80°C until assayed. Routine chemical methods were used to determine serum concentrations of total cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, glucose and electrolytes. The serum concentration of low density lipoprotein cholesterol was estimated using Friedewald's method (22).

Statistical analysis. The results are presented as the mean value ± SD. Values of p < 0.05 were considered significant. The Mann-Whitney *U* test was used to evaluate the differences in variables at the baseline between the hypertensive patients and normotensive subjects. Comparisons of time course curves of variables during RH were analyzed by two-way analysis of variance (ANOVA) for repeated measures. The data were processed using either StatView IV (Brainpower) or Super ANOVA (Abacus Concepts).

RESULTS

Clinical characteristics of normotensive subjects and hypertensive patients. The baseline clinical characteristics of the 13 hypertensive patients treated with imidapril, 12 hypertensive patients treated with amlodipine and 25 normotensive subjects are shown in Table 1. The systolic and diastolic BPs, as well as FVR, were significantly higher in the hypertensive patients than in the normotensive subjects. These variables were similar between the imidapril and amlodipine groups. Body mass index, lipid metabolism, serum glucose and the prevalence of smoking were similar among the three groups.

Reactive hyperemia in normotensive subjects and hypertensive patients. The FBF of the patients with essential hypertension in response to RH, an index of endothelium-dependent vasorelaxation, was significantly smaller than
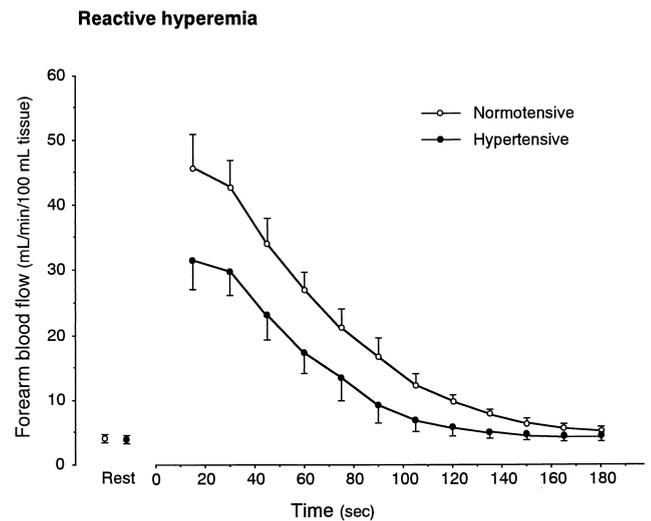

Figure 1. Line graphs show forearm blood flow at rest and during reactive hyperemia in patients with essential hypertension and in normotensive control subjects. Reactive hyperemia was impaired in hypertensive patients compared with normotensive subjects.

that of normotensive subjects, whereas the baseline FBF was similar between the two groups (Fig. 1).

The increase in the FBF after sublingual administration of NTG, an index of endothelium-independent vasodilation, was similar between the normotensive subjects and hypertensive patients (Fig. 2).

Effects of imidapril and amlodipine on clinical characteristics. The effects of imidapril and amlodipine on baseline variables are shown in Table 1. Imidapril significantly reduced systolic BP after two weeks of treatment and diastolic BP after eight weeks of treatment, compared with baseline values (0 weeks). Amlodipine significantly reduced systolic BP and diastolic BP after two weeks of treatment, compared with baseline values. The BP-lowering effects of
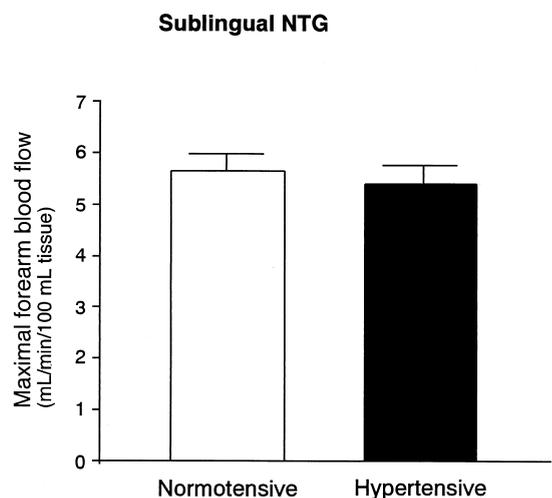

Figure 2. Bar graphs show maximal forearm blood flow after sublingual administration of nitroglycerin (NTG) in patients with essential hypertension and in normotensive control subjects. The NTG-induced vasodilation was similar between the two groups.
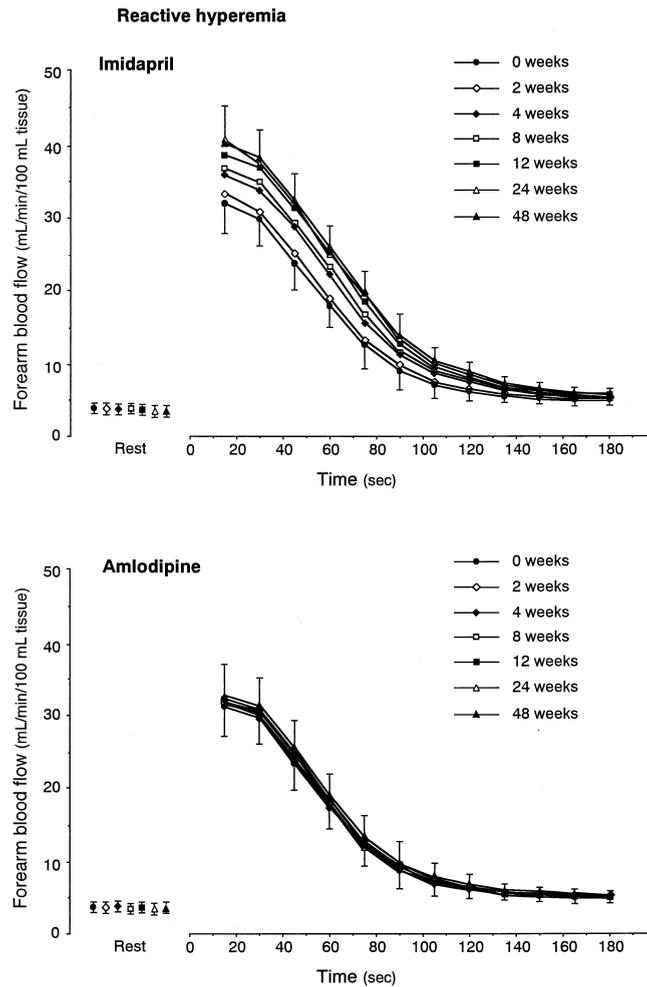
Reactive hyperemia

Figure 3. Line graphs show forearm blood flow at rest and during reactive hyperemia in patients with essential hypertension before (0 weeks) and after 2, 4, 8, 12, 24 and 48 weeks of treatment with the angiotensin-converting enzyme inhibitor imidapril and the calcium antagonist amlodipine. Imidapril augmented RH after 12 weeks of treatment. There was no significant difference in RH at 12, 24 and 48 weeks. Amlodipine did not alter RH for each treatment period.

imidapril and amlodipine were maintained throughout the 48-week treatment period. Heart rate was not significantly modified by imidapril or amlodipine for each treatment period. Both imidapril and amlodipine significantly decreased FVR after two weeks of treatment. Serum levels of lipids and glucose were similar for all treatment periods between the two groups.

Effects of imidapril and amlodipine on RH. The effects of imidapril and amlodipine on endothelial function before and after 2, 4, 8, 12, 24 and 48 weeks of treatment in patients with essential hypertension are shown in Figure 3. Imidapril significantly augmented RH after 12 weeks of treatment, compared with the pretreatment value (0 weeks) (p < 0.001). The ability of imidapril to improve RH was maintained throughout the 48-week treatment period. There was no significant difference in RH after 12, 24 and 48 weeks in the imidapril group. Although imidapril tended to augment RH after the four- and eight-week treatment

periods, this effect did not reach statistical significance. Amlodipine did not alter RH for each treatment period.

The increase in FBF after sublingual administration of NTG was similar for all treatment periods between the two groups (Fig. 4).

Effects of L-NMMA on RH. Intra-arterial infusion of L-NMMA reduced basal FBF from 4.6 ± 1.3 to 2.5 ± 0.5 ml/min per 100 ml tissue in the imidapril group and from 4.4 ± 1.3 to 2.4 ± 0.4 ml/min per 100 ml tissue in the amlodipine group (p < 0.05, respectively) and abolished the augmentation of the FBF response to RH in patients treated with imidapril (p < 0.001). L-NMMA reduced RH in the amlodipine group (p < 0.001). After L-NMMA infusion, RH was similar between the two groups (Fig. 5). L-NMMA infusion did not alter arterial BP or heart rate in any group.

DISCUSSION

The present study confirmed the previous findings that the resistance vessels' endothelial function was impaired in patients with essential hypertension, compared with normotensive control subjects, and demonstrated that ≥12 weeks of treatment with the ACE inhibitor imidapril augmented RH of the forearm artery in patients with essential hypertension.

In the present study, RH, an index of endothelium-dependent vasodilation, was smaller in patients with essential hypertension than in normotensive subjects, whereas the forearm vascular response to NTG, an index of endothelium-independent vasodilation, was similar between the two groups. Our results are consistent with previous studies indicating that endothelium-dependent vasodilation of brachial (8–11), renal (5–7,12) and coronary (23) arteries, as well as small arteries (13), was impaired in patients with essential hypertension, compared with normotensive subjects. These findings suggest that the vascular endothelium, but not smooth muscle cells, was selectively impaired in patients with essential hypertension.

Several lines of evidence from experimental (16–18) and clinical studies (11–15,24) indicate that ACE inhibitors can restore endothelial function in hypertension. In the present study, ≥12 weeks of treatment with imidapril augmented RH in the forearm microvascular circulation of patients with essential hypertension. Our findings are supported by the results of most studies that up to 12 weeks of treatment with ACE inhibitors improves endothelial function in patients with essential hypertension (Higashi et al. [14]; in the renal artery, for 12 weeks, Iwatubo et al. [11] and Higashi et al. [15]; in the brachial artery, for 24 weeks, Mimran et al. [12]; in the renal artery, and Schiffrin et al. [13]; in the small arteries, for two years). These findings suggest that antihypertensive treatment with ACE inhibitors requires at least 12 weeks to restore resistance artery endothelial function. In addition, we confirmed that improvement of the endothelial function of the resistance
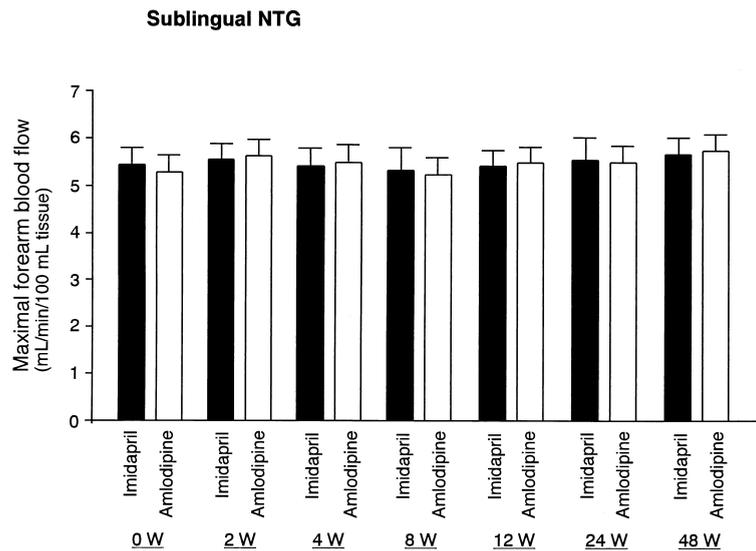
Sublingual NTG

Figure 4. Bar graphs show maximal forearm blood flow after sublingual administration of nitroglycerin (NTG) in patients with essential hypertension before (0 weeks) and after 2, 4, 8, 12, 24 and 48 weeks of treatment with the angiotensin-converting enzyme inhibitor imidapril and the calcium antagonist amlodipine. The NTG-induced vasodilation was similar for all treatment periods.

artery by ACE inhibition was maintained from 12 to 48 weeks after treatment. However, Creager et al. (21) showed that clinically effective antihypertensive therapy with ACE inhibitors for up to seven to eight weeks did not restore impaired endothelium-dependent vasodilation in the forearm circulation. Discrepancies in the reports from their laboratories may be due to the severity of hypertension in selected patients and to differences in the method of assessing endothelial function and the treatment periods.

Mechanisms. Several possible mechanisms by which ACE inhibitors augment RH in patients with essential hypertension were postulated. First, a balance between angiotensin II and NO plays an important role in the regulation of vascular tone (19). Angiotensin II increases vascular superoxide production through activation of membrane-associated NADH/NADPH oxidase, resulting in inactivation of NO and production of toxic peroxynitrite (19). Therefore, ACE inhibitors may relatively increase NO by the inhibition of angiotensin II production. Second, endogenous bradykinin is limited by ACE under physiologic conditions. Bradykinin binds to B_2 receptors on the endothelial cell surface to release NO (20). Angiotensin-converting enzyme inhibitors inhibit the degradation of bradykinin, resulting in increased NO release.

Taddei et al. (25) showed that one-year treatment with the ACE inhibitor lisinopril augmented the FBF response to bradykinin, but not that to acetylcholine, in hypertensive patients, in line with the results of Creager et al. (21). These findings suggest that ACE inhibitors may selectively facilitate the activation of bradykinin B_2 receptors, but not that of other receptors such as the muscarinic receptor, most likely through the inhibition of bradykinin degradation, leading to the accumulation of bradykinin within the vascular endothelium. Recently, Horning et al. (26) showed that shear stress-induced release of bradykinin may be

especially augmented by ACE inhibitors. Taken together, we can hypothesize that the increase in the concentration of endogenous bradykinin by RH-induced shear stress may contribute to the augmentation of the FBF response to RH after treatment with the ACE inhibitor imidapril.

Reactive hyperemia may be mediated by many factors, such as NO, prostaglandin, EDHF and ischemia-induced adenosine release. In the present study, L-NMMA abolished RH by ~50% before ACE inhibitor treatment (Fig. 5). We have previously shown that ACE inhibitor-induced enhancement of RH was not affected by a prostaglandin synthesis inhibitor, suggesting that prostaglandin does not contribute to the beneficial effects of ACE inhibitors on forearm resistance artery endothelial function in patients with essential hypertension (15). The inhibited degradation of bradykinin induced by ACE inhibition has been shown to increase EDHF (27). This vasodilating factor contributes to the augmentation of RH. However, because enhanced RH by imidapril was inhibited substantially by L-NMMA, the increase in NO release may be involved in imidapril-enhanced RH in patients with essential hypertension.

Interestingly, in the present study, the hypotensive effect of the ACE inhibitor imidapril preceded the restoration of FBF response to RH. In addition, although the calcium antagonist amlodipine was equally effective in reducing BP, RH was greater in the imidapril group than in the amlodipine group. These findings suggest that improvement of endothelial dysfunction may not contribute to the mechanism that reduces in BP in patients with essential hypertension. In contrast, there is a possibility that the reduction in BP, per se, may result in improvement of endothelial dysfunction. Several investigators, including ourselves, have reported that ACE inhibitors, but not calcium antagonists or beta-blockers, improve endothelial dysfunction, although all of these drugs have equivalent hypotensive effects (11–
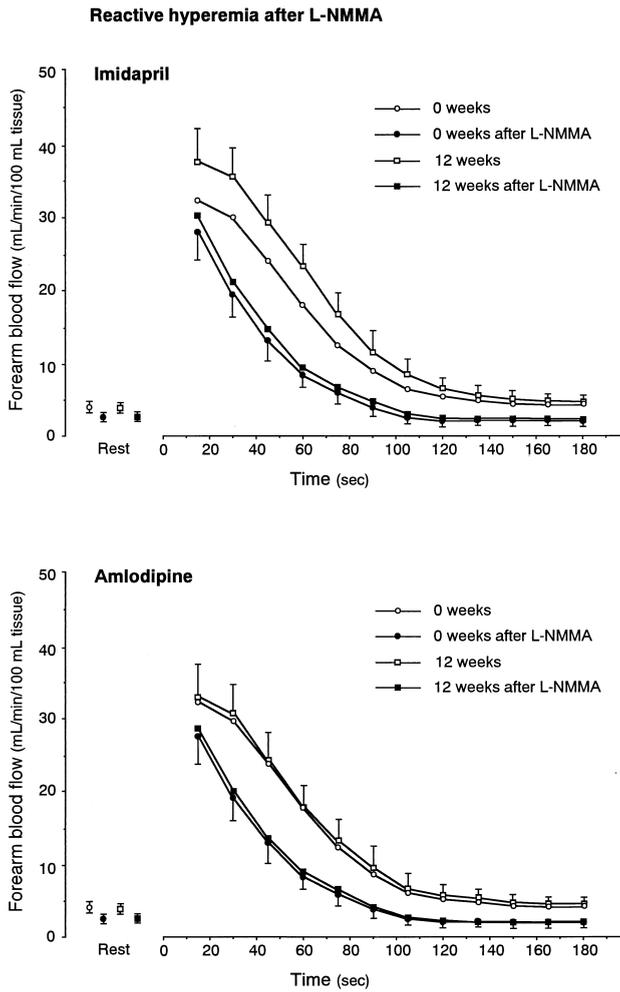
Figure 5. Line graphs show forearm blood flow at rest and during reactive hyperemia (RH) after infusion of N^G-monomethyl-L-arginine (L-NMMA) in patients with essential hypertension before (0 weeks) and after 12 weeks of treatment with the angiotensin-converting enzyme inhibitor imidapril and the calcium antagonist amlodipine. L-NMMA abolished the enhancement of RH in hypertensive patients treated with imidapril.

15). Because the reduction in BP by effective antihypertensive therapy, per se, may not always be accompanied by improvement of endothelial dysfunction in patients with essential hypertension, this possibility is unlikely.

Study limitations. The use of agonists to stimulate NO release, such as acetylcholine or bradykinin, allows us to draw more specific conclusions concerning the role of basal and stimulated release of NO by antihypertensive agents in the forearm circulation. In addition, the use of a bradykinin B_2 receptor antagonist demonstrates more specific conclusions concerning the role of bradykinin-stimulated release of NO by the ACE inhibitor imidapril. Recently, we have demonstrated that noninvasive methods, such as RH, are useful for assessing the resistance vessels' endothelial function, instead of the method of infusion of intra-arterial vasoactive agents (28). Indeed, because this noninvasive technique is simple and reproducible and does not cause adverse effects, it can be used for routine clinical examinations in the future.

We have previously confirmed that NO plays an important role in the augmentation of RH in hypertensive patients receiving ACE inhibitors, including imidapril, over 24 weeks (15). In the present study, the FBF responses to RH augmented by imidapril were similar among the 12-, 24- and 48-week treatment periods. Therefore, the same mechanism may continue at later time points, as well as at 12 weeks. In contrast, not only the endothelial function of peripheral arteries, but also structural changes in the peripheral arteries can affect the FBF response to RH. However, in the present study, the response of FBF to NTG was not changed by the ACE inhibitor imidapril. Therefore, it is unlikely that structural changes in the peripheral arteries were caused by imidapril.

Conclusions. The forearm vascular response to RH was impaired in patients with essential hypertension, compared with normotensive subjects. Twelve or more weeks of treatment with imidapril restored the resistance vessels' endothelial function in the forearm circulation in patients with essential hypertension.

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