Single high dose atorvastatin does not ameliorate endothelial function and large arterial stiffness in dyslipidemic patients without atherosclerosis

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Abstract. – *Background:* Many studies show that statins have beneficial effects on atherosclerotic risk factors and markers such as flow mediated dilatation (FMD). However, studies on early effects of statins on endothelial function of non atherosclerotic humans are limited.

Aim: The purpose of this study was to determine whether a single high dose of atorvastatin could improve endothelial function and large arterial stiffness in statin naïve dyslipidemic non-atherosclerotic patients.

Materials and Methods: Thirty statin naïve dyslipidemic non-atherosclerotic patients from Cardiology Outpatient Clinic were enrolled. Arterial stiffness and endothelial function of patients were evaluated by assessing the finger photoplethysmography and the flow-mediated dilatation (FMD) of the brachial artery before and 24 hour after oral administration of 80 mg atorvastatin.

Results: Stiffness indices and FMD 24 hours after administration of 80mg atorvastatin did not differ from baseline measurements (6.89 ± 1.90 vs 7.06 ± 2.37 p : NS and 9.13 ± 6.07 vs 9.80 ± 6.34 p : NS).

Conclusions: Although it is widely accepted that statins improve endothelial function, evidences of early effect might largely be associated with endothelial injury. Our study suggests that beneficial early effects of statins might not be applicable to patients without atherosclerosis.

Key Words:

Atorvastatin, Flow-mediated dilatation, Stiffness index.

Introduction

Reduction of low density lipoprotein cholesterol (LDLc) with statins decreases cardiovascular mortality and morbidity[1]. Statin treatment induces a reduction in plaque and lipid core volume[2]. Moreover, endothelial function and nitric oxide (NO) bioavailability improves after lipid lowering treatment with statins[3], which is strongly and independently associated with cardiovascular events[4]. However, studies on early effects of statins on endothelial function are limited.

Recently, statins have been shown to upregulate the expression and activity of endothelial NO synthase (eNOS) and prevent their downregulation by oxidized LDL (ox-LDL) *in vitro* through an increase in eNOS mRNA stability[5]. Reduced prevalence of periprocedural myocardial infarction (MI) in elective percutaneous coronary intervention (PCI) by single loading dose of atorvastatin in 24 hours suggested that these drugs may have pleiotropic effect(s) beyond lipid lowering[6]. Additionally, established beneficial effects of preprocedural statin use before PCI seems to be related with reduction of PCI-induced endothelial inflammatory response[7,8]. However, these data do not verify whether statins acutely improve endothelial function in non-atherosclerotic humans.

The purpose of this study was to determine whether a single high dose of atorvastatin could improve the endothelial function and the large arterial stiffness in statin naïve dyslipidemic non-atherosclerotic patients. To elucidate presence of this effect we assessed brachial flow-mediated

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dilatation (FMD) and digital volume pulse (DVP) obtained by measuring infrared light transmission through the finger photoplethysmography of subjects, before and one day after a single high dose of atorvastatin.

Materials and Methods

Study Population

Statin naïve dyslipidemic patients from Cardiology Outpatient Clinic were enrolled. Institutional local Ethics Committee approved the study protocol, and written informed consents were obtained from all patients. Exclusion criteria were presence of more than one additional cardiac risk factor, established atherosclerotic disease, kidney failure, chronic inflammatory or infectious disease, atrial fibrillation or use of nitrates or nitrate donors.

Initial evaluation included collection of demographic and anthropometric measurements. Fasting venous blood samples were drawn and blood pressures of patients were measured. After physical examination all consenting subjects underwent electrocardiography and 2D Doppler transthoracic echocardiography to exclude underlying heart disease including left heart outflow obstruction, left ventricular systolic dysfunction (ejection fraction <50%) or severe heart valve disease.

All subjects fasted from midnight before the procedure and were studied in the morning in semisupine position. Patients were asked to avoid alcohol, tobacco or caffeine use before photoplethysmography and FMD measurements. Arterial stiffness and endothelial function of patients were evaluated by assessing the finger photoplethysmography and the FMD of the brachial artery before and 24 hour after oral administration of 80 mg atorvastatin.

Arterial Stiffness Measurement

Arterial stiffness measurements, stiffness index (SI) and reflection index (RI), were obtained by using a photoplethysmography device (Pulse Trace PCA 2, Micro Medical, Rochester, England). The Pulse Trace probe is attached to left index finger and subjects rest supine or seated for at least 15 min. SI was obtained from subject height and from the time delay between direct and reflected waves (Pulse propagation time [PPT]) in the DVP. Average of three valid measurements obtained over a 10-minute period for each patient was recorded.

Flow-Mediated Dilatation Assessment

Flow-mediated dilatation of the brachial artery was non-invasively examined by 2D high-resolution ultrasound machine (GE Medical Systems Vivid 7 Pro, GE Vingmed AS, Horten, Norway) with a 12-MHz linear array transducer. A sphygmomanometric cuff was first placed above the antecubital fossa. After baseline longitudinal image of brachial artery is acquired, cuff was inflated to at least 50 mmHg above systolic pressure to occlude brachial artery for 5 minutes. Brachial artery diameter was measured 1 minute after cuff deflation. Brachial artery diameter percent change was calculated and recorded as the FMD of the patient. The variability of the diameter measurement was minimized by calculating the average derived from 3 diameter measurements determined along the longitudinal segment of brachial artery. All measurements were done by the same physician.

Laboratory Investigation

Blood samples were obtained after an overnight fasting state from the cubital vein into blood tubes and the serum was immediately separated from the cells by centrifugation at 3000 g for 10 min, stored at $-70°C$, and then analyzed. The levels of triglyceride (TG), total cholesterol, HDL cholesterol, LDL cholesterol, sodium, potassium, urea, creatinine, and fasting glucose were determined using commercially available assay kits (Olympus AU 600 Auto Analyzer, Tokyo, Japan). Complete blood count was analyzed with commercially available assay kits (Beckamn Coulter Hmx, Miami, FL, USA) whereas TSH was analyzed with commercially available assay kits (Roche-Hitachi E170, Raritan, NJ, USA)

Statistical Analysis

Data were expressed as mean ± SD for continuous variables and as number and percent for categorical variables. The Kolmogorov- Smirnov test was used as test of normality. Parameters before and after statin treatments were compared with paired sample *t*-test. A 2-sided $p < 0.05$ was considered statistically significant. The SPSS statistical software 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

Thirty (14 females [47%]) consecutive statin naïve dyslipidemic patients aged 32-68 years (mean±SD: 50.77 ± 8.35) were enrolled. Of subjects 4 (13%) were diabetic, 3(10%) were hypertensive which were newly diagnosed, and were not under medication. Four (13%) patients had low HDLc, and 6 (20%) were smoker. Demographic and biochemical characteristics of patients were summarized on Table I.

Correlation coefficient for intraobserver variability of brachial artery diameter measurements was 0.98 ($p < 0.001$). Pulse waveform analyses and FMD 24 hours after administration of 80 mg atorvastatin did not differ from baseline measurements. Baseline and posttreatment measurements of FMD were closely correlated with each other (r = 0.91, p : 0.001) which was also true for SI (r = 0.57, p : 0.01). Although other lipid parameters were not correlated, TG concentration was positively correlated with SI (r = 0.59, p : 0.001) and correlation of TG with SI persisted after treatment (r = 0.47, p : 0.008) whereas FMD was not associated with lipid parameters. However, there was a weak negative correlation between post-

Table I. Demographic and biochemical characteristics of patients.

Age (years)	50.77 ± 8.35
Waist circumference (cm)	109.87 ± 10.78
Hip circumference (cm)	116.77 ± 9.220
Waist/hip ratio	0.94 ± 0.06
Body mass index (kg/m+)	30.68 ± 5.12
Systolic BP (mmHg)	119.00 ± 13.98
Diastolic BP (mmHg)	77.33 ± 9.89
Total cholesterol (mg/dl)	283.23 ± 30.40
LDLc (mg/dl)	185.08 ± 23.94
HDLc (mg/dl)	55.00 ± 11.44
Triglyceride (mg/dl)	164.73 ± 68.30
Fasting blood glucose (mg/dl)	110.50 ± 39.19
Urea (mg/dl)	31.41 ± 8.98
Creatinine (mg/dl)	0.96 ± 0.19
Na (mmol/L)	139.70 ± 2.37
K (mmol/L)	4.37 ± 0.26
Haemoglobin (g/dl)	14.28 ± 1.93
Leukocyte ($10^3 \times$ /L)	8.20 ± 1.65
TSH (uIU/ml)	1.89 ± 0.72

Abbreviations: BP: Blood pressure; HDLc: High density lipoprotein cholesterol; K: potassium; LDLc: Low density lipoprotein cholesterol; Na: Sodium, TSH: Thyroid stimulating hormone.

treatment FMD and baseline HDLc (r = 0.35, p: 0.03) and MPI was correlated with TG (r = 0.47, p : 0.02).

Discussion

In this study we documented that single high dose statin use did not improve endothelial function or large arterial stiffness in hyperlipidemic patients without atherosclerosis.

Many studies show that statins have benefical effect(s) on atherosclerotic risk factors and markers such as FMD. However, studies on early effects of statins on endothelial function are limited. Recently, a meta-analysis reported that statin therapy improves endothelial function in patients both with and without overt cardiovascular disease[3]. However, in the meta-analysis, there was significant heterogeneity in studies and although little, there was a publication bias. Only one study[9] included in this meta-analysis evaluated effect of single dose statin on endothelial function. However, this study was conducted on patients with coronary artery disease (CAD). Recently a study on 8 normocholesterolemic young male subjects demonstrated improvement in the endothelial function in 24 hours evaluated by venous occlusion plethysmography[10]. However, in another study on healthy male volunteers, administration of cerivastatin led to improvement of FMD in 3rd hour which disappeared in the 6th and 12th hours. Moreover, it was shown that cerivastatin improved endothelial functions in elderly diabetic patients within 3 days[11]. In another study on otherwise healthy hyperlipidemic patients endothelial function was evaluated by venous plethysmography and intra-arterial acetylcholine (Ach) infusion within 3 and within 14 days of statin therapy[12]. Endothelial vasodilator response to Ach was improved within 3 days. However, there was a significant and substantial reduction in LDL in the 3rd day.

In a nested case-cohort subset of the Multi-Ethnic Study of Atherosclerosis, FMD below sex specific median (4.2% for females and 3.6% for males) was associated with higher cardiovascular events. Only three patients in our study had baseline FMD measurements below above-mentioned median. Since endothelial dysfunction was regarded as "ultimate risk of the risk factors" indicating the existence of specific atherogenic vascular *milieu*[13], subjects

Table II. Comparison of parameters before and after statin use*.

	Baseline	Next day
FMD (%)	9.13 ± 6.07	9.80 ± 6.34
RI	56.07 ± 14.28	56.90 ± 14.55
SI	6.89 ± 1.90	7.06 ± 2.37
PPT	234.90 ± 62.26	233.83 ± 57.52
Heart rate (/min)	75.03 ± 8.45	77.93 ± 8.63

Abbreviations: FMD: Flow mediated dilatation; PPT: Pulse Propogation Time; RI: Reflection index; SI: stiffness index. *$p > 0.05$ for all.

in present study may be considered to have lower risk which might be an explanation for indifference of FMD after administration of single high dose atorvastatin.

Increased arterial stiffness is one of the major diagnostic elements for classifying subjects in the high or very high risk categories[14]. We performed finger photoplethysmography because it is simple, rapid and does not require special training. SI is found to be suitable for use in clinical trials[15]. Documentation of absence of difference in SI in our study suggests that a single high dose of atorvastatin may not improve the large arterial stiffness, even though it may not imply ineffectiveness of statins to decrease the large arterial stiffness that develops in the years. A recent meta-analysis could not safely conclude for the effect of statins on arterial stiffness, as estimated by pulse wave velocity (PWV)[16]. Besides our study confirmed association of TG levels with arterial stiffness as previously observed by Aznaouridis et al[17]. Long term prospective and randomized studies to elucidate effects of statins on large arterial stiffness are needed.

Study Limitations

Small and heterogenous study population and absence of control group are main limitations of the present study. Moreover we could not have opportunity to assess ox-LDL concentrations, which would add to the value of the present study.

Conclusion

Although it is widely accepted that statins improve endothelial function, evidences of early ef-

fect might largely be associated with endothelial injury. Our study suggests that beneficial early effects of statins might not be applicable to patients without atherosclerosis.

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