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ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 416–420
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Metabolic syndrome abating the beneficial effect of pravastatin treatment on adhesion of endothelium by monocytes in subjects with hypercholesterolemia

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Received 13 August 2008; accepted 7 October 2008

Abstract

Statin, a potent lipid-lowering agent, ameliorates the interaction of monocytes and endothelium, a critical step in the atherosclerotic process. However, it remains unclear whether this effect of statin depends on different doses or the presence of metabolic syndrome. In this prospectively double-blind study, 21 hypercholesterolemia subjects, with low-density lipoprotein cholesterol between 130 and 170 mg/dL, received low-dose (10 mg/d) or high-dose (40 mg/d) pravastatin treatment for 8 weeks. We assessed the reduction of monocyte adhesion to cultured endothelium between different-dose groups and the relationship to metabolic syndrome. Total cholesterol and low-density lipoprotein cholesterol were significantly decreased after 40-mg pravastatin treatment (−23.3% ± 3.7%, $P < .001$ and −28.8% ± 3.0%, $P < .001$), and the reductions were greater than those in the 10-mg group ($P = .041$ and $P = .045$, respectively). There was no significant difference in monocyte adhesion between high-dose and low-dose pravastatin treatment. When all subjects were divided into an improvement group and a no improvement group, according to the median of change percentage of monocyte adhesion after pravastatin treatment, there were significantly more subjects with metabolic syndrome in the no improvement than the improvement group (6 vs 1 person, $P = .024$). Using logistic regression analysis, metabolic syndrome, rather than dose effect of pravastatin, was an independent predictor of interaction between monocytes and endothelium (95% confidence interval = 0.001-0.865, $P = .041$). Attenuating adhesion between monocytes and endothelium is altered by the presence of metabolic syndrome when hypercholesterolemia subjects receive pravastatin treatment.

1. Introduction

Vascular endothelium is important in keeping normal physiologic conditions of the vessel wall and bloodstream [1,2]. The imbalance in interaction of blood cells to endothelium will induce impairment of endothelial function and development of thrombogenesis, which is associated with abnormal concentrations of cholesterol, particularly low-density lipoprotein (LDL) cholesterol [3,4].

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, have dose-dependent benefits in reducing plasma concentrations of cholesterol and the events of cardiovascular disease [5,6]. However, one of the mechanisms for risk reduction is attenuation of monocyte adhesion to vascular endothelium that may involve not only lowering lipids but also anti-inflammation, which is independent of dosage [7-9].

Metabolic syndrome, associated with inflammation, plays an important role in atherosclerosis and coronary heart disease [10-12]. With pravastatin, the effect on risk reduction of coronary heart disease in subjects with metabolic syndrome is similar to subjects without the syndrome [13]. The effect of statins on the interaction of monocytes and endothelium is not well known in subjects with metabolic syndrome compared with those without metabolic syndrome. We therefore assessed the potency of monocyte adherence to endothelium after pravastatin treatment with different doses in hypercholesterolemic patients.

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doi:10.1016/j.metabol.2008.10.019

2. Methods

2.1. Patients and methods

This study design and findings for dose-dependent effects on lipid profile and insulin resistance have been reported previously [14]. Briefly, nondiabetic subjects with LDL cholesterol between 130 and 170 mg/dL were enrolled. A 75-g oral glucose tolerance test was performed for excluding diabetes. The subjects were also excluded based on the following: (1) age less than 20 or more than 75 years; (2) plasma triglycerides greater than 500 mg/dL; (3) secondary hypercholesterolemia due to hypothyroidism or any other causes; (4) pregnancy; (5) presence of severe systemic disease such as immune disorder, cancer, or acute or chronic inflammation disease; (6) impaired hepatic functions; (7) history of drug or alcohol abuse; and (8) current treatment with antidiabetic, lipid-lowering, or immunosuppressive drugs. The study was approved by the Institutional Review Board of Taichung Veterans General Hospital, and written consent was obtained before randomization.

The 47 subjects received diet control in the 4-week screening period; and afterward, they were randomized and took either 40 mg or 10 mg pravastatin daily with dinner. The subjects received the study drugs for 8 weeks of the active treatment period. The data and blood samples were collected at the beginning and end of the active treatment period in the study.

All blood samples were drawn after overnight fasting. Based on the modified National Cholesterol Education Program criteria, 5 components of metabolic syndrome were assessed; and the latter was diagnosed if 3 or more of the following criteria were present [15]: (1) waist circumference more than 90 cm in men or 80 cm in women; (2) triglycerides equal to or greater than 150 mg/dL; (3) high-density lipoprotein (HDL) cholesterol less than 40 mg/dL in men or 50 mg/dL in women; (4) blood pressure equal to or greater than 130/85 mm Hg or using antihypertensive medications; and (5) fasting glucose between 100 and 125 mg/dL [15-17]. Total cholesterol and HDL cholesterol were measured, and LDL cholesterol level was calculated according to the method of Friedewald et al [18]. C-reactive protein (CRP) was measured by immunochemical assay using purified duck immunoglobulin Y (ΔFc) antibodies (Good Biotech, Taichung, Taiwan) [19].

Additional 10-mL fresh blood samples were mixed with 0.1 mol/L sodium citrate that was prepared for purifying mononuclear cells by Ficoll-Hypaque (density, 1.077; Biochrom, AG, Berlin, Germany) gradient centrifugation (900*g*, 30 minutes). Around 5×10^5 mononuclear cells were added to human umbilical vein endothelial cells, which had been prepared in 24-well plates, for 30 minutes at 37°C. Nonadherent cells were removed by washing twice, and the remaining cells were detached from the plate after treatment with trypsin-EDTA. The cell suspension was analyzed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA) that can discriminate monocytes from endothelial cells. Computer analysis was performed using Lysis II software (Becton Dickinson) [20,21].

All descriptive data were presented as mean ± SEM. Statistical analyses were conducted using *t* test to compare age, lipid profiles, components of metabolic syndrome, CRP, and the counts of monocyte adhesion between the 2 study groups. The changes in lipid profiles, glucose, CRP, and the monocyte adhesion counts before and after treatment were determined by paired *t* test. The Fisher exact test was used to assess differences between sex and patients with metabolic syndrome between the 2 groups. Multivariate logistic regression analyses were used to analyze the relationship of metabolic syndrome or pravastatin doses to improvement of monocyte adhesion. Statistical analysis was performed by SPSS 10.0 (SPSS, Chicago, IL). A *P* value less than .05 was considered statistically significant.

3. Results

Of 47 study subjects, 40 subjects completed an assessment of biochemistry during the study [14]. However, only 21 of these 40 subjects agreed to take part in the monocyte adhesion to endothelium study. The baseline characteristics of the subjects before 40-mg and 10-mg pravastatin treatment are shown in Table 1. There were no significant differences in sex and age between these 2 different-dose groups. The components of metabolic syndrome, CRP, and total and LDL cholesterol were also similar in these 2 groups. The number of monocytes adhesion to endothelium in the 40-mg group was similar to that in the 10-mg group ($P = .543$).

After 8 weeks of pravastatin treatment, the total cholesterol concentration was significantly decreased in the 40-mg group (from 234 ± 6 to 178 ± 6 mg/dL, $P < .001$); and the reduction was more obvious than that in the 10-mg group ($P = .041$). There was also a significant decrease in LDL

Table 1
Baseline clinical characteristics of study subjects

	10 mg/d	40 mg/d	*P*
n	11	10	
Sex (male/female)	8/3	8/2	1.000
Age (y)	63 ± 3	61 ± 3	.686
BMI (kg/m^2)	26.6 ± 1.0	26.7 ± 1.2	.936
Waist (cm)	90.1 ± 1.4	95.6 ± 2.6	.097
Systolic BP (mm Hg)	118 ± 7	124 ± 6	.502
Diastolic BP (mm Hg)	69 ± 3	72 ± 4	.507
Total cholesterol (mg/dL)	230 ± 6	234 ± 6	.653
LDL cholesterol (mg/dL)	149 ± 4	146 ± 4	.557
HDL cholesterol (mg/dL)	47 ± 3	47 ± 3	.896
Triglyceride (mg/dL)	143 ± 19	185 ± 27	.230
Fasting glucose (mg/dL)	94 ± 4	97 ± 4	.539
Glucose at 2-h OGTT (mg/dL)	130 ± 9	135 ± 15	.800
Metabolic syndrome (persons)	2	5	.183
CRP (mg/L)	2.99 ± 0.70	2.18 ± 0.30	.310
Monocyte adherence (cell)	2759 ± 593	2286 ± 467	.538

BMI indicates body mass index; BP, blood pressure; OGTT, oral glucose tolerance test.

Table 2
The changes (percentage) of laboratory characteristics after pravastatin treatment between the 2 study groups

	10 mg/d (mean ± SEM, P^a)	40 mg/d (mean ± SEM, P^a)	P^b
Total cholesterol	−10.4 ± 4.6, .043	−23.3 ± 3.7, <.001	.041
LDL cholesterol	−14.7 ± 5.7, .036	−28.8 ± 3.0, <.001	.045
HDL cholesterol	6.3 ± 3.8, .302	6.2 ± 4.9, .341	.987
Triglyceride	−15.4 ± 8.7, .068	−20.6 ± 7.7, .028	.659
Fasting glucose	2.8 ± 2.7, .433	−2.5 ± 1.4, .116	.100
CRP	−12.8 ± 8.9, .094	−6.3 ± 14.8, .721	.712
Monocyte adherence	−2 ± 21, .107	184 ± 101, .142	.103

[a] In comparison with baseline.
[b] In comparison between groups.

cholesterol concentration after 40-mg pravastatin treatment (from 146 ± 4 to 103 ± 3 mg/dL, $P < .001$), and the reduction was more pronounced than that in the 10-mg pravastatin treatment ($P = .045$). The changes in HDL cholesterol, triglyceride, glucose, and CRP were not significantly different between the 2 study groups. The change in monocyte adhesion to endothelium after treatment was not significant in either the 40-mg group ($P = .142$) or the 10-mg group ($P = .107$). There was no significant difference in change of monocyte adhesion between the 2 different-dose treatments ($P = .103$) (Table 2).

Because there was no significant difference in change of monocyte adhesion to endothelium between the groups given different pravastatin doses, all the study subjects were divided into 2 groups by median (8%) of reduction in percentage of monocyte adhesion after pravastatin treatment to assess the associated factors for improvement of monocyte adhesion. There were no significant differences in sex and age between the improvement group (n = 11) and the no improvement group (n =10). The monocyte adhesion significantly decreased after pravastatin treatment in the improvement group (−36% ± 4%, $P < .001$), and the reduction percentage was significantly greater than that in the no improvement group ($P = .023$). The clinical characteristics in these 2 groups are shown in Table 3. There were no significant differences in the reduction of plasma concentration of total cholesterol and LDL cholesterol after pravastatin treatment between the 2 groups ($P = .106$ and $P = .074$, respectively). The improvement in monocyte adhesion was not significantly associated with change of each criterion of metabolic syndrome, including HDL cholesterol ($P = .624$), triglyceride ($P = .699$), and glucose ($P = .249$), after pravastatin treatment. There was only 1 subject with metabolic syndrome in the improvement group, whereas there were 6 subjects with metabolic syndrome in the no improvement group. The difference was significant ($P = .024$). Using logistic regression analysis, metabolic syndrome (95% confidence interval [CI]= 0.001-0.865, $P = .041$), but not dose effect of pravastatin (95% CI= 0.829-1.032, $P = .163$), is the independent predictor for improvement of interaction between monocytes and endothelium (Table 4). Furthermore, we also found the reduction of monocyte adhesion to endothelium to be significantly greater in subjects without metabolic syndrome (n = 14) than in those with metabolic syndrome (n =7) ($P = .003$).

Table 3
Baseline and alteration of clinical characteristics between improvement of monocyte adhesion group and no improvement of monocyte adhesion group

	Improvement of monocyte adhesion	No improvement of monocyte adhesion	P
n	11	10	
Baseline data			
Sex (male/female)	9/2	7/3	.635
Age (y)	64 ± 3	59 ± 3	.257
BMI (kg/m^2)	25.9 ± 0.8	27.4 ± 1.3	.357
Metabolic syndrome (persons)	1	6	.024
Change (%) during study in:			
Total cholesterol	−11.6 ± 4.8	−22.0 ± 3.8	.106
LDL cholesterol	−15.4 ± 6.0	−28.1 ± 2.7	.074
HDL cholesterol	4.7 ± 3.9	7.8 ± 4.8	.624
Triglyceride	−19.8 ± 8.1	−15.2 ± 8.8	.699
Fasting glucose	2.1 ± 2.4	−1.8 ± 2.1	.249
CRP	−16.3 ± 7.9	−1.7 ± 16.2	.434

Table 4
The relationship of metabolic syndrome or dose of pravastatin to improvement of monocyte adhesion using logistic regression

	95% CI	P
Risk itself without adjusting		
With metabolic syndrome	0.006-0.745	.028
High dose of pravastatin	0.883-1.002	.059
After adjusting for age, sex, and CRP		
With metabolic syndrome	0.001-0.865	.041
High dose of pravastatin	0.829-1.032	.163

4. Discussion

Pravastatin is a potent 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, and its lipid-lowering effect is dependent on dosage [22,23]. In our study, we found that the plasma concentrations of total cholesterol and LDL cholesterol were decreased in subjects after high-dose (40 mg) pravastatin treatment during the 8-week period; and the reduction was significantly different to that in the low-dose (10 mg) pravastatin treatment. Statins not only have hypolipidemic effects, but they can also inhibit interactions between leukocytes and endothelium [7,8,24,25]. The inhibition of molecular adhesion may not be related to their effect on lipid lowering [24]. Our study directly showed that there was no significant difference in the reduction of monocyte adhesion to endothelium between the 40-mg and the 10-mg pravastatin treatment groups, although the plasma concentrations of total cholesterol and LDL cholesterol were improved significantly after high-dose pravastatin treatment compared with low-dose treatment.

To further elucidate the underlying cause, all study subjects were divided into the improvement of monocyte adhesion group and the no improvement of monocyte adhesion group. After pravastatin treatment, we found significantly more subjects with metabolic syndrome in the no improvement group. Therefore, metabolic syndrome may greatly influence the beneficial effects of pravastatin on interactions of monocyte and endothelium. The changes in monocyte adhesion were not significantly related to changes of single components of metabolic syndrome, including fasting plasma concentrations of glucose, triglyceride, or HDL cholesterol, in our study. The improvement of monocyte adhesion was not significantly related to the reduction of total cholesterol or LDL cholesterol.

Previous studies have demonstrated that the benefit of statins on cardiovascular prevention is dose dependent in subjects with metabolic syndrome [6,26]. Results of the secondary prevention trial indicated greater effects of pravastatin in subjects with higher CRP levels [9]. However, Sattar et al [13] demonstrated similar effects of pravastatin in prevention of cardiovascular disease in subjects with or without metabolic syndrome. We assume that benefits of atherosclerotic prevention by statins in subjects with metabolic syndrome cannot be exactly explained by attenuating monocyte activation [13]. In addition, only monocytes, but not endothelium, obtained from study subjects who were exposed to pravastatin treatment might not reflect the in vivo condition. Thus, although the monocyte adhesion improvements between different doses of pravastatin did not reach statistically significant difference, large studies are necessary before claiming that metabolic syndrome abates the benefits of statin treatment on adhesion of endothelium by monocyte.

In conclusion, the improvement of monocyte adhesion to cultured endothelium, independent of the dose effect of pravastatin, is greater in hypercholesterolemic subjects without metabolic syndrome than in those with metabolic syndrome.

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