Original Article

Angiotensin I–Converting Enzyme Inhibitor Improves Reactive Hyperemia in Elderly Hypertensives with Arteriosclerosis Obliterans

Masashi OKURO[1)], Shigeto MORIMOTO[1)], Takashi TAKAHASHI[2)], Kohya OKAISHI[1)], Takeshi NAKAHASHI[1)], Hiroshi MURAI[1)], Kunimitsu IWAI[1)], Tsugiyasu KANDA[2)], and Masayuki MATSUMOTO[1)]

Endothelial function in elderly hypertensive patients with arteriosclerosis obliterans has not been evaluated. We examined whether antihypertensive drugs improve vasodilatory response to reactive hyperemia of the limbs in elderly hypertensive patients (83±8 [SD] years) without (n=46, 0.9 ≤ ankle-brachial pressure index ≤1.4) and with (n=24) arteriosclerosis obliterans (ankle-brachial pressure index <0.2). Patients were randomized for treatment with monotherapy of either temocapril (14 with and 26 without arteriosclerosis obliterans) or amlodipine (10 with and 20 without arteriosclerosis obliterans) for 6 months. Blood flows of the forearms and legs were measured by strain-gauge plethysmography. The vasodilatory response to the release of compression of the forearms and thighs at 200 mmHg or 20 mmHg more than systolic blood pressure for 5 min and to sublingual administration of nitroglycerin (0.3 mg) was assessed. The maximum reactive hyperemic flow in 35 legs with arteriosclerosis obliterans was significantly (p<0.001) decreased compared to the value in legs in the control hypertensive subjects. Moreover, maximum reactive hyperemic flow in the forearms of patients with arteriosclerosis obliterans was significantly (p=0.002) decreased compared to that in the control subjects. Blood pressure was similarly decreased by treatment with temocapril or amlodipine. Response to nitroglycerin (0.3 mg) was not changed by either drug. Treatment with temocapril significantly improved maximum reactive hyperemic flow of not only the legs and forearms in control hypertensives but also the legs and forearms in patients with arteriosclerosis obliterans, and attenuated the worsening of activity of daily living in these patients, although treatment with amlodipine did not. These results suggest that the angiotensin-converting enzyme inhibitor temocapril has a beneficial effect on endothelial function in elderly patients with arteriosclerosis obliterans. (*Hypertens Res* 2006; 29: 655–663)

***Key Words*: antihypertensive drug, arteriosclerosis obliterans, elderly, hypertension, plethysmography**

Introduction

Arteriosclerosis obliterans has become one of the major health problems in the elderly, since the incidence of this disease increases with age (*1*), and since the disease not only causes decreased activity of daily living and quality of life, but is also a serious life-threatening condition due to its progressive nature and/or the high mortality of associated arterial lesions of cardiovascular organs (*2*). Arteriosclerosis obliterans frequently follows an inexorable downhill course (*3*, *4*), and, importantly, the Consensus Document of the European

From the Departments of [1)]Geriatric Medicine and [2)]General Medicine, Kanazawa Medical University, Ishikawa, Japan.

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Address for Reprints: Shigeto Morimoto, M.D., Ph.D., Department of Geriatric Medicine, Kanazawa Medical University, 1–1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920–0293, Japan. E-mail: shigeto@kanazawa-med.ac.jp

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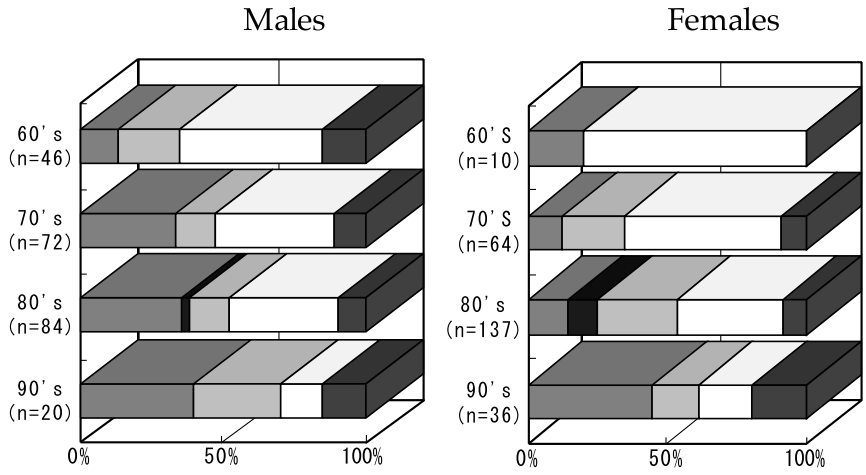

Fig. 1. *Prevalence of subjects with ABI>1.4 (), 0.9≤ABI≤1.4 (), 0.5≤ABI<0.9 (), 0.2≤ABI<0.5 (), and ABI<0.2 () in males (*n*=222) and females (*n*=247) in their 60s, 70s, 80s and 90s respectively, in a hospital for the elderly.*

Working Group on Critical Limb Ischemia has concluded that there is no optimal medical therapy for this disease (*5*).

On the other hand, arteriosclerosis obliterans is highly associated with hypertension (*6*), and hypertension itself is a major risk for arteriosclerosis obliterans (*7–9*). It has been shown by microscopic examination that the endothelium of lesions of arteriosclerosis obliterans is impaired (*10*), and that endothelial function assessed by maximum reactive hyperemic flow is significantly decreased in patients with arteriosclerosis obliterans and/or hypertension (*11*). Among antihypertensive agents, angiotensin I–converting enzyme (ACE) inhibitors (*12*, *13*) and calcium antagonists (*13*, *14*) are known to maintain or even increase limb blood flow and to improve tolerability to exercise in patients with arteriosclerosis obliterans. Moreover, ACE inhibitors have been reported to have beneficial effects on endothelial function, as assessed by reactive hyperemia in middle-aged patients with essential hypertension (*15*) and in elderly patients with hypertension (*16*). However, there has been little study of the effects of these antihypertensive agents on endothelial function in this hypertension-associated disease.

In this study, we evaluated the effects of an ACE inhibitor, temocapril, and a calcium antagonist, amlodipine, on vasodilator responses in the limbs of elderly patients with hypertension with or without associated arteriosclerosis obliterans. These drugs were given as monotherapy for 6 months. We examined whether reactive hyperemia was impaired, and whether the antihypertensive agents affected the vasodilatory responses in these elderly subjects.

Methods

Study Population

The study was conducted in Sengi Hospital, which serves as both a hospital and a long-term care facility for the elderly; medical and care services are often combined in single facilities in Japan (*17*). Prior to the selection of subjects, we surveyed ankle-brachial pressure index (ABI) in 469 elderly inpatients aged 65 years or older (222 males and 247 females, including 46 males and 10 females in their 60s, 72 males and 64 females in their 70s, 84 males and 127 females in their 80s, and 20 males and 36 females in their 90s, with a mean [±SD] age of 82±9 years) in the hospital. ABI was measured as described below (*18*). Our classification of ABI was essentially based on the report of Resnick *et al*. (*18*), who used the three categories of ABI>1.4 (at least one leg), 0.9≤ABI≤1.4 (both legs), and ABI<0.9 (at least one leg). However, since about half (48%) of our subjects had an ABI<0.9, we further separated the category of ABI<0.9 into three classes, namely, 0.5≤ABI<0.9, 0.2≤ABI<0.5, and ABI<0.2 (at least one leg, respectively). The preliminary survey revealed that the numbers (%) of subjects with ABI>1.4, 0.9≤ABI≤1.4, 0.5≤ABI<0.9, 0.2≤ABI<0.5, and ABI<0.2 were 51 (12%), 191 (41%), 97 (21%), 16 (3%), and 114 (24%), respectively. Increases in age-related prevalence in those with ABI<0.2 were prominent both in males and females (Fig. 1). Moreover, the survey also disclosed that the percentage of subjects with 0.5≤ABI<0.9 was very small. Furthermore, none of the 113 subjects with 0.2≤ABI<0.9 showed any characteristic features of arteriosclerosis obliterans, such as intermittent claudication, pain at rest, or ulcer/gangrene of the lower extremities, although 12 (11%) of the 114 subjects with ABI<0.2 showed characteristic features of arteriosclerosis obliterans, *i.e.*, pain at rest (8 patients) and ulcer/gangrene (4 patients). Accordingly, we adopted rather strict criteria, using ABI<0.2 for selection of hypertensive patients with arteriosclerosis obliterans. The numbers (%) of subjects with hypertension, defined as a systolic blood pressure (SBP) of 140 mmHg or higher and/or diastolic blood pressure (DBP) of 90

mmHg or higher in the sitting position on at least three separate occasions and/or current use of antihypertensive drugs, were 19 (37%) out of 51 subjects with ABI>1.4, 67 (35%) out of the 191 with 0.9≤ABI≤1.4, 35 (36%) out of the 97 with 0.5≤ABI<0.9, 5 (31%) out of the 16 with 0.2≤ABI<0.5, and 57 (50%) out of the 114 with ABI<0.2. From among these hypertensive patients, we selected elderly patients with mild (140–159/90–99 mmHg) to moderate (160–179/100–109 mmHg) hypertension (mean±SD age: 83±8 years; age range: 68–94 years) without (n=46, 0.9≤ABI≤1.4) or with (n=24) arteriosclerosis obliterans (ABI <0.2, two legs in 11 subjects and one leg in 13 patients). Subjects showing pain at rest or ulcer/gangrene as features of arteriosclerosis obliterans, or those with diabetes mellitus (fasting blood glucose >7 mmol/l or treated with or current use of an antidiabetic drug) or hypercholesterolemia (total cholesterol >5.66 mmol/l) were excluded by clinical and laboratory examinations. None of the subjects had undergone amputation due to arteriosclerosis obliterans. Patients were randomized to treatment with initial doses of 2 mg temocapril (14 patients with and 26 patients without arteriosclerosis obliterans; Sankyo Co., Ltd., Tokyo, Japan) or 2.5 mg amlodipine (10 patients with and 20 patients without arteriosclerosis obliterans; Sumitomo Pharmaceutical Ltd., Osaka, Japan) once daily for 6 months. When the blood pressure was not decreased by 20/10 mmHg or to below 150/90 mmHg, the dose of drug was doubled. Blood pressure control was achieved in all hypertensive subjects with monotherapy of either drug. All patients had either never been treated (n=35) or had discontinued antihypertensive drugs—in this case, β-blockers (n=2), ACE inhibitors (n=17), or calcium antagonists (n=16)—for at least 4 weeks before the study. Biochemical factors were measured in blood collected in the morning after overnight fasting. The study protocol was approved by the ethical committee of the hospital. All subjects were Japanese, and only those who gave informed consent were enrolled in the study.

Measurement of Ankle and Arm Blood Pressures

At each ABI measurement, right arm blood pressures and bilateral ankle blood pressure (posterior tibial artery), measured by handheld Doppler ultrasonography (Imex Medical Systems, Golden, USA), were taken with the subject supine. If the absent pulse was verified, ankle blood pressure measures were taken on the dorsalis pedis. The means of the 2 measurements for each leg and for the arm were used to calculate ABI, and the lower of the 2 values was used to define ABI for each individual (*18*).

Blood Flow Measurements of the Forearm and Legs

The study was conducted in a dark, quiet, temperature-controlled (at 23°C) room. Subjects rested for 30 min in the supine position before the study. Blood flow of the right forearm and of both legs was measured by strain gauge plethysmography (model EC6; De Hokanson, Inc., Bellevue, USA) (*19*, *20*). Hand and foot circulations were excluded by wrist and ankle cuffs inflated to suprasystolic pressure. Mercury-in-silastic strain gauges that had been electrically calibrated were placed on the widest part of the right forearm and both lower legs at 5 cm and 8 cm below the antecubital and popliteal creases, respectively. Blood flows of forearm or legs were calculated from the rate of increase in forearm or leg volumes, while venous return was prevented by inflating the cuffs at upper arm or thigh to a venous-occlusion pressure of 50 mmHg. Flow measurements were recorded for 9 s every 15 s, and an average of 4 measurements was used for analysis. Endothelium-dependent vasodilatation was assessed by ischemia-induced reactive hyperemia. After a baseline recording of 4 min, ischemia was induced for 5 min by inflating the upper arm cuff to 200 mmHg or 20 mmHg more than SBP. Immediately after cuff deflation, maximal hyperemic blood flow was measured (peak flow), followed by continuous measurements for 3 min. At least 15 min after the last measurement, blood flow was measured to confirm that it had returned to the basal level. Then, endothelium-independent vasorelaxation was assessed after sublingual administration of nitroglycerin at 0.3 mg by one puff of a spray device (Miokol Spray; Toa Eiyo, Tokyo, Japan), and blood flows were measured for 5 min. The blood flow is expressed as ml of blood per min per 100 ml of limb volume (*15*, *20*, *21*).

Other Measurements

We evaluated activity of daily living according to the following four states of ambulation: walking, using a wheelchair, sitting on the bed, and bedridden. We also recorded known risk factors for arteriosclerosis obliterans, including dementia (Mini-Mental State Examination score≤23), chronic stage of stroke (motor deficit and evidence of cerebral hemispheric stroke on CT or MRI), and chronic ischemic heart disease (previous myocardial infarction or angina pectoris).

Statistical Analyses

Data are expressed as the mean±SD. Differences in changes of blood pressure and vasodilatory responses by treatment with antihypertensive drugs were assessed by analysis of variance with repeated measurements. Changes in ABI and activity of daily living were assessed by non-parametric Wilcoxon test. A value of $p<0.05$ was regarded as significant. Differences among the four groups were analyzed by Kruskal-Wallis χ^2 and Mann-Whitney U test for multiple comparisons with post-hoc Bonferroni correction. The statistical significance of p values was set at 0.008 for the analysis among four groups. Data were analyzed on a microcomputer running SPSS (SPSS Inc., Chicago, USA).

Table 1. Comparison of Clinical Factors between Hypertensive Patients with and without Arteriosclerosis Obliterans

	Control subjects		Arteriosclerotic patients	
	Amlodipine (n=20)	Temocapril (n=26)	Amlodipine (n=10)	Temocapril (n=14)
Clinical background				
Age (years)	82±7	83±8	83±7	83±8
Sex (male/female)	7/13	10/16	4/6	5/9
Total cholesterol (mmol/l)	4.89±0.32	4.86±0.30	4.85±0.29	4.87±0.28
Fasting blood glucose (mmol/l)	5.06±0.34	5.03±0.20	5.04±0.31	5.04±0.29
Dementia (n (%))	13 (65)	16 (62)	6 (60)	8 (57)
Chronic stage of stroke (n (%))	4 (20)	8 (31)	2 (20)	3 (21)
Ischemic heart disease (n (%))	7 (35)	8 (31)	3 (30)	3 (21)
Before				
SBP (mmHg)	161±12	162±13	162±14	162±12
DBP (mmHg)	82±7	84±8	83±7	82±6
Heart rate (bpm)	68±5	69±7	67±6	68±7
ABI	1.03±0.09	1.04±0.10	0.11±0.010$^{\#}$	0.10±0.01$^{\#}$
Activity of daily living (n (%))				
Walking	11 (55)	15 (57)	6 (60)	8 (57)
Using a wheelchair	5 (25)	6 (23)	2 (20)	4 (29)
Sitting on the bed	2 (10)	2 (8)	1 (10)	1 (7)
Bedridden	2 (15)	3 (12)	1 (10)	1 (7)
Six months				
SBP (mmHg)	138±10†	137±11†	138±10†	139±12†
DBP (mmHg)	72±7†	74±8†	72±9†	74±8†
Heart rate (bpm)	69±5	71±7	69±6	69±8
ABI	0.99±0.05	1.05±0.03	0.10±0.01$^{\#}$	0.13±0.01$^{\#,\dagger}$
Activity of daily living (n (%))				
Walking	10 (50)	15 (57)	4 (40)	10 (72)
Using a wheelchair	3 (15)	5 (19)	1 (10)	2 (14)
Sitting on the bed	4 (20)	3 (12)	2 (20)	0 (0)
Bedridden	3 (15)	3 (12)	3 (30)	2 (14)

SBP, systolic blood pressure; DBP, diastolic blood pressure; bpm, beats per minute; ABI, ankle-brachial pressure index. Values are mean±SD. $^{\#}p<0.008$: significant difference *vs*. control groups by Kruskal-Wallis analysis. *$p<0.05$ and $^{\dagger}p<0.01$: significant difference *vs*. before.

Results

Table 1 shows the backgrounds of the groups of hypertensive elderly patients with and without arteriosclerosis obliterans treated with amlodipine or temocapril. There was no statistically significant difference among the four groups in age, male/female ratio, circulating concentration of total cholesterol or glucose, activity of daily living, or prevalence of dementia, chronic phase of stroke, or chronic ischemic heart disease. Of course, the ABI values in the two groups with arteriosclerosis obliterans were significantly ($p<0.008$) reduced compared to those in the two control groups (Table 1).

The values of basal blood flow (Fig. 2a), nitroglycerin-induced increase in blood flow (Fig. 2b), and maximum reactive hyperemic flow (Fig. 2c) in the 35 affected legs of the 24 hypertensive patients with arteriosclerosis obliterans were significantly ($p<0.001$ in each) decreased compared to the respective values in the 92 legs of 46 age- and sex-matched elderly hypertensive control subjects. Although the basal and nitroglycerin-induced increases in blood flow in the right forearm were similar in the two hypertensive groups, maximum reactive hyperemic flow in the right forearm in the 24 patients with arteriosclerosis obliterans was significantly ($p<0.01$) decreased compared to that in the 46 control subjects.

Baseline values (ml/min/100 ml) of blood flow in the legs and forearms before treatment in patients with arteriosclerosis obliterans (1.8±0.4 and 3.9±0.5 in the amlodipine-treated group and 1.9±0.5 and 4.0±0.4 in the temocapril-treated group) and patients without arteriosclerosis obliterans (3.2±0.4 and 4.1±0.4 in the amlodipine-treated group and 3.3±0.4 and 4.2±0.5 in the temocapril-treated group) were

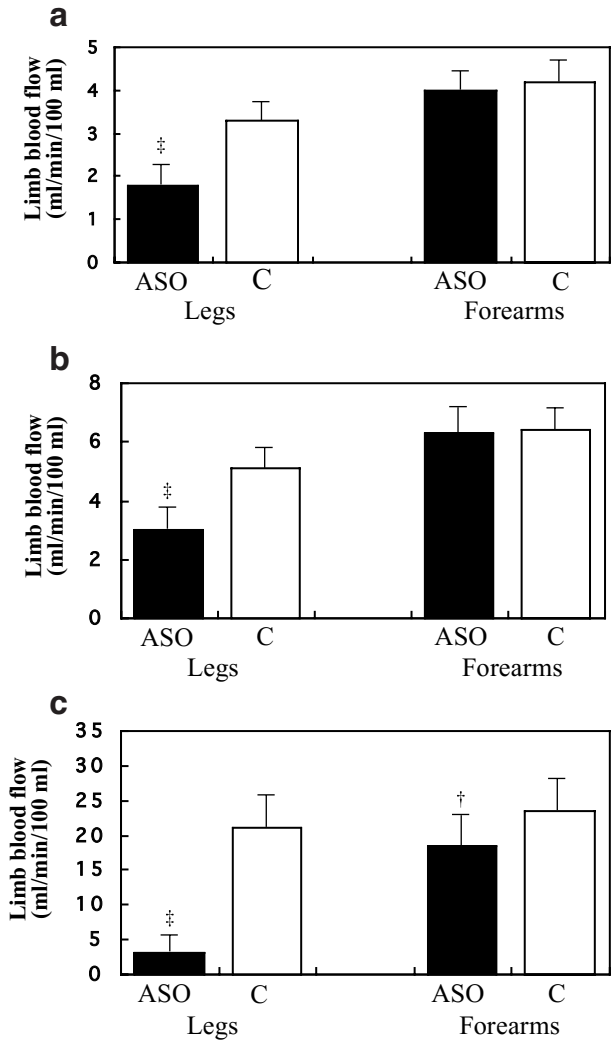

Fig. 2. *Comparison of basal blood flow (a), nitroglycerin-induced increase in blood flow (b), and maximum reactive hyperemic flow (c) between legs with arteriosclerosis obliterans (ASO,* n = *35, solid bars) and legs in control hypertensive patients (C,* n = *92, open bars) (left), and between the right forearms in ASO patients (*n = *24) and control patients (*n = *46) (right).* †p < *0.01,* ‡p < *0.001: significant differences between hypertensive elderly patients with and without ASO.*

similar in the two drug groups. Basal blood flow in the affected legs of patients with arteriosclerosis obliterans ($n=14$ in the amlodipine-treated group and $n=21$ in the temocapril group) was again significantly lower compared to that in the legs of patients without arteriosclerosis obliterans in the respective drug groups.

Blood pressure was well controlled by 2.5 mg ($n=6$ in the group with arteriosclerosis obliterans and $n=12$ in the control group) or 5 mg ($n=4$ in the group with arteriosclerosis obliterans and $n=8$ in the control group) amlodipine, or 2 mg ($n=11$ in the group with arteriosclerosis obliterans and $n=22$ in the control group) or 4 mg ($n=3$ in the group with arteriosclerosis obliterans and $n=5$ in the control group) temocapril. Monotherapy of either amlodipine or temocapril for 6 months produced a similar reduction of blood pressure (Table 1).

Treatment with temocapril slightly but significantly increased the ABI value in patients with arteriosclerosis obliterans, although amlodipine did not significantly change the ABI value in patients with arteriosclerosis obliterans (Table 1). On the other hand, basal blood flow and nitroglycerin-induced increase in blood flow (Fig. 3a) in the legs and forearm were not significantly changed by either drug. Treatment with temocapril significantly improved maximum reactive hyperemic flow (ml/min/100 ml) of not only the legs (21.8 ± 3.8 *vs*. 24.9 ± 3.7, $p<0.001$) and forearm (24.2 ± 3.6 *vs*. 27.3 ± 3.6, $p<0.01$) in control hypertensive subjects, but also in the affected legs (2.8 ± 2.2 *vs*. 5.8 ± 2.5, $p<0.001$) and forearms (18.2 ± 3.8 *vs*. 22.3 ± 2.9, $p<0.01$) of patients with arteriosclerosis obliterans, although treatment with amlodipine did not affect maximum reactive hyperemic flow (Fig. 3b). Moreover, the activities of daily living were slightly but significantly decreased in control subjects and patients with arteriosclerosis obliterans treated with amlodipine for 6 months, but not in the other two groups treated with temocapril (Table 1).

Discussion

As a diagnostic criterion for arteriosclerosis obliterans, ABI < 0.9 is often used (*18*, *22*). According to one report, the prevalence of asymptomatic arteriosclerosis obliterans defined by an ABI value < 0.9 was only 3.4% in subjects aged 65 years or more who were inhabitants of rural communities in Japan (*22*). However, our preliminary survey in 469 inpatients of an elderly hospital aged 65 years or older revealed that about half of the elderly subjects would have been diagnosed with arteriosclerosis obliterans if this criterion had been adopted. Moreover, subjects with $0.2\leq$ABI<0.9 did not show any characteristic features of arteriosclerosis obliterans. Furthermore, only 3% of the total subject group had $0.2\leq$ABI<0.5. According to these findings, we selected subjects with ABI < 0.2 as subjects with arteriosclerosis obliterans in this study. Furthermore, subjects with apparent rest pain or ulcer/gangrene were excluded from the present study, and thus most of the remaining subjects would be classified as stage I or II in Fontaine classification. However, we did not adopt the Fontaine classification for staging of the severity of arteriosclerosis obliterans, since many of the inpatients of the elderly hospital were not capable of complaining about intermittent claudication or rest pain of the lower extremities, and in some cases not capable of walking, due to rather high prevalence of dementia and chronic phase of stroke (Table 1). Instead, we analyzed the activity of daily living in the four groups, and found no statistically significant difference in this parameter among the four groups. This observation suggests that decreased activity of daily living due to arteriosclerosis

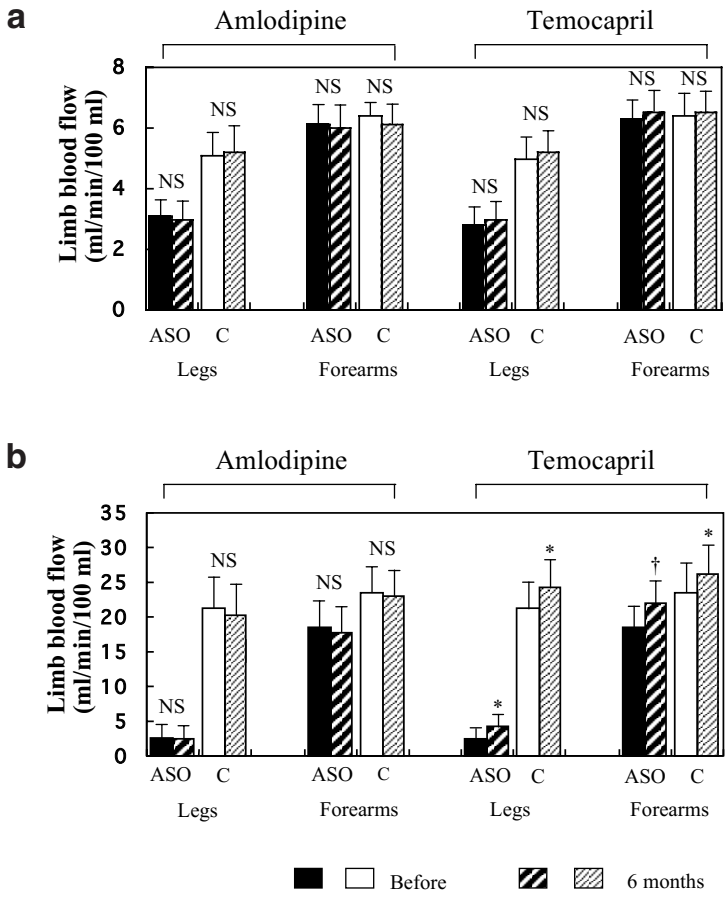

Fig. 3. *Changes of blood flow in legs and forearms in response to sublingual administration of nitroglycerin (a), and of reactive hyperemic response (b), before and after 6 months of treatment with amlodipine or temocapril. ASO, patients with arteriosclerosis obliterans; C, control patients.* *$p < 0.05$, †$p < 0.01$: *significant differences between before and after 6 months of treatment; NS: not significant.*

obliterans itself was not a direct cause of the differences seen in the basal and reactive hyperemic blood flow in this study.

There may be two possible mechanisms which could explain the decreased reactive hyperemia in the diseased legs. Since we measured ankle blood pressure at the lower part of the main coarctation sites of arteriosclerosis obliterans (*23*), the first possibility is that the endothelial function had already reached an almost maximal level because of the decreased blood stream due to coactation of the upper part of the legs which would have resulted in a decrease in reactive hyperemia after complete occlusion by the cuff. The second possibility is that the decreased reactive hyperemia in the diseased legs was one of the general findings of the reduced vasodilatory endothelial function represented by those in upper extremities, since vasodilatory responses to reactive hyperemia were significantly decreased not only in the diseased legs but also in the forearms of patients with arteriosclerosis obliterans. The results of several reports may lend support to this latter mechanism. Postischemic hyperemia in human limbs is thought to be partly mediated by nitric oxide (NO), since inhibition of NO synthesis by N^G-monomethyl-L-arginine has been shown to decrease reactive hyperemic flow (*24*), although the participation of other mechanisms, including endothelium-dependent vasodilating substances such as prostaglandins (*25*) and endothelium-stimulating substances such as adenosine (*26*), has also been reported. Impaired endothelial function in hypertensive patients has been demonstrated by several investigators (*15*, *27–29*). Moreover, aging itself is also a causal factor for decreased endothelial function (*30*). Furthermore, a generalized decrease of endothelial function was also reported in patients with thromboangiitis obliterans (Buerger's disease), another occlusive arterial disease (*31*). In the present study, on the other hand, treatment with temocapril slightly but significantly increased the ABI value, and improved the reactive hyperthermia even in the legs with arteriosclerosis obliterans without any significant change in basal blood flow, suggesting that this ACE inhibitor improves not only the generalized decrease in endothelial function in patients with arteriosclerosis obliterans, but also might improve the decreased reactivity of the endothelial function

due to defatigation by continuous stimulation of the decreased blood stream.

Our results also showed that the reactive hyperemia in the legs and forearms in control hypertensive subjects as well as in the affected legs and forearms of patients with arteriosclerosis obliterans was improved by 6 months of treatment with an ACE inhibitor, temocapril, but not by a calcium antagonist, amlodipine. Moreover, significant decreases in the activity of daily living observed both in control subjects and patients with arteriosclerosis obliterans treated with amlodipine for 6 months were apparently attenuated by treatment with the ACE inhibitor. Treatment with amlodipine and temocapril produced a similar blood pressure reduction after 6 months of treatment. Both drugs are long-acting antihypertensive agents that are usually given once a day. Therefore, it is unlikely that the difference in duration of normotension can explain the results obtained in this study. ACE inhibitors reduce circulating and tissue levels of angiotensin II (*32*), the most potent vasoconstrictor, which causes sequential production of other vasoconstricting factors such as endothelin (*33*) and prostaglandin H_2 (*34*) from endothelial cells, and production of superoxide, an inactivator of NO, *via* the stimulation of reduced nicotine amide adenine dinucleotide oxidase (*35*). Moreover, ACE inhibitors induce accumulation of bradykinin (*36*), which causes the release of endothelium-derived relaxing factor from the endothelium (*37*) and the ACE inhibitor–induced increase in reactive hyperemia (*38*). These mechanisms may participate in the improvement of vasodilatory responses to reactive hyperemia in temocapril-treated elderly patients with hypertension. Interestingly, it has also recently been reported that brachial flow-mediated vasodilation is significantly correlated with coronary endothelial function and fibrinolytic activity in response to bradykinin in 14 diabetic and 63 non-diabetic subjects (*39*). The results of our study are partly compatible with previous reports demonstrating the beneficial effects of ACE inhibitors on endothelial function as assessed by reactive hyperemia in middle-aged patients with essential hypertension (*15*) and in elderly patients with hypertension (*16*). Moreover, treatment with temocapril might improve the decreased reactivity of endothelial function due to defatigation by continuous stimulation of the decreased blood stream.

In the present study, on the other hand, treatment with amlodipine had no effects on reactive hyperemia in the legs and forearms of elderly hypertensive patients with and without arteriosclerosis obliterans. Since amlodipine (*40*) and other calcium antagonists (*41*) are reported to enhance *in vitro* endothelial synthesis of NO, the reason for the difference in the vasodilatory response between the two antihypertensives is uncertain. However, our results are partly compatible with the clinical observations that treatment with amlodipine failed to improve forearm reactive hyperemia (*15*) and L-arginine–induced increase in renal plasma flow (*42*) in patients with essential hypertension.

In the present study, however, we did not measure factors related to the renin-angiotensin system or bradykinin. Further studies including evaluations of local or systemic levels of these factors are required to elucidate the precise mechanism of the efficacy of ACE inhibitors for improvement of vasodilatory responses to reactive hyperemia, especially in elderly hypertensive patients with arteriosclerosis obliterans, since ACE inhibitors suppress local and systemic formation of angiotensin II (*32*) and degradation of bradykinin (*36*) and since local bradykinin has been reported to play a role in the ACE inhibitor–induced improvement of endothelial function in humans (*38*). Moreover, because they were asymptomatic, despite their rather low ABI values, the majority of elderly inpatients with arteriosclerosis obliterans in this study were not treated with anti-platelet drugs, anticoagulants, or prostaglandins, which are standard prescriptions for younger patients with this disease. The efficacies of these drugs for prevention of progression and treatment of arteriosclerosis obliterans in oldest–old patients like our subjects have not been examined.

Reactive hyperemia-induced increase in forearm blood flow is a frequently used marker of endothelium-dependent vasorelaxation, especially because its measurement is noninvasive (*43*, *44*). However, it has certain limitations compared with the reference method of forearm blood flow measurement during intra-arterial infusion of acetylcholine. Hyperemic blood flow is not exclusively dependent on the endothelium, because in addition to endothelium-derived vasoactive agents, other local metabolic factors may contribute to vasodilatation after ischemia. Furthermore, the placement of the arm occlusion (upper *vs*. lower arm) and the age of the investigated subjects may influence the correlation of hyperemic forearm blood flow with endothelium-mediated vasorelaxation.

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