Clin. Cardiol. 24, 705–709 (2001)

Clinical Investigations

Effects of Low-Dose Aspirin on Endothelial Function in Hypertensive Patients

HIDEAKI MONOBE, M.D., HIROSHI YAMANARI, M.D., KAZUFUMI NAKAMURA, M.D., TOHRU OHE, M.D.

Department of Cardiovascular Medicine, Okayama University Medical School, Okayama, Japan

Summary

Background: It has been reported that administration of low-dose aspirin significantly reduces the frequency of major cardiovascular events in patients with hypertension and coronary artery disease. It is generally considered that the preventative effects of long-term aspirin administration on major cardiovascular events are due to the inhibition of platelet aggregation.

Hypothesis: It is not known whether administration of low-dose aspirin restores endothelium-dependent vasodilatation, and this study was undertaken to prove or disprove this question in patients with hypertension.

Methods: Flow-mediated endothelium-dependent dilatation and glyceryl trinitrate-induced endothelium-independent dilatation were investigated in 18 hypertensive patients and 10 normotensive control subjects. In the hypertensive patients, flow-mediated dilatation was investigated and cyclic guanosine monophosphate plasma (cGMP) was measured before and at 8 weeks after the administration of 162 mg of aspirin.

Results: Flow-mediated dilatation before aspirin administration was more reduced in the hypertensive patients than in the control subjects ($6.4 \pm 2.0\%$ vs. $11.3 \pm 2.3\%$, $p < 0.0001$). Glyceryl trinitrate-induced dilatation before aspirin administration was similar in hypertensive patients and control subjects. Flow-mediated dilatation after aspirin administration was improved compared with that before aspirin administration ($10.4 \pm 3.5\%$ vs. $6.4 \pm 2.0\%$, $p < 0.0004$). The cGMP product after aspirin administration was significantly higher than that before aspirin administration.

Conclusions: Administration of low-dose aspirin may restore the endothelium-dependent vasodilatation in hypertensive patients. Furthermore, increased nitric oxide production may play a partial role in the improvement in endothelial function induced by administration of low-dose aspirin.

Key words: endothelial function, hypertension, aspirin, nitric oxide, cyclic guanosine monophosphate plasma

Address for reprints:

Hiroshi Yamanari, M.D.
Department of Cardiovascular Medicine
Okayama University Medical School
2-5-1 Shikata-cho
Okayama 700-8558, Japan
e-mail: hyama@mx31.tiki.ne.jp

Received: October 3, 2000
Accepted with revision: February 15, 2001

Introduction

In patients with hypertension, endothelium-dependent dilatation assessed by the response to acetylcholine or to increased blood flow (flow-mediated dilatation) is impaired not only in peripheral resistant vessels but also in conduit arteries such as the epicardial coronary artery or the brachial artery.[1–4] Recent studies[5,6] have shown that acute inhibition of cyclooxygenase in hypertensive patients restored endothelium-dependent vasomotion to reduce cyclooxygenase-dependent contract prostanoids such as thrombxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2), or superoxide anion impaired nitric oxide (NO) production.

Aspirin in various dosages ranging from 75 to 1,500 mg/day has been used to reduce the incidence of stroke and myocardial infarction when given long-term to healthy individuals or patients with previous cardiovascular events.[7,8] However, high-dose aspirin prevents the formation of prostacyclin in vascular endothelial cells as well as TXA_2 in platelets via cyclooxygenase inhibition. The Hypertension Optimal Treatment (HOT) study[9] showed that low-dose aspirin (75 mg/day) significantly reduced the frequency of major cardiovascular events in patients with hypertension. It is generally considered that long-term aspirin administration prevents major cardiovascular events through inhibition of platelet aggregation. However, it is still unknown whether administration of low-dose aspirin restores endothelium-dependent vasodilatation to reduce TXA_2 and PGH_2 or superoxide anion without inhibition of prostacyclin.

The purpose of the present study was to evaluate the effects of low-dose aspirin administration on endothelial function in hypertensive patients. We also investigated whether NO participated in the changes in endothelium-dependent vasodilatation.

Methods

Subjects

The subjects included 18 (10 men, 8 women, age 65 ± 8 years, range 52–79 years) patients with essential hypertension. The duration of hypertension in the patients ranged from 2 to 20 years (average 10 ± 5 years). All patients were treated with manidipine (20–40 mg/day) for at least 6 months, and 14 of 18 patients were also treated with metoprolol (60–120 mg/day) for at least 6 months. None of the patients had ischemic heart disease, arteriosclerosis obliterans, cerebrovascular disease, or diabetes mellitus. All patients had normal renal function as evidenced by normal serum creatinine and negative results of urinalysis. Five patients had hyperlipidemia, and they had been treated with simvastatin or pravastatin for more than 6 months. Patients smoking more than five cigarettes per day were excluded from the study; four patients smoked less than five cigarettes per day. The patients took their antihypertensive and antihyperlipidemic medications as usual in the morning of each day during the study period. The age- and gender-matched control subjects (6 men, 4 women; average age 60 ± 8 years, age range, 51–72 years) were normotensive. Normalcy was determined by careful examination of history, physical examination, and laboratory analysis. Four subjects in the control group smoked less than five cigarettes per day. This study was performed after informed consent had been obtained from each subject according to the Declaration of Helsinki.

Study Design

The ultrasound method for measuring endothelium-dependent and -independent arterial dilatation has been described in detail by another group.[10] B-mode ultrasound images of the target artery were obtained using a commercially available device (SONOS 1500, Hewlett-Packard, Andover, Mass., USA) equipped with a 7.5 MHz linear array transducer. In all patients, first and second scans were performed, respectively, before and at 8 weeks after the administration of 162 mg/day of aspirin (Lion Corp., Tokyo, Japan). Both scans were taken in patients and controls at rest, during reactive hyperemia, again at rest, and after sublingual administration of glyceryl trinitrate (GTN). The patients and control subjects rested in recumbent position for at least 15 min before scans. The right brachial artery was scanned in longitudinal section 2 to 5 cm above the elbow, wherever the clearest ultrasound image could be obtained. The center of the artery was identified when the clearest picture of the anterior and posterior intimal layers was obtained. The transmit focus zone was set to the depth of near wall. Depth and gain settings were set to optimize images of the lumen/arterial wall interface. Images were magnified using a zoom function, and machine operating parameters were not changed during the study.

When a satisfactory transducer position was found, the skin was marked. A resting scan was obtained, and the velocity of arterial flow was measured with a pulse-Doppler signal at a 60° angle to the vessel, with the gate length (0.5 mm) in the center of the artery. Increased flow was then induced by inflation of a pneumatic cuff placed around the forearm to a pressure of 200 mmHg for 5 min, followed by release. A hyperemic scan was performed continuously for 30 s before and 90 s after deflation of the cuff, including a repeated recording of flow velocity for the first 15 s after the cuff was released. Three hundred μg of GTN was sublingually administered by one puff of a spray device (Myocor Spray, Toa Eiyo Co., Ltd., Tokyo, Japan), and the last scan was performed 3 to 4 min after the administration of GTN. All scans were recorded on super-VHS videotape together with an electrocardiogram (ECG) at lead II.

Blood was drawn from the right median cubital vein of each patient and each control subject for measurement of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and C-reactive protein (CRP) before and after administration of aspirin. Immediately after both the first resting scan and the hyperemic scan, blood was drawn for measurement of plasma cGMP both at the time of first and second scans. Plasma cGMP levels were measured as an indirect assay of NO. The ratio of plasma cGMP at the time of the hyperemic scan to that at the time of the first resting scan (cGMP product) was calculated to represent the production of cGMP during reactive hyperemia.

Assays

Samples of venous blood were placed in tubes containing ethylene diamine tetraacetic acid (EDTA)-Na (1 mg/ml). The tubes containing EDTA were promptly chilled in an ice bath. Plasma and serum were immediately separated by centrifugation at 3,100 g for 10 min at 4°C and at 1,000 g for 10 min at room temperature, respectively, and were stored at −80°C until assayed. Serum concentrations of LDL-C, HDL-C, and CRP were determined by routine chemical methods. The plasma level of cGMP was measured by an enzyme immunoassay using a cGMP kit (R & D Systems, Inc., Minneapolis, Minn., USA).

Data Analysis

The end-diastolic images in the scan images recorded on super-VHS videotape were preserved as digitized images in an offline image analyzing computer (Cardio 500, Kontron Elektronik, Munich, Germany). The diameters of the vessels in the digitized images were measured using the software installed in Cardio 500 for automatic detection of vessel wall (Fig. 1).[11] Arterial diameter was measured from a fixed anatomic marker in all scans of the same patients. Three cardiac cycles were measured for each scan and the measurements were averaged. Measurements were taken from the anterior "m" line (media–adventitia interface) anteriorly to the leading edge of the intima–lumen interface of the posterior wall at end diastole, incident with the R wave on the ECG. Endothelial function was assessed by comparing endothelium-dependent vasodilatation in response to flow (induced by reactive hyperemia) with endothelium-independent response to sublingual

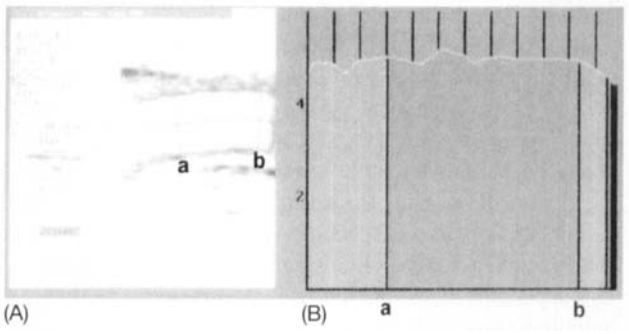

FIG. 1 Measurement of vessel diameter in the control subjects using an analyzing computer, Cardio 500. (A) Digitized vascular echo image, and (B) the result of distance measurement. The average value of the distance between line a and line b was used to calculate vessel size.

administration of GTN. The vessel diameters in the digitized images obtained after reactive hyperemia (flow-mediated dilatation) and the sublingual administration of GTN (GTN-induced dilatation) were expressed as a percentage of the diameter of the artery in the first resting scan (considered as 100%). Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle) by the heart rate and the cross-sectional area of the vessel.

$$\left(\frac{\pi D^2}{4}\right)$$

The flow velocity used in the calculation was measured in the center of the artery. Reactive hyperemia was calculated as the maximal flow recorded in the first 15 s after cuff deflation divided by the flow during the resting scan.

Interobserver variability was determined by calculating the mean and standard deviation (SD) of the difference in the two observers' results from 20 arterial studies. The interobserver variability for measurement of flow-mediated dilatation was 0.3 ± 1.8 %.

TABLE I Characteristics of the study patients

	Hypertensive patients		Control subjects
	Before aspirin	After aspirin	
Mean BP (mmHg)	93 ± 16	98 ± 11	90 ± 9
Heart rate (/min)	64 ± 7	62 ± 8	71 ± 13
LDL-C (mg/dl)	108 ± 27	108 ± 24	126 ± 19
HDL-C (mg/dl)	54 ± 16	53 ± 14	58 ± 14
CRP (mg/dl)	0.53 ± 0.12[a]	0.34 ± 0.11	0.11 ± 0.19
cGMP product	1.07 ± 0.29	3.09 ± 2.40[b]	2.72 ± 1.90[b]
Vessel size (mm)	4.22 ± 0.6	4.15 ± 0.49	4.06 ± 0.43
Reactive hyperemia (%)	323 ± 118	367 ± 130	386 ± 132

[a] $p < 0.02$ vs. control subjects.

[b] $p < 0.05$ vs. before aspirin.

Abbreviations: BP = blood pressure, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, CRP = C-reactive protein, cGMP = cyclic guanosine monophosphate.

Statistical Analysis

Data are expressed as means ± SD. Comparison between patients with hypertension and control subjects was performed by an unpaired Student's *t*-test. A paired Student's *t*-test was used to analyze differences in the parameters before and after administration of aspirin. A p value < 0.05 was considered to be statistically significant.

Results

Basal-Line Characteristics

Mean blood pressures and heart rates during the first resting scan were similar in the hypertensive patients and control subjects, as were LDL-C and HDL-C. However, CRP was higher in the hypertensive patients than in the control subjects (Table I).

Comparison of Vascular Responses in the Hypertensive Patients and Controls before Aspirin Administration

The degrees of reactive hyperemia and the vessel sizes at the resting scan were similar in the hypertensive patients and control subjects. Flow-mediated dilatation was more reduced in the hypertensive patients than in the control subjects (6.4 ± 2.0%, 11.3 ± 2.3%, respectively, $p < 0.0001$) (Fig. 2). Glyceryl trinitrate-induced dilatation and vessel diameter at the first resting scan were similar in hypertensive patients and control subjects (12.7 ± 3.7%, 15.1 ± 3.0%, respectively) (Fig. 2).

Comparison of Vascular Responses in Hypertensive Patients after Aspirin Administration

Vessel sizes at the time of the resting scan, the degrees of reactive hyperemia, mean blood pressures, heart rates, and ser-

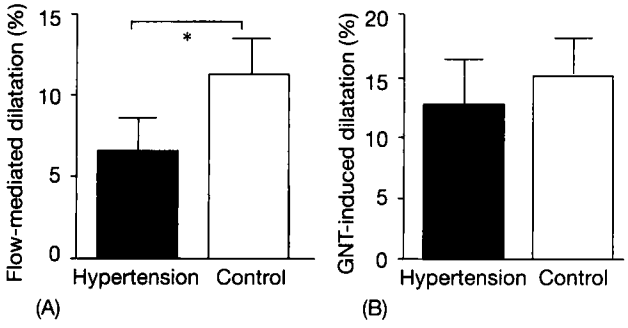

FIG. 2 (A) Flow-mediated dilatation in hypertensive patients before aspirin administration and in control subjects. It was significantly impaired compared with that in control subjects. (B) Glyceryl trinitrate (GTN)-induced dilatation in hypertensive patients and in control subjects before aspirin administration. There were no differences between GTN-induced dilatations in hypertensive patients and in control subjects before aspirin administration. * $p < 0.0001$. GNT = glyceryl trinitrate.

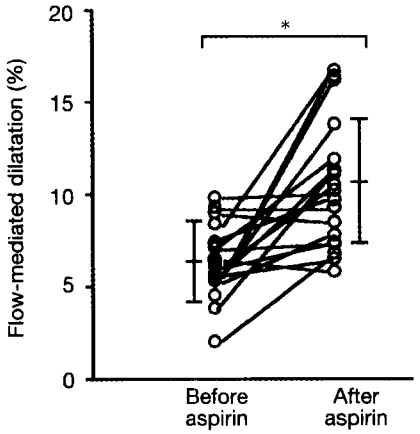

FIG. 3 Flow-mediated dilatation before and after administration of low-dose aspirin in hypertensive patients; it was improved compared with that before administration of aspirin. * $p < 0.0004$.

um CRP, LDL-C, and HDL-C concentrations were similar before and after aspirin administration (Table I). Flow-mediated dilatation was improved after aspirin administration compared with that before aspirin administration ($10.4 \pm 3.5\%$ vs. $6.4 \pm 2.0\%$, $p < 0.0004$) (Fig. 3). The cGMP product after aspirin administration was significantly higher than that before aspirin administration, and was similar in hypertensive patients and control subjects after aspirin administration (Table I). There was no correlation between the change in cGMP product and the change in flow-mediated dilatation before and after aspirin administration.

Discussion

The major findings of the present study were that (1) flow-mediated dilatation was more impaired in the patients with hypertension than in the control subjects, (2) flow-mediated dilatation in the patients with hypertension was improved after administration of low-dose aspirin, and (3) cGMP product after aspirin administration was significantly higher than that before aspirin administration. These data suggest that the increased level of NO production as a result of cyclooxygenase inhibition may play a role in the improvement in endothelial function induced by administration of low-dose aspirin.

Endothelium-dependent vasodilatation is reduced in patients with hypertension compared with that in normotensive control subjects. Several mechanisms have been proposed to explain the impaired endothelium-dependent vasodilatation in hypertension, including alterations in endothelial signal transduction, reduced availability of L-arginine, modification of the expression of endothelial NO synthase (eNOS), altered availability of cofactors for eNOS, increased destruction of NO by reactive oxygen species, intimal thickening, reduced response of vascular smooth muscle to NO, and production of endothelium-dependent contracting factors.[12–16] It has been reported that cyclooxygenase inhibition due to administration of aspirin restored endothelium-dependent vasodilatation in patients with hypertension and hypercholesterolemia, but not in the normotensive controls.[5,6,16] These findings suggested that cyclooxygenase-dependent substances such as TXA_2, PGH_2, and superoxide anions caused NO destruction and that reduced bioavailability of NO led to impaired endothelium-dependent vasodilatation. However, in a previous study, only the administration of a single high-dose of aspirin was used to investigate the role of cyclooxygenase inhibition in endothelium-dependent dilatation. Since high-dose aspirin is known to inhibit the production of prostacyclin as well as TXA_2 or endoperoxide, low-dose aspirin administration that did not inhibit the production of prostacyclin has generally been used to prevent the occurrence of cardiovascular diseases. However, it is not known whether low-dose aspirin reduces NO destruction as a result of inhibition of cyclooxygenase-dependent substances and restores the bioavailability of NO. In the present study, administration of aspirin at a dosage of 162 mg/day for 8 weeks improved flow-mediated vasodilatation in hypertensive patients. The plasma cGMP level indicated that NO production had increased significantly after aspirin administration. These findings suggest that at least one mechanism of the improved flow-mediated vasodilatation is the effect of restored NO availability.

Several investigators have examined the role of cyclooxgenase-dependent factors in endothelium-dependent dilatation in patients with hypertension using a single high dose of a cyclooxygenase inhibitor. However, their results have been far from consistent. Taddei *et al.*[5] and Husain *et al.*[6] reported that cyclooxygenase inhibition due to the administration of indomethacin or aspirin restored endothelial-dependent vasodilatation. In contrast, Imaizumi *et al.*[17] found that endothelium-dependent forearm blood flow in response to administration of acetylcholine did not change despite aspirin administration. The discrepancies between the results of previous studies may be due to the rather small number of patients and the wide spectrum of hypertension. Various degrees of improvement in endothelial-dependent vasodilatation were found in previous studies. In the present study, administration of low-dose aspirin restored endothelium-dependent vasodilatation in hypertensive patients up to that in the control subjects. This effect of administration of low-dose aspirin may be due to the reduction of cyclooxygenase-dependent contract prostanoids following the preservation of prostacyclin. However, the change of cyclooxygenase-dependent prostanoids after aspirin administration was not evaluated in the present study. Therefore, further study is required to clarify the participation of prostanoids on the effect of administration of low-dose aspirin in hypertension.

Study Limitations

There are several limitations to the present study. The number of patients investigated was relatively small; thus, care must be taken in the extrapolation of our results to other populations. The flow-mediated dilatation in the patients with hypertension was measured during administration of antihyper-

tensive and antihyperlipidemic medications in the present study, and therefore we could not completely exclude the possible effects of those medications on the flow-mediated dilatation. However, these medications were not changed during the present study. Therefore, the results of the present study suggest that the difference between flow-mediated dilatations before and after aspirin administration was caused by administration of low-dose aspirin.

Conclusion

Administration of low-dose aspirin may restore the endothelium-dependent vasodilatation in hypertensive patients. Furthermore, increased nitric oxide production may play a partial role in the improvement in endothelial function induced by administration of low-dose aspirin.

References

1. Panza JA, Quyyumi AA, Brush JE, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22–27
2. Linder L, Kiowski W, Buhler FR, Luscher TF: Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. *Circulation* 1990;81:1762–1767
3. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE: Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994;24:1468–1474
4. Antony I, Lerebours G, Nitenberg A: Loss of flow-dependent coronary artery dilatation in patients with hypertension. *Circulation* 1995;91:1624–1628
5. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A: Cyclooxygenase inhibition restores nitric oxide activity in essential hypertension. *Hypertension* 1997;29:274–279
6. Husain S, Andrews NP, Mulcahy D, Panza JA, Quyyumi AA: Aspirin improves endothelial dysfunction in atherosclerosis. *Circulation* 1998;97:716–720
7. Becker RC: Antiplatelet therapy in coronary heart disease: Emerging strategies for the treatment and prevention of acute myocardial infarction. *Arch Pathol Lab Med* 1993;117:89–96
8. Juul-Moller S, Edvardsson N, Jahnmatz B, Rosen A, Sorensen S, Omblus R: Double-blind trial of aspirin in primary prevention of myocardial infarction in patients with stable chronic angina pectoris: The Swedish Angina Pectoris Aspirin Trial (SAPAT) Group. *Lancet* 1992;340:1421–1425
9. Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, Menard J, Rahn KH, Wedel H, Westerling S: Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: Principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet* 1998;351:1755–1762
10. Celermajer DS, Sorensen KE, Gooch VM, Spoegelhalter DJ, Miller OI, Sullivan I, Lloyd JK, Deanfield JE: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111–1115
11. Hausleiter J, Jost S, Nolte CWT, Dirschinger J, Kastrati A, Stiel GM, Wunderlich W, Fischer F, Linderer T, Hausmann D, Schomig A: Comparative in-vitro validation of eight first- and second-generation quantitative coronary angiography systems. *Coron Artery Dis* 1997;8:83–90
12. Busse R, Fleming I: Endothelial dysfunction in atherosclerosis. *J Vasc Res* 1996;33:181–194
13. Shimokawa H, Vanhoutte PM: Endothelium and vascular injury in hypertension and atherosclerosis. In *Handbook of Hypertension* (Eds. Zanchetti A, Marcia G), p. 1001–1065. New York: Elsevier Science, 1997
14. Harrison DG: Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest* 1997;100:2153–2157
15. Shimokawa H: Primary endothelial dysfunction: Atherosclerosis. *J Moll Cell Cardiol* 1999;31:23–37
16. Noon JP, Walker BR, Hand MF, Webb DJ: Impairment of forearm vasodilatation to acetylcholine in hypercholesterolemia is reversed by aspirin. *Cardiovasc Res* 1998;38:480–484
17. Imaizumi T, Yoshida M, Hirooka Y: Defective endothelium-dependent vasodilation in patients with essential hypertension. In *Recent Progress in Cardiovascular Mechanics* (Ed. Hosoda S), p. 333–348. Switzerland: Harwood Academic Publishers, 1994