International Journal of
Clinical & Experimental Hypertension

Research Article

Effects of Amlodipine plus Atorvastatin on Arterial Function in Essential Hypertension: A Randomized Controlled Study -

Yan Yang[1#], Ya-qiong Wang[2#], Zhe Hu[3], Yu-jing Shen[4] and Ping-jin Gao[1,5*]

[1]State Key Laboratory of Medical Genomics, Shanghai Key Laboratory of Vascular Biology, Department of Hypertension, Shanghai Institute of Hypertension, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[2]Department of Nephrology, Zhongshan Hospital, Fudan University, Shanghai Medical Center of Kidney, Shanghai Institute of Kidney and Dialysis, Shanghai Key Laboratory of Kidney and Blood Purification, Hemodialysis quality control center of Shanghai, Shanghai, China
[3]Department of Hypertension, Ruijin Hospital North, Shanghai Jiaotong University School of Medicine, Shanghai, China
[4]Echocardiography Department, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China
[5]Laboratory of Vascular Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[#]Authors contributed equally to this article.

***Address for Correspondence:** Ping-jin Gao, Shanghai Institute of Hypertension, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, No.197, Ruijin 2nd Road, Shanghai, 200025, China, Tel: +862-164-370-045; Fax: +862-154-654-498; E-Mail: gaopingjin@sibs.ac.cn; gaopingjincn@163.com

Submitted: 30 January 2018; **Approved:** 14 February 2018; **Published:** 16 February 2018

Cite this article: Yang Y, Wang YQ, Hu Z, Shen Yj, Gao Pj. Effects of Amlodipine plus Atorvastatin on Arterial Function in Essential Hypertension: A Randomized Controlled Study. Int J Clin Exp Hypertens. 2018;1(1): 007-014.

ABSTRACT

We aimed to investigate the potential effects of fix-dose atorvastatin plus amlodipine treatment and amlodipine alone treatment for 24 weeks on blood pressure, arterial stiffness and endothelial function in patients with hypertension and hypercholesterolemia. In a single-blinded, randomized, placebo-controlled and parallel design, 60 hypertensive and hypercholesterolemic patients were allocated to receive atorvastatin 10 mg/day plus amlodipine 5 mg/day or amlodipine 5 mg/day for 24 weeks. Central blood pressure was reduced significantly greater in atorvastatin plus amlodipine group than in amlodipine group after 12 and 24 weeks' treatment. Both amlodipine and atorvastatin plus amlodipine therapy significantly improved Flow-Mediated Dilation (FMD) compared to baseline ($p < 0.01$), the effect of atorvastatin plus amlodipine therapy was even greater after 24 weeks($p < 0.05$). Atorvastatin plus amlodipine therapy significantly decreased Heart Rate-Adjusted Augmentation Index (AIx@HR75), carotid-femoral and brachial-ankle Pulse Wave Velocity (PWV) when compared with baseline in both 12 weeks and 24 weeks' administration, while amlodipine therapy not. FMD improvement was independently correlated with change in TC (β = -0.416, $P = 0.004$), while arterial stiffness improvement assessed with AIx@HR75 and baPWV, was correlated with change in central SBP (β = 0.772, $P < 0.001$, and β = 0.420, $P = 0.003$, respectively) in multivariate linear stepwise model. Fixed-dose amlodipine and atorvastatin treatment for 24 weeks reduced central BP and arterial stiffness, improved endothelial function greater than amlodipine therapy. Our findings suggested decrease in TC was the independent protective factor for endothelial function improvement and decrease in central SBP was the independent protective factor for arterial stiffness reduction during the follow-up period.

Keywords: Central aortic pressure; Endothelium-dependent flow-mediated dilation; Arterial stiffness; Essential hypertension

ABBREVIATIONS

ABPM: Ambulatory Blood Pressure Monitor; AIx@HR75: Heart Rate-Adjusted Augmentation Index; ALT: Alanine Transaminase; ASCOT: The Anglo-Scandinavian Cardiac Outcomes Trial; AST: Aspartate Transaminase; BMI: Body Mass Index; BP: Blood Pressure; CAP: Central Aortic Pressure; CCB: Calcium Channel Blocker; CK: Creatine Kinase; FMD: Flow-Mediated Dilation; HDL-C: Human High Density Lipoprotein Cholesterol; IMT: Intima-Media Thickness; LDL-C: Human Low Density Lipoprotein Cholesterol; PP: Pulse Pressure; PWV: Pulse Wave Velocity; SBP: Systolic Blood Pressure; TC: Cholesterol; TG: Triglycerides

INTRODUCTION

Previous epidemiologic studies highlighted that hypertension and dyslipidemia cluster in populations. [1,2]. It is estimated that more than 160 million have hypertension, and that half of them have more than two risk factors worldwide. The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) demonstrated that combined intervention with Blood Pressure (BP) and cholesterol reduced the overall incidence of cardiovascular complications more than intervention with BP only. [3] Statins, also known as HMG-CoA reductase inhibitors are widely prescribed, were demonstrated to reduce cardiovascular events [4-6].

Endothelial dysfunction is one of the initial pathological processes of atherosclerosis [7] and associated with increased cardiovascular risk factors as well as target organ damage in the early stage of hypertension. [8] Arterial stiffness has evolved as early target organ damage in hypertension and is revealed as independent predictor of cardiovascular and all-cause mortality. [9,10] There were evidences that statins improved impaired endothelial function [11,12], but their effects on BP or arterial stiffness beyond the lipid-lowing properties are controversial. [13-15] Amlodipine, as a widely used Calcium Channel Blocker (CCB), whether it is beneficial on endothelial function is still unclear [16-19].

Despite the numerous clinical researches of statin or amlodipine have studied their effects on BP and arterial function, the results are contradictory. The aim of this study was to investigate in detail the potential effects of fix-dose atorvastatin plus amlodipine treatment and amlodipine alone treatment for 24 weeks (6 months) on peripheral and central BP, arterial stiffness and endothelial function in patients with mild hypertension and hypercholesterolemia.

METHODS

Study population and design

This study evaluated effects of amlodipine +/- atorvastatin using a randomized, single-blinded, placebo-controlled and parallel design (registered at Clinicaltrial.Gov, NCT01922687; registered at Ruijin Hospital, ID: Ruijin2010No.14). The diagnosis of hypertension was defined as a BP of at least 140mmHg systolic or 90mmHg diastolic by sphygmomanometer, as well as a mean 24-h BP of at least 130 mmHg systolic or 80 mmHg diastolic by Ambulatory Blood Pressure Monitor (ABPM), or use of anti-hypertensive agents for controlling BP. We included untreated patients or those on a single antihypertensive drug (except CCB) but with an uncontrolled BP on conventional measurement, ranging from 140 to 179 mmHg systolic or from 90 to 109 mmHg diastolic, with fasting total cholesterol concentration ranging from 4.14 to 6.22 mmol/ L (160 to 240 mg/ dL) and endothelium-dependent Flow-Mediated Dilatation (FMD) below 10%. We excluded patients with severe hypertension; secondary hypertension; Low-Density Lipoprotein Cholesterol (LDL-C) below 2.59 mmol/ L (100mg/ dL); renal dysfunction (defined as eGFR < 60ml/min/1.73m^2); current treatment with specific drugs or diets, such as fibrates (especially gemfibrozil), amiodarone, grapefruit juice; stroke within 2 years of randomization; unstable angina; acute myocardial infarction; known contra-indications to a dihydropyridine CCB or statins. We excluded patients with BP higher than 160/100 mmHg after amlodipine or amilodipine plus atovarstatin due to safety concerns of the patients. In addition, no patient had taken any lipid-lowering agent, angiotensin converting enzyme inhibitor, or angiotensin receptor blocker during 1 months preceding our study. 65 patients with mild to moderate hypertension were considered eligible for this study and were assigned to receive placebo amlodipine in 1 month preceding. 4 patients had BP higher than 160/100 mmHg after amlodipine or amilodipine plus atovarstatin and one other patient who declined to follow up were withdrawn from the study. Thus, data from a total of 60 patients were analyzed (Figure 1). Patients were randomly assigned to: atorvastatin 10mg/day plus amlodipine 5mg/ day (1 tablet Caduet®), or amlodipine 5mg/day for 24 weeks. At the beginning of the study, and 6, 12, 18, 24 weeks of follow-up, the liver

function (including the level of aspartate transaminase, AST and alanine transaminase, ALT) and the concentration of Creatine Kinase (CK) were assayed. At the beginning and 12, 24 weeks of follow-up, the ABPM, the indices of vascular structure and function, including FMD, Pulse Wave Velocity (PWV) and AIx@HR75 were performed, respectively. At the beginning and 24 weeks of follow-up, carotid IMT were obtained. After 24 weeks of follow-up, the investigator telephoned the patients 7 to 10 days after the end of trial visit to establish if any adverse events occurred after the final intake of trial medication. The flow chart of this study was shown in figure 1. Prior informed consent was obtained from all patients for participation in the study. This study was approved by the Ethics Committee of Ruijin Hospital.

Brachial flow-mediated dilation (FMD) assessment

Brachial FMD was determined using high-resolution ultrasonography (HD11XE ultrasound system, Philips, USA) of brachial artery according to guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery [20,21]. Assessment of brachial FMD was performed at baseline, after 12 weeks and 24 weeks follow-up study.

Participants were asked to abstain from food, consumption of vitamin E or C, and smoking for ≧ 6 hours before the scan. A sphygmomanometer BP cuff was positioned around the right upper arm, and the brachial artery was imaged above the antecubital fossa. After baseline images of the right brachial artery were obtained for 2 minutes, the cuff was occluded for 5 minutes at an occlusion cuff pressure of ≧ 50 mmHg above the participant's systolic BP to occlude the right brachial artery flow. Images of the right brachial artery were captured 45 ~ 60 s after cuff deflation. Brachial diameter measurements were obtained in end-diastole, identified by the onset of the R-wave. FMD was expressed as the percentage of increase in the brachial artery diameter (media-adventitial interface to the media-adventitial interface) with reactive hyperemia: FMD (%) = [(peak brachial artery diameter after cuff deflation-diameter at rest)/diameter at rest]*100%. After 15 minutes, the endothelium-independent response was assessed by the change in artery diameter at 3 minutes after a 0.4mg dose of sublingual Nitroglycerin (NTG).

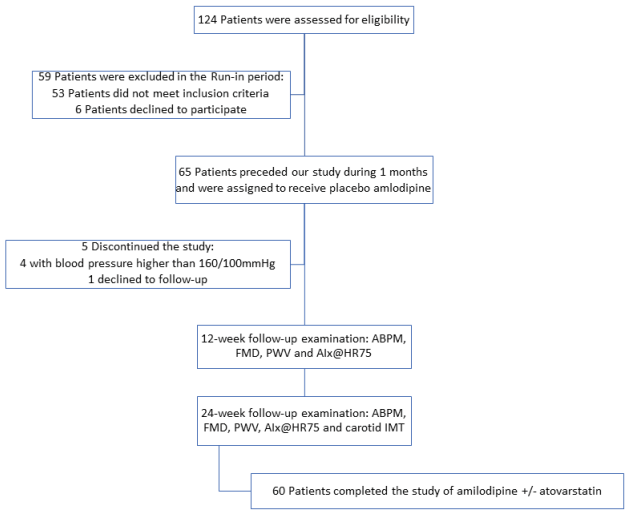

Figure 1: Flow Chart.

All scans were recorded by a computer for analysis. It was accepted that 10% as the normal lower range of FMD in our study. FMD ≥ 10% was defined as normal and FMD < 10% was defined impaired.

To evaluate reproducibility for resting brachial artery diameter, peak diameter, and FMD, ultrasound studies from 20 participants were scanned on 2 separate days more than 2 weeks apart. The intra-observer variation coefficients of brachial artery diameter at rest, peak diameter and FMD were 3.7%, 2.9% and 6.9% respectively.

Central aortic pressure (CAP) measurement

CAP was performed by pressure tonometry using the integrated software (SphygmoCor; AtCor Medical, Sydney, Australia) of the radial pulse. The augmentation index at a heart rate of 75 bpm (AIx@HR75) was a measure of systemic arterial stiffness, calculated as the difference between the second and first systolic peaks, expressed as a percentage of the Pulse Pressure (PP), correcting for a heart rate of 75 bpm.

Pulse wave velocity (PWV) measurement

Pulse Wave Velocity (PWV) was measured automatically with a vascular testing device simultaneously (VP-2000; Omron Healthcare) according to the methods described previously [22]. Briefly, cf-PWV was calculated from measurement of the pulse transit time between the two recording sites, namely the femoral and common carotid arteries; as ba- PWV was between brachial and tibial arteries. During preprocessing analysis, the gain of each waveform was adjusted to generate a signal of equal magnitude for the two waveforms.

Statistical analysis

Data were stored and analyzed using the SPSS 13.0 statistical package (SPSS Inc, Chicago, IL). Data were presented as mean ± SD for continuous variables and the frequencies of subjects in each category for categorical variables. Differences between studied groups were compared by independent t tests or paired t tests, and comparisons of proportion among groups were performed using $\chi 2$ test. The comparison of endothelium-dependent FMD between atorvastatin plus amlodipine (1 tablet Caduet®) and amlodipine was prospectively designated as the primary end-point of the study, all other comparisons were considered secondary. Multivariate linear regression analysis was performed with forward selection followed by backward elimination of covariates, resulting in an equation in which only covariates that significantly increase the predictability of the dependent variable were included. All covariates included in the final model were tested for interactions with each other. Age, sex, BMI, change in central Systolic Blood Pressure (SBP) and change in Total Cholesterol (TC) during the follow-up period were selected as independent variables; FMD improvement, AIx@HR75 improvement and ba-PWV improvement during 24 weeks' follow-up period were the dependent variables. A p value of < 0.05 was defined as statistically significant.

RESULTS

The mean age of our subjects was 60 ± 8 years and the male: female proportion was 24:36. The mean Body Mass Index (BMI) was 25.1 ± 3.2 kg/m^2. There are 5 patients with type 2 diabetes (8.3%). Age, sex, and BMI were matched among subjects. Baseline values before each treatment were compared between the two groups, no significant difference was noted in baseline 24-h or clinic BP values (Table 1). The central Diastolic Blood Pressure (DBP) in atorvastatin plus amlodipine group was marginal significantly higher than

amlodipine group (82.5 ± 7.7 vs. 78.0 ± 8.9 mmHg, $P = 0.050$). 2 of 30 (6.7%) and 3 of 30 (10.0%) had diabetes in the amlodipine group and atorvastatin plus amlodipine group, respectively. There were no significant differences in mean baseline lipoprotein levels, fasting glucose, renal function, indices of vascular structure and function, including FMD, as reported in table 1.

Effects on blood pressure and lipids

Atorvastatin plus amlodipine, or amlodipine all significantly reduced peripheral SBP and DBP after both 12 weeks and 24 weeks' administration compared with baseline. However, atorvastatin plus amlodipine significantly reduced BP to a greater extent than amlodipine alone in clinic and central systolic BP, as well as ambulatory diastolic BP after 12 weeks ($P < 0.05$, Table 2 and Figure 2), and clinic DBP after 24 weeks' administration (all $P < 0.05$, Table 2). There was no difference in central BP after 12 or 24 weeks' administration of amlodipine compared with baseline. However, atorvastatin plus amlodipine significantly reduced central SBP, DBP, PP and CAP after 12 and 24 weeks' administration compared with baseline (all $P < 0.05$, Table 2). The effect of atorvastatin plus amlodipine therapy on central SBP Changes were even greater than amlodipine therapy after 12 weeks' administration ($P = 0.029$, Figure 2), while the effect was borderline statistical significantly greater after 24 weeks' administration ($P = 0.052$, Figure 2). There were no significant changes in central DBP between atorvastatin plus amlodipine therapy and amlodipine therapy after 12 or 24 weeks' administration (Figure 2).

Table 1: Baseline characteristics of study participants.

Variable	All	AML group	AML+ATO group	*P* value
	(N = 60)	(N = 30)	(N = 30)	
Age (yrs)	60.1 ± 7.9	61.3 ± 9.1	58.9 ± 6.6	0.25
Male, n (%)	24 (40)	12 (40)	12 (40)	1.0
Diabetes, n (%)	5 (8.3)	2 (6.7)	3 (10.0)	0.64
Body mass index (kg/m^2)	25.2 ± 3.2	25.3 ± 3.1	25.0 ± 3.3	0.76
TC (mmol/l)	5.58 ± 0.86	5.74 ± 0.99	5.41 ± 0.68	0.14
TG (mmol/l)	2.19 ± 1.08	2.17 ± 1.03	2.21 ± 1.15	0.90
HDL-C (mmol/l)	1.31 ± 0.34	1.36 ± 0.35	1.27 ± 0.33	0.31
LDL-C (mmol/l)	3.58 ± 0.78	3.77 ± 0.84	3.38 ± 0.67	0.06
Fasting glucose (mmol/l)	5.78 ± 0.88	5.59 ± 0.59	5.98 ± 1.08	0.11
Serum creatinine (μmol/l)	69.0 ± 14.4	66.2 ± 14.7	71.9 ± 13.8	0.13
Clinic SBP (mmHg)	148.9 ± 12.4	148.3 ± 12.7	149.4 ± 12.2	0.73
Clinic DBP (mmHg)	83.1 ± 11.2	81.3 ± 11.6	85.0 ± 10.8	0.21
Clinic mean BP (mmHg)	105.0 ± 9.1	103.6 ± 9.0	106.5 ± 9.1	0.23
Clinic PP (mmHg)	65.8 ± 15.4	67.0 ± 16.7	64.5 ± 14.1	0.52
Clinic HR (bpm)	73.0 ± 8.3	74.0 ± 8.7	72.2 ± 7.8	0.41
24-h SBP (mmHg)	134.1 ± 13.1	134.6 ± 13.8	133.6 ± 12.7	0.79
24-h DBP (mmHg)	82.2 ± 7.9	80.8 ± 8.7	83.6 ± 6.8	0.17
24-h mean BP (mmHg)	99.5 ± 9.0	98.3 ± 10.1	100.6 ± 7.7	0.33
24-h PP (mmHg)	51.9 ± 12.2	53.8 ± 14.1	50.0 ± 9.8	0.24
24-h HR (bpm)	69.5 ± 7.2	70.4 ± 8.1	68.5 ± 6.1	0.31
Central SBP (mmHg)	132.8 ± 18.0	131.0 ± 20.6	134.4 ± 15.4	0.47
Central DBP (mmHg)	80.3 ± 8.5	78.0 ± 8.9	82.5 ± 7.7	0.050
Central PP (mmHg)	52.6 ± 17.0	53.2 ± 20.2	52.0 ± 13.6	0.79
CAP (mmHg)	15.6 ± 9.4	15.0 ± 11.2	16.2 ± 7.5	0.62
AIx@HR75 (%)	25.3 ± 9.4	23.6 ± 11.4	26.9 ± 6.7	0.18
FMD (%)	6.73 ± 2.48	6.73 ± 2.48	5.84 ± 2.57	0.18
cf-PWV (m/s)	9.27 ± 2.16	9.19 ± 2.31	9.35 ± 2.04	0.79
ba-PWV (m/s)	16.7 ± 2.4	16.8 ± 2.2	16.6 ± 2.6	0.74
CCA-IMT (mm)	0.71 ± 0.12	0.71 ± 0.13	0.70 ± 0.12	0.77

AML group: Amlodipine group; AML+ATO group: Atorvastatin plus Amlodipine group; TC: Total Cholesterol; TG: Triglycerides; HDL-C: Human High Density Lipoprotein Cholesterol; LDL-C: Human Low Density Lipoprotein Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PP: Pulse Pressure; HR: Heart Rate; CAP: Central Aortic Pressure; Aix@HR75: Heart Rate-Adjusted Augmentation Index; FMD: Flow-Mediated Dilation; cf-PWV: Carotid-Femoral Pulse Wave Velocity; ba-PWV: Brachial-Ankle Pulse Wave Velocity; CCA-IMT: Common Carotid Artery Intima-Media Thickness.

Atorvastatin plus amlodipine therapy significantly lowered TC, Triglycerides (TG) and LDL-C when compared with baseline or amlodipine alone therapy after 12 and 24 weeks (all $P < 0.05$, Table 2). There were no differences in TC, TG or high density lipoprotein cholesterol (HDL-C) after 12 or 24 weeks' administration of amlodipine compared with baseline. However, LDL-C was reduced significantly compared with baseline after both 12 weeks ($P < 0.05$, Table 2) and 24 weeks ($P < 0.01$, Table 2) amlodipine administration.

Effects on FMD and other vascular function

Both amlodipine and atorvastatin plus amlodipine therapy significantly improved FMD compared to baseline ($P < 0.01$, Table 2). The effect of atorvastatin plus amlodipine therapy on FMD was even greater than amlodipine therapy after 24 weeks ($P = 0.007$, Figure 2), but there was no significant difference in FMD change after 12 weeks' administration ($P = 0.19$, Figure 2). However, brachial artery dilator responses to nitroglycerin were not significantly different between amlodipine and atorvastatin plus amlodipine treatment groups (data not shown).

Atorvastatin plus amlodipine therapy significantly decreased AIx@HR75, cf-PWV and average ba-PWV when compared with baseline in both 12 weeks and 24 weeks' administration (all $P < 0.05$, Table 2). The effects of atorvastatin plus amlodipine therapy on AIx@HR75 were even greater than amlodipine therapy after 24 weeks ($P = 0.007$, Figure 2). Changes in average ba-PWV were more significant in atorvastatin plus amlodipine group than in amlodipine group both at week 12 and week 24 follow-up visit ($P = 0.01$ and 0.005, respectively). Carotid IMT had no change with atorvastatin plus amlodipine or amlodipine therapy during the follow-up period ($P > 0.05$, Table 2). When compared with baseline, amlodipine therapy did not significantly change arterial stiffness assessed with AIx@HR75, cf-PWV and ba-PWV.

Independent risk factors of FMD and arterial stiffness improvement during follow-up

As FMD improved in both amlodipine and atorvastatin plus amlodipine treatment groups, we analyzed the independent risk factors of FMD changes during the 24 weeks' follow-up period. The FMD improvement was adversely related to change in TC ($r = -0.416$, $P = 0.001$) and change in central SBP ($r = -0.238$, $P = 0.050$) in univariate analysis. While in multivariate analysis, FMD improvement only had correlation with change in TC (â = -0.416, $P = 0.004$) during follow-up period. There was no significant correlation in FMD improvement with age, sex or BMI (Table 3).

Arterial stiffness improvement assessed with AIx@HR75 was related to change in TC (r=0.310, P=0.02) and change in central SBP ($r = 0.637$, $P < 0.001$) in univariate analysis during 24 weeks' follow-up period. Arterial stiffness improvement was independently correlated with change in central SBP (â = 0.772, $P < 0.001$) and age (â = 0.225, $P = 0.02$) in multivariate linear stepwise model (Table 4).

As arterial stiffness assessed with baPWV, its improvement was related to age (r = 0.221, P = 0.049) and change in central SBP ($r = 0.420$, $P = 0.001$) in univariate analysis, while independently correlated

Table 2: Blood pressure, lipids and vascular changes during the follow-up period in amlodipine and atorvastatin plus amlodipine treatment groups.

	AML group (n = 30)			AML+ ATO group (n = 30)		
	Baseline	Week 12	Week 24	Baseline	Week 12	Week 24
Clinic SBP (mmHg)	148.3 ± 12.7	133.3 ± 14.7 **	135.2 ± 18.4 **	149.4 ± 12.2	122.9 ± 24.0 **	127.6 ± 13.0 **
Δ Clinic SBP (mmHg)		-14.6 ± 10.9	-14.0 ± 13.5		-24.6 ± 22.9†	-19.9 ± 13.8
Clinic DBP (mmHg)	81.3 ± 11.6	72.1 ± 8.7**	74.4 ± 7.2 **	85.0 ± 10.8	72.9 ± 8.1 **	72.2 ± 7.4 **
Δ Clinic DBP (mmHg)		-8.8 ± 6.9	-6.7 ± 9.1		-11.8 ± 10.6	-12.1 ± 9.6†
Clinic PP (mmHg)	67.0 ± 16.7	61.1 ± 16.9**	60.7 ± 18.7 **##	64.5 ± 14.1	50.1 ± 21.1 **†	55.4 ± 13.9 **
Clinic HR (bpm)	74.0 ± 8.7	76.4 ± 10.4	77.9 ± 8.7 *#	72.2 ± 7.8	75.6 ± 8.5 *	74.6 ± 8.8
24-h SBP (mmHg)	134.6 ± 13.8	128.5 ± 12.2**	127.4 ± 11.3**	133.6 ± 12.7	125.4 ± 9.0 **	125.6 ± 9.3 **
Δ 24-h SBP (mmHg)		-6.7 ± 10.9	-7.2 ± 10.5		-8.9 ± 10.5	-9.0 ± 9.3
24-h DBP (mmHg)	80.8 ± 8.7	77.3 ± 7.6 *	77.5 ± 7.6 **	83.6 ± 6.8	79.8 ± 6.4 **	79.6 ± 6.7 **
Δ 24-h DBP (mmHg)		-3.9 ± 7.3	-3.6 ± 6.0		-4.4 ± 6.0†	-4.7 ± 5.4
24-h PP (mmHg)	53.8 ± 14.1	51.2 ± 12.4 *	49.9 ± 11.7 **	50.0 ± 9.8	45.9 ± 8.6 **	46.1 ± 7.2 **
24-h HR (bpm)	70.4 ± 8.1	70.7 ± 6.6	72.3 ± 6.4	68.5 ± 6.1	70.9 ± 5.7 **	69.4 ± 7.2
Central SBP (mmHg)	131.0 ± 20.6	125.8 ± 16.3	128.5 ± 18.9	134.4 ± 15.4	118.7 ± 13.9 **	120.9 ± 13.2 **
Central DBP (mmHg)	78.0 ± 8.9	75.7 ± 7.2	76.5 ± 6.6	82.5 ± 7.7	76.1 ± 9.4 **	76.5 ± 8.2 **
Central PP (mmHg)	53.2 ± 20.2	50.1 ± 16.7	51.3 ± 19.9	52.0 ± 13.6	42.5 ± 9.9 **	44.4 ± 12.2 **
CAP (mmHg)	15.0 ± 11.2	13.2 ± 8.8	15.1 ± 9.6	16.2 ± 7.5	11.5 ± 5.5 **	12.2 ± 5.7 *
AIx@HR75 (%)	23.6 ± 11.4	20.5 ± 10.5	25.2 ± 9.3	26.9 ± 6.7	23.2 ± 6.8 **	22.7 ± 8.5 **
FMD (%)	6.73 ± 2.48	11.4 ± 5.1 **	9.2 ± 4.7 **	5.84 ± 2.57	12.6 ± 4.9 **	11.5 ± 3.4 ** †
cf-PWV (m/s)	9.19 ± 2.31	9.16 ± 2.16	9.11 ± 2.06	9.35 ± 2.04	8.68 ± 2.14 *	8.38 ± 1.71 *
ba-PWV (m/s)	16.8 ± 2.2	16.8 ± 2.9	16.7 ± 3.2	16.6 ± 2.6	15.0 ± 2.7 ** †	14.9 ± 2.1 ** †
CCA-IMT	0.71 ± 0.13		0.70 ± 0.13	0.70 ± 0.12		0.67 ± 0.13
TC (mmol/l)	5.74 ± 0.99	5.50 ± 0.83	5.51 ± 0.89	5.41 ± 0.68	3.84 ± 0.48 ** ‡	4.03 ± 0.67 **‡
TG (mmol/l)	2.17 ± 1.03	2.16 ± 0.87	2.19 ± 1.08	2.21 ± 1.15	1.61 ± 0.89 ** †	1.49 ± 0.68 ** ‡
HDL-C (mmol/l)	1.36 ± 0.35	1.36 ± 0.34	1.41 ± 0.54	1.27 ± 0.33	1.32 ± 0.34	1.31 ± 0.33
LDL-C (mmol/l)	3.75 ± 0.84	3.36 ± 0.60*	3.28 ± 0.82 *	3.38 ± 0.67	2.05 ± 0.33 ** ‡	2.10 ± 0.63 **‡

AML group: Amlodipine group; AML+ATO group: Atorvastatin plus Amlodipine group; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PP: Pulse Pressure; HR: Heart Rate; CAP: Central Aortic Pressure; Aix@HR75: Heart Rate-Adjusted Augmentation Index; FMD: Flow-Mediated Dilation; cf-PWV: Carotid-Femoral Pulse Wave Velocity; ba-PWV: Brachial-Ankle Pulse Wave Velocity; CCA-IMT: Common Carotid Artery Intima-Media Thickness; TC: Total Cholesterol, TG: Triglycerides; HDL-C: Human High Density Lipoprotein Cholesterol; LDL-C: Human Low Density Lipoprotein Cholesterol
*P < 0.05 **P < 0.01: Week 12 or week 24 compared with baseline
†P < 0.05 ‡P < 0.01: AML+ATO group compared with AML group (at the same follow-up period)
# P < 0.05 ## P < 0.01: Week 24 compared with Week 12

with change in central SBP (â = 0.420, P = 0.003) in multivariate linear stepwise model during 24weeks follow-up period (Table 5).

Safety profile

There was no serious adverse event was reported in either of the two treatment groups. No significant changes in biochemical parameters were observed after amlodipine or atorvastatin plus amlodipine therapy (P < 0.05) (data not shown).

DISCUSSION

The results of this randomized, single-blinded, placebo-controlled and parallel designed 24 weeks' follow-up study demonstrated that both atorvastatin plus amlodipine and amlodipine alone treatment significantly reduced peripheral SBP and DBP, however, only atorvastatin plus amlodipine significantly reduced central arterial BP. Both amlodipine and atorvastatin plus amlodipine therapy significantly improved endothelial function, but the effect of atorvastatin plus amlodipine therapy on FMD was even greater than amlodipine therapy. Atorvastatin plus amlodipine therapy significantly decreased arterial stiffness assessed by AIx@HR75, cf-PWV and average ba-PWV, instead of amlodipine therapy. Our findings suggested that decrease in TC was an independent protective factor for endothelium-dependent FMD and decrease in central systolic BP was an independent protective factor for arterial stiffness during the follow-up period.

The effects of amlodipine on endothelial function are controversial. Although some researchers found FMD was improved by amlodipine, [16,17] while others did not. [18,19] In the present study, we found that in patients with hypertension, both atorvastatin plus amlodipine and amlodipine alone therapy improved endothelium-dependent FMD. Amlodipine may improve endothelial function through anti-inflammatory, anti-oxidant [23] and improvement in nitric oxide (NO) availability. [24] There were evidences that statins improved endothelial function [11,12] and it seemed to be mediated by an increased bioavailability of nitric oxide, [25] anti-inflammation and down regulation of angiotensin type 1 receptor expression. [26,27] It was showed that atorvastatin plus amlodipine further improved FMD with 24 weeks therapy than amlodipine, but not with 12

weeks. Hoshiga M et al found that aggressive atorvastatin improved endothelial function in patients with stable coronary disease at 6 months, but not at 1 month. [28] FMD improvement was related to change in central SBP and lipid, but only the decrease in TC was independent protective factor in endothelial function improvement during 24 weeks follow up period. It may suggest that long term of statin therapy is beneficial to endothelial function in patients with hypertension and hyperlipidemia.

Central arterial BP and arterial stiffness is known to be stronger predictor of future cardiovascular events than brachial BP [29,30] as the heart, kidney, and major arteries are exposed to aortic rather than brachial pressure. [31] The influence of atorvastatin on central arterial BP and arterial stiffness is controversial. Kanaki AI et al found in patients with hypertension and hypercholesterolemia, 26 weeks' atorvastatin treatment improved both central arterial BP and arterial stiffness. [13] The ASCOT-LLA study found that atorvastatin treatment was associated with less augmentation of the carotid

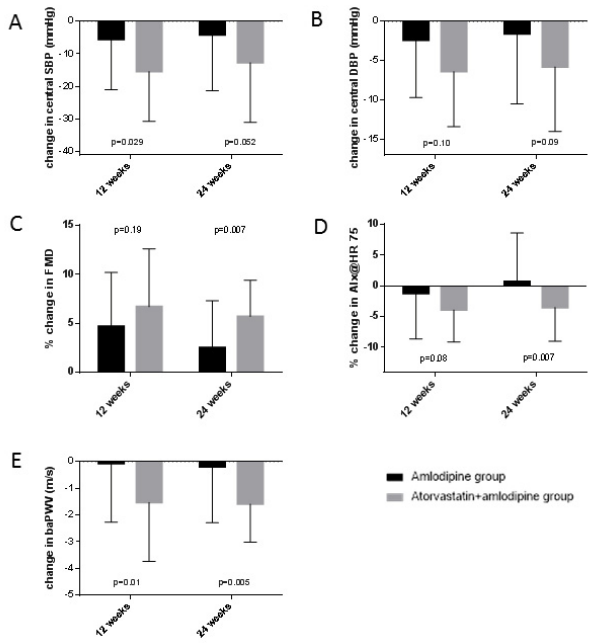

Figure 2: Changes in central blood pressure (A and B) and vascular function variations (C, D and E) in amlodipine group (n = 30) versus atorvastatin plus amlodipine treatment group (n = 30) at 12 weeks and 24 weeks' follow-up visit SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FMD: Flow-Mediated Dilation; Aix@HR75: heart rate-adjusted augmentation index; ba-PWV: Brachial-ankle pulse Wave Velocity

Table 3: Univariate analysis and multivariate linear model of FMD improvement during follow-up period.

Variables	Univariate CorrelationCoefficient	*P*	Multivariate beta-Coefficient (SE)	*P*
Age (yrs)	- 0.10	0.37		
Sex (male)	0.05	0.22		
Body mass index (kg/m^2)	- 0.206	0.06		
Change in TC (mmol/l)	- 0.416	0.001	- 0.416 (0.584)	0.004
Change in central SBP (mmHg)	- 0.238	0.050		

FMD: Flow-Mediated Dilation; TC: Total Cholesterol; SBP: Systolic Blood Pressure.

Table 4: Univariate analysis and multivariate linear model of AIx@HR75 improvement during follow-up period.

Variables	Univariate Correlation Coefficient	*P*	Multivariate beta-Coefficient (SE)	*P*
Age (yrs)	0.175	0.11	0.225 (0.082)	0.02
Sex (male)	0.062	0.33		
Body mass index (kg/m^2)	0.024	0.44		
Height (cm)	- 0.04	0.39		
Change in TC (mmol/l)	0.310	0.02		
Change in central SBP (mmHg)	0.637	<0.001	0.772 (0.083)	<0.001

Aix@HR75: Heart Rate-Adjusted Augmentation Index; TC: Total Cholesterol; SBP: Systolic Blood Pressure.

Table 5: Univariate analysis and multivariate linear model of ba-PWV improvement during follow-up period.

Variables	Univariate Correlation Coefficient	*P*	Multivariate beta-Coefficient (SE)	*P*
Age (yrs)	0.221	0.049		
Sex (male)	0.109	0.21		
Body mass index (kg/m^2)	0.204	0.06		
Change in TC (mmol/l)	0.065	0.32		
Change in central SBP (mmHg)	0.420	0.001	0.420 (0.014)	0.003

ba-PWV: brachial-ankle pulse wave velocity; TC: total cholesterol; SBP: systolic blood pressure.

BP waveform after 12 to 18 months in hypertensive patients. [14] However, in another sub study of ASCOT, CAFE-LLA study showed that atorvastatin did not influence central aortic BP or augmentation index during 3.5 years of follow-up period. [15] The different indices of arterial stiffness or aortic BP measurement, the distinct characteristics of the study participants and the complex factorial design might account for the controversial results of these relevant studies. The present study was designed to study the beneficial effects of atorvastatin and amlodipine on overall BP measurement, including peripheral BP and central arterial BP, as well as various arterial stiffness indices. Moreover, the participants in our study were patients with mild to moderate hypertension and hypercholesterolemia, with the most common use of statins in the real world. It was showed that atorvastatin plus amlodipine significantly reduced central arterial BP and arterial stiffness in 12 weeks and 24 weeks treatment compared to baseline, however, amlodipine did not change central BP or arterial stiffness. The independent protective factor of arterial stiffness improvement in our study was decrease in central SBP. Many studies have revealed that statins reduced cardiovascular events in patients with diabetes or coronary artery disease. [4-6] The decrease of central arterial BP and arterial stiffness might suggest the improvement of arteriosclerosis by fixed-dose atorvastatin plus amlodipine, thus partly explain the beneficial effects.

Although carotid IMT seemed to decrease after 24 weeks' follow-up period, neither atorvastatin plus amlodipine nor amlodipine therapy reached the statistically significant decrease of IMT in the present study. However, fixed-dose amlodipine and atorvastatin was found to decrease mean IMT in type 2 diabetic patients after 12 months follow-up period. [32] Our result might be due to the follow-up period was not long enough to see the differences.

Some limitations of our study should be acknowledged. The follow-up period is 24 weeks totally, thus, the beneficial effects of atorvastatin plus amlodipine or amlodipine on endothelial function and arterial stiffness should be confirmed in studies with longer follow-up periods. The determination of endothelium-dependent vasodilation could be considered another possible limitation of this study. The occluding cuff on the upper arm and caliper measurements were used in FMD measurement. Although there is no clear consensus, a majority of studies performed now use a forearm cuff position, and automatic measurement might enhance accuracy and reduce FMD variability. In addition, since the participants in our study were enrolled from the hypertension clinic in Ruijin Hospital and the relative small sample sizes, we are unable to extrapolate these findings to other groups.

In conclusion, the present study revealed that fixed-dose amlodipine and atorvastatin treatment for 24 weeks reduced central BP and arterial stiffness, improved endothelial function greater than amlodipine therapy in patients with mild to moderate hypertension and hyperlipidemia. These effects may represent a potential mechanism of cardiovascular risk reduction with statin use. Our findings suggested that decrease in TC was an independent protective factor for endothelial function improvement and decrease in central SBP was an independent protective factor for arterial stiffness reduction during the follow-up period.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov NCT01922687

REFERENCES

1. Kannel WB. Fifty years of Framingham Study contributions to understanding hypertension. J Hum Hypertens. 2000; 14: 83-90. https://goo.gl/SF5rnX
2. Thomas F, Bean K, Guize L, Quentzel S, Argyriadis P, Benetos A. Combined effects of systolic blood pressure and serum cholesterol on cardiovascular mortality in young (<55 years) men and women. Eur Heart J. 2002; 23: 528-535. https://goo.gl/QTLg2H
3. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial--Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. Lancet. 2003; 361: 1149-1158. https://goo.gl/QrHgQL
4. Liu Z, Xu Y, Hao H, Yin C, Xu J, Li J, et al. Efficacy of high intensity atorvastatin versus moderate intensity atorvastatin for acute coronary syndrome patients with diabetes mellitus. Int J Cardiol. 2016; 222: 22-26. https://goo.gl/6J9MEG
5. Kunt A, Ozcan S, Kuçuker A, Odabaşi D, Sami Kunt A. Efficacy of chronic statin therapy on major cardiac events after coronary artery bypass grafting: low-dose versus high-dose. Med Glas (Zenica). 2015; 12: 196-201. https://goo.gl/XKgTkd
6. Collins R, Armitage J, Parish S, Sleigh P, Peto R; Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet. 2003; 361: 2005-2016. https://goo.gl/cC3VrV
7. Widlanksy ME, Gokce N, Keaney JF, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003; 42: 1149-1160. https://goo.gl/k6dfkp
8. Yang Y, Xu JZ, Wang Y, Tang XF, Gao PJ. Brachial flow-mediated dilation predicts subclinical target organ damage progression in essential hypertensive patients: a 3-year follow-up study. J Hypertens. 2014; 32: 2393-2400. https://goo.gl/Sx2xMN
9. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010; 55: 1318-1327. https://goo.gl/U6zk6j
10. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. Eur Heart J. 2010; 31: 1865-1871. https://goo.gl/FdaT6X
11. Rauch U, Osende JI, Chesebro JH, Fuster V, Vorchheimer DA, Harris K, et al. Statins and cardiovascular diseases: the multiple effects of lipid-lowering therapy by statins. Atherosclerosis. 2000; 153: 181-189. https://goo.gl/HyBtKX
12. de Jongh S, Lilien MR, op't Roodt J, Stroes ES, Bakker HD, Kastelein JJ. Early statin therapy restores endothelial function in children with familial hypercholesterolemia. J Am Coll Cardiol. 2002; 40: 2117-2121. https://goo.gl/xFrFSB
13. Kanaki AI, Sarafidis PA, Georgianos PI, Kanavos K, Tziolas IM, Zebekakis PE, et al. Effects of low-dose atorvastatin on arterial stiffness and central aortic pressure augmentation in patients with hypertension and hypercholesterolemia. Am J Hypertens. 2013; 26: 608-616. https://goo.gl/KeHrQz
14. Manisty C, Mayet J, Tapp RJ, Sever PS, Poulter N, McG Thom SA, et al. Atorvastatin treatment is associated with less augmentation of the carotid pressure waveform in hypertension: a substudy of the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT). Hypertension. 2009; 54: 1009-1013. https://goo.gl/4uUZuJ
15. Williams B, Lacy PS, Cruickshank JK, Collier D, Hughes AD, Stanton A, et al. Impact of statin therapy on central aortic pressures and hemodynamics: principal results of the Conduit Artery Function Evaluation-Lipid-Lowering Arm (CAFE-LLA) Study. Circulation. 2009; 119: 53-61. https://goo.gl/gw7tBJ
16. Schiffrin EL, Pu Q, Park JB. Effect of amlodipine compared to atenolol on small arteries of previously untreated essential hypertensive patients. Am J Hypertens. 2002; 15: 105-110. https://goo.gl/NbUHWv
17. Koh KK, Han SH, Ahn JY, Chung WJ, Lee Y, Shin EK. Amlodipine improves endothelial function and metabolic parameters in patients with hypertension. Int J Cardiol. 2009; 133: 23-31. https://goo.gl/iXYWRt
18. Ghiadoni L, Magagna A, Versari D, Kardasz I, Huang Y, Taddei S, et al. Different effect of antihypertensive drugs on conduit artery endothelial function. Hypertension. 2003; 41: 1281-1286. https://goo.gl/7uSZHV
19. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol. 2000; 35: 60-66. https://goo.gl/5o73dq
20. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. J Am Coll Cardiol. 2002; 39: 257-265. https://goo.gl/4jcP5e
21. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, et al. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. Eur J Cardiovasc Prev Rehabil. 2011; 18: 775-789. https://goo.gl/k46Jca
22. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension.1995; 26: 485-490. https://goo.gl/uvVciZ
23. Toma L, Stancu CS, Sanda GM, Sima AV. Anti-oxidant and anti-inflammatory mechanisms of amlodipine action to improve endothelial cell dysfunction induced by irreversibly glycated LDL. Biochem Biophys Res Commun. 2011; 411: 202-207. https://goo.gl/D8Bt7U
24. He X, Zhang HL, Zhao M, Yang JL, Cheng G, Sun L, et al. Amlodipine ameliorates endothelial dysfunction in mesenteric arteries from spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2011; 38: 255-261. https://goo.gl/orQBTJ
25. John S, Schlaich M, Langenfeld M, Horst Weihprecht, Gerd Schmitz, Gottfried Weidinger, et al. Increased bioavailability of nitric oxide after lipid-lowering therapy in hypercholesterolemic patients: a randomized, placebo-controlled, double-blind study. Circulation. 1998; 98: 211-216. https://goo.gl/xiCxAf
26. Koh KK, Quon MJ, Waclawiw MA. Are statins effective for simultaneously treating dyslipidemias and hypertension? Atherosclerosis. 2008; 196: 1-8. https://goo.gl/PSi9Lr

27. Osende JI, Ruiz-Ortega M, Blanco-Colio LM, Egido J. Statins to prevent cardiovascular events in hypertensive patients. The ASCOT-LLA study. Nephrol Dial Transplant. 2004; 19: 528-531. https://goo.gl/iYqNNw

28. Hoshiga M, Arishiro K, Nakakoji T, Miyazaki N, Negoro N, Okabe T, et al. Switching to aggressive statin improves vascular endothelial function in patients with stable coronary artery disease. J Atheroscler Thromb. 2010; 17: 705-711. https://goo.gl/h5c1uA

29. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. Hypertension. 2007; 50: 197-203. https://goo.gl/BDV9En

30. Pini R, Cavallini MC, Palmieri V, Marchionni N, Di Bari M, Devereux RB, et al. Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. J Am Coll Cardiol. 2008; 51: 2432-2439. https://goo.gl/3FmyND

31. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. Eur Heart J. 2014; 35: 1719-1725. https://goo.gl/a54VTe

32. Tanaka M, Nishimura R, Nishimura T, Toshihide Kawai, Shu Meguro, Junichiro Irie, et al. Effect of single tablet of fixed-dose amlodipine and atorvastatin on blood pressure/lipid control, oxidative stress, and medication adherence in type 2 diabetic patients. Diabetol Metab Syndr. 2014; 6: 56. https://goo.gl/cUjDjg