Lipids, Lipoproteins and Fibrinolytic Parameters During Amlodipine Treatment of Hypertension

Joseph Eberendu Ahaneku,*[a, 1] Kazuyuki Sakata,[c] Tetsumei Urano,[a] Yumiko Takada,[b] and Akikazu Takada[a]

[a]Department of Physiology and [b]Department of Pathophysiology, Hamamatsu University School of Medicine, 3600, Handa-cho, Hamamatsu-shi, Shizuoka 431–3192, Japan, and [c]Department of Cardiology, Shizuoka General Hospital, 4–27–1, Kita-andou, Shizuoka-shi, Shizuoka 420–0881, Japan

(Received May 29, 2000; Accepted July 10, 2000)

In order to determine the safety or otherwise of amlodipine, we evaluated lipid and lipoprotein indices as well as fibrinolytic parameters in Japanese hypertensive patients undergoing amlodipine treatment. Lipids, lipoproteins, tissue plasminogen activator (t-PA), total and free plasminogen activator inhibitor-1 (PAI-1) and t-PA-PAI-1 complexes were determined in twenty-seven patients with essential hypertension before and after 3 months of amlodipine treatment. Plasma renin and noradrenaline levels were also determined. The mean systolic and diastolic blood pressures and heart rate were reduced, while the plasma renin and noradrenaline levels remained unchanged after amlodipine therapy. Triglycerides, very low density lipoprotein- cholesterol (VLDLC), total and free PAI-1 levels were significantly reduced, while the levels of total cholesterol (TC), high density lipoprotein-cholesterol (HDLC), low density lipoprotein-cholesterol (LDLC), HDLC/TC ratio, t-PA and t-PA-PAI-1 complex did not change from their pretreatment values after amlodipine treatment. There was a significant positive correlation between HDLC and t-PA-PAI-1 complex levels after amlodipine treatment. The findings from the present study show that amlodipine is effective for the treatment of hypertension and does not cause reflex tachycardia in Japanese patients. We also found that amlodipine treatment is a safe antihypertensive agent characterized by beneficial lipid changes and enhanced fibrinolysis in Japanese hypertensive patients. The direct relationship between lipid levels and fibrinolytic function seen in this study has added to our understanding and knowledge of the pathogenesis of atherosclerosis during antihypertensive pharmacotherapy.

Key words —— amlodipine therapy, lipid, lipoprotein, fibrinolytic function, hypertensive patient

INTRODUCTION

Amlodipine belongs to the 1,4-dihydropyridine family and is structurally related to nifedipine. The clinical pharmacology of the antihypertensive action of amlodipine involves a direct relaxant effect on vascular smooth muscle. The effectiveness of amlodipine in lowering raised blood pressures in hypertensive patients has been demonstrated in various populations.[1,2] Studies on lipid and lipoprotein atherogenic side effect profiles of amlodipine[3] and other calcium channel blockers[4–6] have been documented, and some of the results from these previous reports are controversial. Some of the previous researchers[3,5] showed that amlodipine or nifedipine either had a beneficial or no effect on lipid and lipoprotein levels, while Trost and Weildmann[6] observed that calcium antagonist therapy may induce adverse changes in lipid levels. Information on the effects of calcium channel antagonists on fibrinolytic function in hypertensive patients is scanty. The few reports available show that isradipine either increased fibrinolytic activity[7,8] or had no effect on fibrinolysis.[8] Meanwhile, detailed reports on fibrinolytic function during amlodipine treatment of hypertension are yet to emerge.

The present study was therefore designed to comprehensively evaluate lipid and lipoprotein indices, as well as fibrinolytic parameters, in Japanese hypertensive patients undergoing amlodipine therapy. The results are intended to reveal the safety or otherwise of amlodipine therapy.

*To whom correspondence should be addressed: Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, P.M.B. 5001 Nnewi, Anambra State, Nigeria. Tel.: +234-46-463663; Fax: +234-46-460124; E-mail: chsnne@infoweb.abs.net

[1]Present address: Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, P. M. B. 5001 Nnewi, Anambra State, Nigeria.

MATERIALS AND METHODS

Patient Selection —— Twenty-seven (17 males and 10 females) Japanese patients aged 45–79 years with mild to moderate essential hypertension, who were referred for cardiac catheterization because of chest pain and/or electrocardiographic abnormalities which revealed normal coronary artery without spasm and normal cardiac function, were selected into this study. All the hypertensive patients were newly diagnosed and had not been previously exposed to antihypertensive therapy. The diagnosis of essential hypertension was based on the WHO criteria for mild to moderate essential hypertension as described previously.[9] All the patients had their diastolic and systolic blood pressures measured in the sitting position throughout the study period. The patients were not on any dietary regulation throughout the study period. The female patients were neither pregnant nor lactating mothers nor on any oral contraceptives. Upon selection into the study, the patients were placed on oral intake of 5–10 mg amlodipine per day for the entire study period of 3 months. All the patients that had diabetes mellitus or any of the complications of hypertension such as heart failure, stroke and renal failure were excluded from this study. The patients were not on any other drugs throughout the 3-month study period. In this study, each patient was his/her own control. Before and at the end of 3 months of amlodipine therapy, blood samples were collected from all study patients. Informed consent was obtained from each patient before selection into the study. The study protocol was approved by the ethical committee of Shizuoka General Hospital, Shizuoka, Japan.

Coronary Angiography —— Coronary angiography with the acetylcholine provocation test was performed using the standard Judkins technique in all patients.

Sample Collection, lipid, Lipoprotein and Fibrinolytic Function Determination —— At the beginning and after 3 months of amlodipine therapy, after at least 12 h of overnight fasting, blood samples were collected after the patients had been lying undisturbed in a supine position for at least 10 min. Blood was drawn by venipuncture. After collection, the first few milliliters of blood were always discarded. The blood was immediately drawn into three separate polypropylene syringes for the measurement of lipids, lipoproteins, fibrinolytic parameters, renin activity and noradrenaline levels. Blood samples intended for lipid and lipoprotein estimations were collected into sterile containers and allowed to clot, centrifuged, and thereafter the sera were separated and analyzed immediately. For the determination of fibrinolytic parameters, 9 parts of blood were mixed with 1 part of 3.13% sodium citrate solution. Plasma was separated by centrifugation at 3300 rpm for 15 min and was immediately frozen and stored at –70°C before analysis. The levels of total cholesterol (TC) and triglycerides (TG) were determined by enzymatic methods[10,11] (Determiner TC, TG diagnostic kits from Kyowa Medex, Tokyo), then direct measurement of high-density lipoprotein cholesterol (HDLC) in serum[12] was applied (Determiner HDLC diagnostic kits from Kyowa Medex, Tokyo) using an automated procedure on a Hitachi 7450 (Hitachi Medical, Tokyo, Japan). The values of low density lipoprotein-cholesterol (LDLC) and very low density lipoprotein-cholesterol (VLDLC) were evaluated as previously described.[3] Plasma levels of the antigens of tissue plasminogen activator (t-PA), total and free plasminogen activator inhibitor-1 (PAI-1), and t-PA-PAI-1 complexes were determined by enzyme immunoassay (EIA) as described previously.[13–15] Briefly, t-PA antigens were measured employing a sandwich method by using monoclonal anti-t-PA antibody as a first antibody and polyclonal anti-t-PA antibody as a second antibody. The t-PA-PAI-1 complex in plasma was measured using monoclonal anti-PAI-1 antibody as a first antibody. The t-PA part of the t-PA-PAI-1 complex combined with anti-PAI-1 antibody was then complexed with anti-t-PA antibody, so that only t-PA-PAI-1 complex was detected and measured. In order to measure total PAI-1, excess amounts of t-PA were added to the plasma prior to the assay, which converted all the PAI-1 to the form complexed with t-PA. The concentration of free PAI-1 in the plasma was calculated by subtraction of the concentration of t-PA-PAI-1 complex from that of total PAI-1. The assays of t-PA, PAI-1 and t-PA-PAI-1 complexes were performed in duplicate. Plasma noradrenaline concentration was determined by high performance liquid chromatography. Plasma renin level was assayed using a Gammacoat ^{125}I plasma renin activity radioimmunoassay kit (INCSTAR, Stillwater, MN U.S.A.).

Stastistical Analysis —— Changes in the measured variables before and after 3 months of amlodipine therapy were evaluated by a paired t-test. The relationships between lipids, lipoproteins and fibrinolytic parameters were explored using Spearman's rank correlation coefficient.

Table 1. Physical Characteristics, Blood Pressure and Hormonal Levels During Amlodipine Treatment of Hypertension

	Before therapy (n=27)	After therapy (n=27)	p Value (n=27)
Age (years)	62.5 (9.07)	62.5 (9.06)	0.99 NS
BMI (kg/m^2)	22.7 (2.84)	22.7 (2.85)	0.90 NS
Systolic BP (mmHg)	158.7 (12.2)	136.3 (12.9)	0.001**
Diastolic BP (mmHg)	98.5 (18.7)	84.4 (6.3)	0.001**
Heart rate (beats/min)	75.2 (7.1)	73.1 (6.8)	0.05*
Noradrenaline (ng/l)	229.4 (125.0)	240.4 (118.6)	0.53 NS
Renin (ng/dl)	0.73 (1.47)	0.88 (0.81)	0.61 NS

Values are expressed as mean ± standard deviation; standard deviation is given in parentheses. BMI = Body mass index; BP = Blood pressure; *$p < 0.05$; **$p < 0.001$; NS = Not significant. n = Number of patients.

Table 2. Levels of Lipids, Lipoproteins and Fibrinolytic Parameters before and after 3 Months of Amlodipine Treatment

	Before therapy (n=27)	After therapy (n=27)	p Value (n=27)
Total cholesterol (mmol/l)	5.07 (0.094)	5.0 (0.095)	0.55 NS
HDL-cholesterol (mmol/l)	1.40 (0.31)	1.37 (0.35)	0.39 NS
LDL-cholesterol (mmol/l)	3.03 (0.91)	3.10 (0.89)	0.56 NS
Triglycerides (mmol/l)	1.39 (0.89)	1.15 (0.54)	0.05*
VLDL-cholesterol (mmol/l)	0.28 (0.18)	0.23 (0.11)	0.05*
HDLC/TC	0.29 (0.07)	0.28 (0.07)	0.37 NS
t-PA (ng/ml)	7.20 (3.6)	7.40 (3.8)	0.79 NS
Total PAI-1(ng/ml)	15.7 (11.1)	11.7 (7.9)	0.01**
Free PAI-1 (ng/ml)	10.2 (10.2)	7.2 (6.9)	0.05*
t-PA-PAI-1complex (ng/ml)	5.2 (3.0)	5.3 (3.9)	0.91 NS

Values are expressed as mean ± standard deviation, the standard deviation is given in parentheses. *$p < 0.05$; **$p < 0.01$; NS = Not significant. HDL = High density lipoprotein; LDL = low density lipoprotein; VLDL = Very low density lipoprotein; TC = Total cholesterol; HDLC = High density lipoprotein cholesterol. t-PA = Tissue plasminogen activator; PAI-1 = Plasminogen activator inhibitor-1. n = Number of patients

RESULTS

As shown in Table 1, the mean systolic and diastolic blood pressures were significantly decreased ($p < 0.001$) after amlodipine treatment. Heart rate was also decreased ($p < 0.05$) after amlodipine therapy. The mean values of age, body mass index, renin and noradrenaline recorded after amlodipine treatment were not significantly different from their pretreatment values.

In Table 2, the mean values of triglycerides, VLDL-cholesterol ($p < 0.05$), total PAI-1 ($p < 0.01$) and free PAI-1 ($p < 0.05$) were significantly reduced after amlodipine treatment. The values of total cholesterol, HDL-cholesterol, LDL-cholesterol, HDLC/TC ratio, t-PA and t-PA-PAI-1 complex were not significantly different before and after amlodipine treatment. HDL-cholesterol positively correlated with t-PA-PAI-1 complex levels ($r = 0.446$; $p < 0.05$) after amlodipine treatment. No significant correlation was observed between the other lipid components and the fibrinolytic parameters.

DISCUSSION

The reduction in systolic and diastolic blood pressure showed that amlodipine was an effective antihypertensive agent in the treatment of hypertension in Japanese patients. The effectiveness of amlodipine therapy in reducing raised arterial blood pressurre has also been reported in Caucasian whites[1] and African hypertensives.[2] A decrease in heart rate observed in this study showed that amlodipine treatment was not associated with reflex tachycardia. Previous studies involving amlodipine therapy in a different population[2] and nitrendipine therapy in Japanese patients[16] showed that amlodipine had no effect and nitrendipine caused a non-significant increase in heart rate in hypertensive patients. The non-significant variation in the mean value of renin demonstrates that amlodipine has no direct effect on the renin-angiotensin system. The non-significant change in the mean values of total cholesterol, HDL-cholesterol, LDL-cholesterol and HDLC/TC ratio after amlodipine treatment in this study is consistent with previous findings by others.[3,16] However, the reduction in the mean values of triglycerides and VLDL-cholesterol after amlodipine treatment, which is at variance with reports by previous workers,[3,16] suggests that amlodipine therapy has beneficial lipid changes in Japanese hypertensive patients. A previous study in

the same population involving a different calcium channel blocker, nitrendipine, indicated a non-significant increase in serum triglyceride level in Japanese hypertensive patients.[17] This observation suggests that different calcium channel blockers seem to have variable effects on lipid levels in hypertensive patients, but this needs to be investigated further.

The significant reduction in total and free PAI-1 and the slight but non-significant increase in t-PA and t-PA-PAI-1 levels observed in this study showed that amlodipine caused enhanced fibrinolysis in Japanese hypertensive patients. The enhanced fibrinolytic activity may be due to a direct action of calcium channel blockade on vascular endothelium.[8] A previous report on nitrendipine treatment in Japanese hypertensive patients showed that nitrendipine caused impaired fibrinolysis (an increase in total PAI-1 activity),[17] further demonstrating the differential effects of these calcium channel blockers on the fibrinolytic system. Concerning the relationship between lipid levels and the fibrinolytic system in hypertensive patients, this study has shown that beneficial lipid change was related to enhanced fibrinolysis after amlodipine treatment in Japanese hypertensive patients, as confirmed by the positive correlation between HDL-cholesterol and t-PA-PAI-1 levels. The reductions in triglyceride and VLDL-cholesterol levels which coincided with similar reductions in total and free PAI-1 levels after amlodipine treatment in this study futher support the view that there is a complex interplay between triglyceride or VLDL-cholesterol and fibrinolytic function (PAI-1 levels) in hypertension.[18,19]

In conclusion, the results from this study show that amlodipine is effective for the treatment of hypertension and it does not cause reflex tachycardia in Japanese patients. The present study also showed that amlodipine treatment is a safe antihypertensive agent characterized by beneficial lipid changes (decreases in triglyceride and VLDL-cholesterol) and enhanced fibrinolysis (decreased total and free PAI-1) in Japanese hypertensive patients. The direct relationship between lipid levels and fibrinolytic function observed in this study has improved our understanding and knowledge of the pathogenesis of atherosclerosis during antihypertensive pharmacotherapy.

Acknowledgements This work was partly supported by the Grant-in-Aid for Scientific Research No. 09670041 from the Ministry of Education, Science and Culture, Japan and by the Smoking Research Foundation Grant for Biomedical Research and Ito Memorial Research Foundation.

REFERENCES

1) Varrone J., the investigators of study AML-NY-86-002, *J. Cardiovasc. Pharmacol.*, **17**, (suppl. 1), 530–533 (1991).
2) Ahaneku J.E., Taylor G.O., Walker O., Agbedana E.O., Sowunmi A., Salako L.A., *Eur. J. Clin. Pharmacol.*, **46**, 249–251 (1994).
3) Ahaneku J.E., Taylor G.O., Agbedana E.O., Walker O., Sowunmi A., Salako L.A., *J. Int. Med. Res.*, **232** 489–491 (1992).
4) Krone W., Nagele H., *Am. Heart. J.*, **116**, 1729–1733 (1988).
5) Ohman K.P., Weiner L., Von Schneck H., Karlberg B.E., *Eur. J. Clin. Pharmacol.*, **29**, 149–154 (1985).
6) Trost B.N., Weidmann P., *J. Hypertens.*, **5**, (suppl. 4), S81–S104 (1987).
7) Gleerup G., Winther K., *J. Hypertens.*, **4**, 168s–171s (1991).
8) Gleerup G., Hedner T., Hjorting-Hansen E., Winther K., *J. Cardiovasc. Pharmacol.*, **18**, (suppl. 3), S34–S36 (1991).
9) Ahaneku J.E., Agbedana E.O., Taylor G.O., *Acta. Med. Okayama.*, **49** (5), 267–270 (1995).
10) Allian C.C., Poon L.S., Chan C.S.G., Richmond W., Fu P.C., *Clin. Chem.*, **20**, 470–475 (1974).
11) Fossati P., Prencipe L., *Clin. Chem.*, **28**, 2077–2080 (1974).
12) Sugiuchi H., Uji Y., Okabe H., Irie T., Uekama K., Kayahara N., Miyauchi K., *Clin. Chem.*, **41**, 717–723 (1995).
13) Takada A., Shizume K., Ozawa T., Takahashi S., Takada Y., *Thromb. Res.*, **42**, 63–72 (1995).
14) Takada Y., Takada A., *Thromb. Res.,* (suppl. VIII), 15–22 (1988).
15) Rydzewski A., Takada Y., Takada A., *Thromb. Res.*, **55**, 285–289 (1988).
16) Osterloh I., *Am. Heart. J.*, **118**, (5 pt2), 1114–1119 (1988).
17) Sakata K., Shirotani M., Yoshida H., Urano T., Takada Y., Takada A., *Am. Heart. J.*, **137** (6), 1094–1099 (1999).
18) Cigolini M., Targher G., Seidel J.J.C., Tonoli M., Schiavon R., Agostion G., De Sandre G., *J. Hypertens.*, **13** (6), 659–666 (1995).
19) Stiko-Rahm A., Wiman B., Hamstein A., Nilsson J., *Arteriosclerosis*, **10** (6), 1067–1073 (1995).