

Improvement of Endothelial Function by Amlodipine and Vitamin C in Essential Hypertension

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Background : The effects of antihypertensive agents on endothelial function have not been fully evaluated in human hypertension and data on the forearm circulation of humans are controversial. The aim of this study was (1) to evaluate the endothelial function in hypertensive patients (2) to investigate whether vitamin C administration has any benefit on the endothelial function and (3) to determine whether treatment with calcium antagonist improves endothelial dysfunction in hypertensive patients.

Methods : The endothelial function was estimated using venous occlusion plethysmography (VOP) in 8 hypertensive patients and 8 healthy volunteers. The patients in the hypertension group were treated with amlodipine, then examined again. The change of forearm blood flow (FBF) was measured with acetylcholine infusion through brachial artery and also with intra-arterial vitamin C.

Results : Forearm blood flow response to acetylcholine was significantly enhanced with intra-arterial infusion of vitamin C in hypertensive group before antihypertensive treatment. Co-infusion of L-NMMA, an inhibitor of nitric oxide synthase, blunted forearm blood flow response to acetylcholine. After treatment with amlodipine for 2 months in hypertensive group, endothelium-dependent vasorelaxation to acetylcholine was significantly improved compared to pretreatment, and vitamin C did not affect the improved endothelial function by amlodipine treatment.

Conclusion : Vitamin C (acutely) and amlodipine (chronically) improved endothelial function in hypertensive patients. These results suggest that increased oxidative stress, at least in part, may be involved in the decreased endothelial function in hypertension.

Key Words : Plethysmography, Endothelium, Hypertension, Ascorbic acid, Amlodipine

INTRODUCTION

The endothelium plays an important role in maintaining vascular tone and function. The main endo-

thelium derived factor is nitric oxide (NO) which is not only a potent vasodilator but also inhibits platelet aggregation, smooth muscle cell migration and proliferation, monocyte adhesion and adhesion molecule expression, thus protecting the vessel wall against the development of atherosclerosis and thrombosis.

Essential hypertension is associated with alterations in endothelial function. Endothelium-dependent vasodil-

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ation in response to substances, such as acetylcholine, bradykinin and substance P and reactive hyperemia, was reduced in brachial^{1,3)}, coronary⁴⁾, renal arteries^{5,6)} and femoral arteries⁷⁾ in patients with essential hypertension. Impairment of endothelial function has been shown to play an important role in the development and maintenance of hypertension⁸⁾. Therefore, an important aim of antihypertensive therapy would be not only to normalize blood pressure values but also to reverse endothelial dysfunction by restoring NO availability.

Several studies have demonstrated the restoration of endothelial function in essential hypertensive patients through the administration of antihypertensive agents⁸⁻¹⁰⁾, while others have shown that effective antihypertensive therapy did not restore impaired endothelium-dependent vasodilation in the forearm circulation of hypertensive patients^{11,12)}.

The effects of antihypertensive agents on endothelial function have not been fully evaluated in human hypertension and data on the forearm circulation of humans are controversial. It may be clinically important to select an appropriate antihypertensive agent that is effective in improving endothelial dysfunction in patients with established essential hypertension.

Recently, the role of superoxide anion and its interaction with nitric oxide has been investigated¹³⁾. Under physiological conditions, these oxygen-free radicals are potent chemical inactivators of NO^{14,15)} and the balance between NO and superoxide is more important than the absolute levels of either alone¹⁶⁾.

Vitamin C is an important antioxidant in human plasma, capable of scavenging oxygen-free radicals and sparing other endogenous antioxidants from consumption¹⁷⁾.

Therefore, the aim of this study was (1) to evaluate the endothelial function in hypertensive patients (2) to investigate whether vitamin C administration has any benefit on the endothelial function and (3) to determine whether treatment with calcium antagonist improves endothelial dysfunction in hypertensive patients.

METHODS

Subjects

Eight hypertensive patients (age range 35 to 73 years) were recruited. They had a clinical blood pressure reading (the average of 3 different sphygmomanometric measurements, each performed on 3 separate days)

of >140/90 mmHg. The possibility of secondary causes of hypertension was excluded by standard clinical and laboratory tests. Exclusion criteria were 1) evidence of overt atherosclerotic disease, i.e., coronary artery disease, peripheral vascular disease, stroke, etc. 2) having other risk factors of atherosclerosis, i.e., current smoking and smoking within 1 year, severe hypercholesterolemia (> 240 mg/dL) and diabetes mellitus 3) advanced organ failure 4) malignancy. In all patients, a noninvasive 24-hour blood pressure monitoring was performed at baseline and after treatment. Eight normal volunteers were enrolled who had normal blood pressure. Exclusion criteria were the same as hypertensive group.

Study design

Subjects were randomized to receive amlodipine 5-10 mg daily for at least 2 months. It was only the hypertensives that underwent the treatments. Clinical profiles of the subjects are shown in Table 1. Forearm vascular function was studied before and after antihypertensive treatment. Subjects were required to refrain from drinking alcohol or caffeine-containing beverage for 12 hours before the study.

The protocol of the study was approved by the ethics committee of our institution and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Table 1. Clinical characteristics of normal control and hypertensive patients

	NC	HT
Number	8	8
Age	50 ± 4	56 ± 3
Sex (m:f)	2:6	3:5
Cholesterol	182 ± 20	191 ± 14
SBP*	115 ± 4	169 ± 8
DBP*	65 ± 3	98 ± 3

Values are mean ± SEM, * $p < 0.05$ (NC vs HT)
NC, normal control; HT, hypertensive patients group

Venous occlusion plethysmography

The examination was done at supine position. The brachial artery of the nondominant arm was cannulated with 20 gauge cannula. A mercury-filled silastic strain gauge was placed around the thickest part of the forearm. The size of the strain gauge was selected to be about 2 cm less than the maximal forearm circum-

ference. The strain gauge was connected to plethysmograph (Hokanson EC-R5, Issaquah, Washington) to record the forearm volume change. A rapid cuff inflator (Hokanson E-10) was used to inflate the arm cuff to 40 mmHg instantaneously, thus occluding venous return from the forearm. A wrist cuff was inflated to 20 mmHg above the systolic pressure to cut off the arterial flow to hand. Intra-arterial pressure was measured continuously (Transpac; Abbot Laboratories) throughout the study. Drug infusions were administered using a constant rate infusion pump. The measurement of forearm volume change was repeated 7 times for each stage. Between infusions, the cuffs were deflated, allowing at least 15 min for forearm blood flow to recover from the preceding infusion and before further baseline measures were recorded. All solutions were prepared aseptically from sterile stock solutions or ampoules immediately before infusion into brachial artery. Acetylcholine and L-NMMA had been diluted in distilled water and filtered through 0.22 µm filter.

Evaluation of endothelium-dependent vasodilation and effect of vitamin C

An intra-arterial infusion of 5% dextrose solution was begun at 1 mL/min and continued throughout drug infusion. Basal measurement was obtained during the infusion of dextrose solution. Acetylcholine (Sigma chemical) was infused intra-arterially at 7.5, 15 and

30 g/min. Acetylcholine was infused for 5 minutes for each dose level and forearm blood flow was measured during the last 2 minutes of each dose. After 15 minutes of wash-out period, intra-arterial infusion of vitamin C at 24 mg/min was done for 10 minutes. With continued infusion of vitamin C at the same rate, acetylcholine was infused at the same incremental doses as the previous stage and measurement of forearm blood flow was made during the last 2 minutes of each dose. After another 15 minutes of rest period, intra-arterial infusion of vitamin C at the same rate, plus infusion of L-NMMA (NG-monomethyl-L-arginine, Sigma chemical), an inhibitor of nitric oxide synthesis, was begun at 100 g/min and continued for 5 minutes. Then infusion of acetylcholine at incremental dose and measurement of forearm blood flow were done as in previous stages. (Figure 1).

Statistical analysis

Forearm blood flow was measured and forearm blood flow changes during acetylcholine infusion were expressed as percentage changes from the baseline immediately preceding each drug administration. Forearm blood flow changes were compared between normal and hypertensive groups with and without vitamin C infusion. Comparison was also made between before and after antihypertensive treatment in hypertensive group and forearm blood flow was compared in hypertensive group with and without vitamin C infusion. Repeated measures ANOVA were used in comparison with SAS ver. 6.12 All values were expressed in mean ± standard error of mean and $p < 0.05$ was considered significant.

Methods - VOP protocol

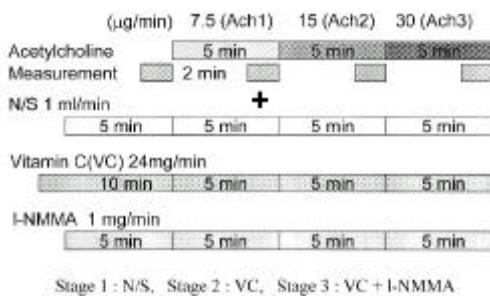


Figure 1. Methods ; venous occlusion plethysmography Acetylcholine was infused intra-arterially at 7.5, 15 and 30 g/min for 5 minutes for each dose level and forearm blood flow was measured during the last 2 minutes of each dose. Forearm blood flow response to acetylcholine was assessed during the infusion of dextrose solution (stage 1), intra-arterial infusion of vitamin C (stage 2) and intra-arterial co-infusion of vitamin C and L-NMMA (NG-monomethyl-L-arginine (stage 3).

RESULTS

Eight hypertensive patients and eight normotensive controls (age range 35 to 73 years) were recruited. There were not any significant differences in cholesterol levels between normotensive controls and hypertensive patients. Only systolic and diastolic blood pressure differed in the two groups (Table 1). After 2-month antihypertensive treatment, there were significant decreases in systolic and diastolic blood pressure (mean blood pressure ; 169/98 mmHg 131/74 mmHg, $p < 0.05$).

Absolute FBF data recorded in the infused limbs at baseline and during the infusion of acetylcholine at three dose levels before and after antihypertensive treatment are presented in Table 2.

Table 2. Absolute forearm blood flow responses in normal control and hypertensive patients

	NC	HT	post-treatment
Basal	4.2 ± 0.6	5.7 ± 0.4	4.9 ± 0.3
Ach1	9.2 ± 1.8	7.2 ± 0.6	9.7 ± 3.0
Ach2	12.9 ± 1.9	12.1 ± 1.2	13.8 ± 3.2
Ach3	17.6 ± 1.9	15.9 ± 1.6	26.1 ± 4.3

Values are means ± SEM in ml/100 ml forearm/min. NC, normal control; HT, hypertensive patients group; Ach1, acetylcholine infusion with 7.5 µg/min; Ach2, acetylcholine infusion with 15 µg/min; Ach3, acetylcholine infusion with 30 µg/min

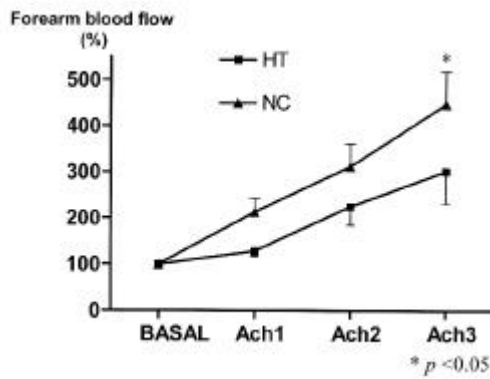


Figure 2. FBF responses to three doses of acetylcholine in normal control and hypertensive patients. Values are mean ± SEM. Ach1, Ach2 and Ach3 represent 7.5, 15 and 30 µg/min, respectively.

FBF in HT and NC groups

Endothelium-dependent vasorelaxation (vasodilatory response to acetylcholine) was significantly greater in normal control group compared to hypertensive group before antihypertensive treatment (maximum forearm blood flow in NC : 448 ± 63%, HT: 302 ± 58%, $p < 0.05$, Figure 2).

FBF response to intra-arterial vitamin C infusion

Forearm blood flow response to acetylcholine was significantly enhanced with intra-arterial infusion of vitamin C in hypertensive group before antihypertensive treatment (maximum forearm blood flow in Vit C (-) : 302 ± 58%, Vit C (+) : 446 ± 43%, $p < 0.05$, Figure 3A). Such an enhanced response was not observed in normal

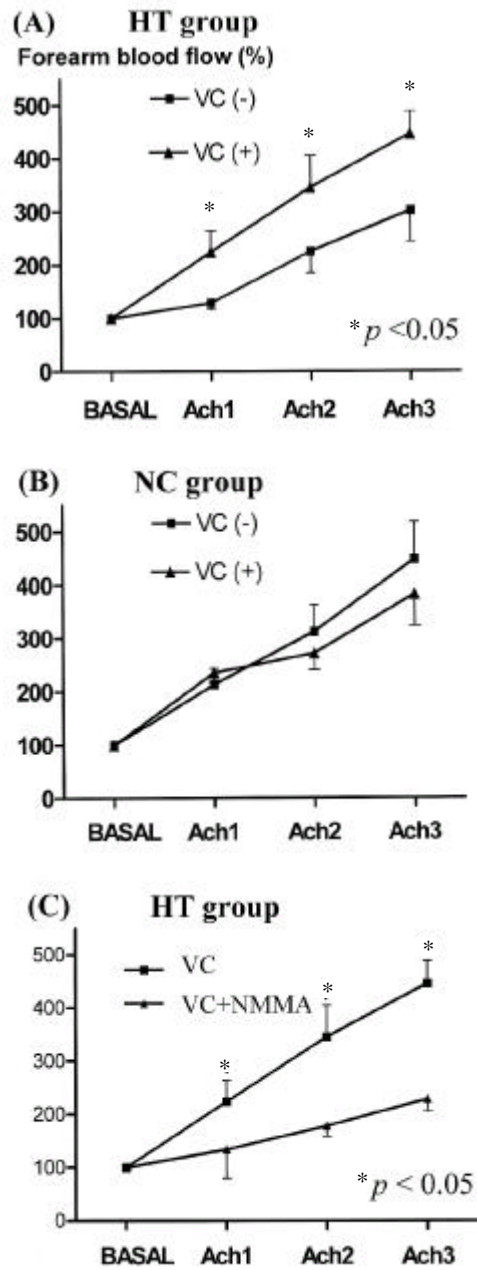


Figure 3. Effect of vitamin C and NMMA on the FBF response to acetylcholine.

control group (maximum forearm blood flow in Vit C (-) : 448 ± 63%, Vit C (+) : 383 ± 51%, $p = 0.11$, Figure 3B). Co-infusion of L-NMMA, an inhibitor of nitric oxide synthase, blunted forearm blood flow response to acetylcholine (maximum forearm blood flow in Vit C (+) :

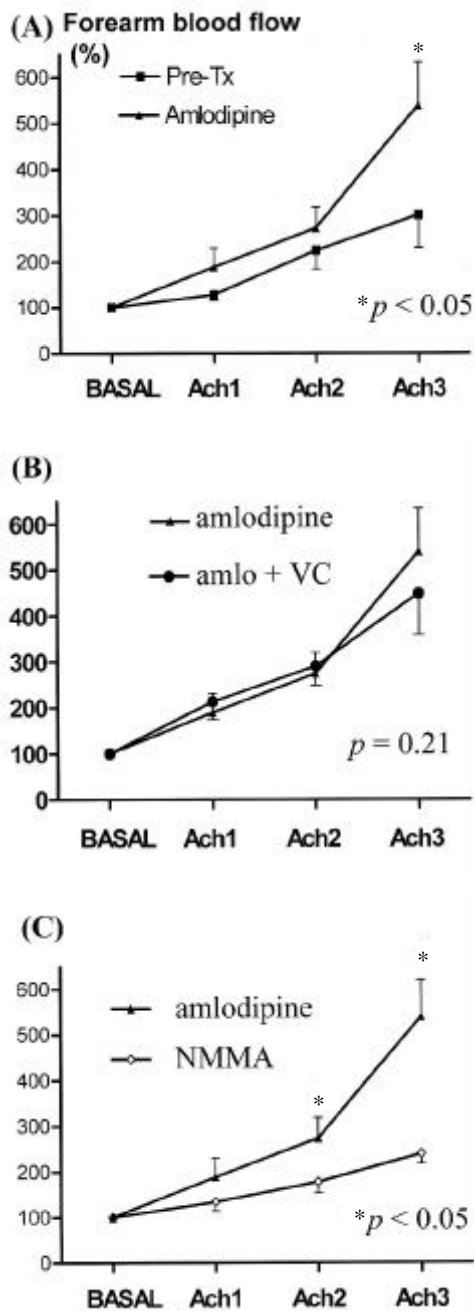


Figure 4. Effect of amlodipine treatment on the vasodilatory response to acetylcholine (A). Intra-arterial infusion of vitamin C in amlodipine treated hypertensive group did not change the forearm blood flow response to acetylcholine (B). Co-infusion of L-NMMA, an inhibitor of nitric oxide synthase, blunted forearm blood flow response to acetylcholine (C).

446 ± 43%, Vit C + L-NMMA (+) : 229 ± 23%, *p* < 0.05, Figure 3C).

FBF after antihypertensive treatment in HT group

After antihypertensive treatment with amlodipine for 2 months in hypertensive group, endothelium-dependent vasorelaxation (vasodilatory response to acetylcholine) was significantly improved in amlodipine treated group compared to before treatment (maximum forearm blood flow in before treatment : 302 ± 58%, after treatment: 539 ± 81%, *p* < 0.05, Figure 4A). Intra-arterial infusion of vitamin C in amlodipine treated hypertensive group did not change the forearm blood flow response to acetylcholine (maximum forearm blood flow in Vit C (-) : 539 ± 81%, Vit C (+) : 448 ± 78%, *p* = 0.21, Figure 4B).

Co-infusion of L-NMMA, an inhibitor of nitric oxide synthase, blunted forearm blood flow response to acetylcholine in amlodipine treated hypertensive group (maximum forearm blood flow in L-NMMA (-) : 539 ± 81%, L-NMMA (+) : 240 ± 22%, *p* < 0.05, Figure 4C).

DISCUSSION

The present study demonstrates that the endothelium-dependent vasodilation is impaired in essential hypertensive patients as compared with normotensive control subjects. In addition, short-term, intra-arterial administration of the antioxidant vitamin C restores endothelium-dependent vasodilation in patients with essential hypertension. A nitric oxide (NO) synthase inhibitor, L-NMMA, blunted the improvement of endothelium-dependent vasodilation. These findings suggest that oxygen-derived free radicals may decrease the bioavailability of endothelium-derived nitric oxide and impair endothelium-dependent vasodilation in patients with essential hypertension. These results were consistent with previous observations that, in essential hypertensive patients, impaired endothelium-dependent vasodilation of forearm circulation could be improved by the antioxidant vitamin C^{3, 18}.

Solzbach et al demonstrated that impaired endothelium-dependent vasodilatory function of human coronary arteries in hypertensive patients could be improved by the administration of the antioxidant vitamin C¹⁹.

Assessment of the clinical relevance of the present results should take into account the fact that superoxide anion production has been found to cause endothelial dysfunction in the presence of several cardiovascular

risk factors. In patients with coronary artery disease, both single dose (2 g PO) and long-term (500 mg/d) administration of vitamin C reverse endothelial vasomotor dysfunction in the brachial circulation^{20, 21}.

With regard to mechanism, it has been proposed that ascorbic acid improves NO action by scavenging superoxide anion and preventing inactivation of NO. This explanation is attractive because atherosclerosis and hypercholesterolemia are linked to excess generation of superoxide, and superoxide reacts with NO and eliminates its biological activity²².

In addition to scavenging superoxide anion or inhibiting LDL oxidation, vitamin C could alternatively improve NO action by sparing intracellular glutathione which, together with vitamin C, is the primary regulator of intracellular redox state.

In essential hypertension, impaired endothelium-dependent vasodilation seems to be a primary phenomenon and the endothelial vasomotor dysfunction is not normalized by the mere reduction of blood pressure^{12, 23, 24}.

Several investigators have addressed the possibility that antihypertensive treatment could restore or at least improve endothelial function.

Calcium antagonists have been shown to be effective in reversing endothelial dysfunction of angiographically normal and stenotic epicardial coronary vessels in essential hypertension²⁵. In agreement with these results, calcium antagonists also show a beneficial effect on endothelial function in the forearm microcirculation²⁶. But several other studies have demonstrated that the treatment with long-term calcium antagonist did not improve forearm vasodilator response to reactive hyperemia^{9, 10}. Hirooka et al also reported that single dose administration of nifedipine did not alter forearm vasodilation with acetylcholine in hypertensive patients²⁷.

In our findings, prolonged (8 weeks of oral treatment) amlodipine administration could improve endothelium-dependent vasodilation in essential hypertensive patients. Intra-arterial infusion of vitamin C to the treated patients did not increase forearm vasodilation in response to acetylcholine. L-NMMA blunted the effect of amlodipine on endothelium-dependent vasodilation. These data indicate that amlodipine is effective in improving endothelial dysfunction in essential hypertension and amlodipine appears to act specifically on the NO pathway by a mechanism that is probably related to antioxidant activity. Experimental data indicate that calcium antagonists exert an antioxidant effect and

therefore, could protect endothelial cells against free radical injury and diminish oxidative breakdown of NO²⁸.

An insufficient blood pressure reduction with an antihypertensive drug with antioxidant activity would not improve the endothelial function. So, a sufficient blood pressure reduction with an antihypertensive drug with antioxidant activity would be required. All the hypertensive patients in this study have normalized their systolic and diastolic blood pressure.

CONCLUSION AND IMPLICATIONS

Even though the relative importance of the various possible mechanisms leading to depressed endothelial function in essential hypertension remains to be elucidated, our study shows that vitamin C or amlodipine result in demonstrable improvement by a mechanism that is probably related to antioxidant activity.

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