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Statin Reduce the Platelet P-Selectin Expression in Atherosclerotic Ischemic Stroke

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Abstract. **Recently, it has been demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitor or statin can regulate the thrombogenesis beyond its lipid lowering effect. In this study, we investigated the beneficial effect of statin to reduce the platelet P-selectin expression in atherosclerotic ischemic stroke. Thirty-two (28 men, 4 women; mean age 59.8 ± 9.6 years) patients with atherosclerotic ischemic stroke were assigned to receive simvastatin 20 mg per day for 12 weeks and discontinued for another 12 weeks. Then, administration of simvastatin was discontinued for the following 12 weeks. Using whole blood flow cytometry, we evaluated the change of platelet P-selectin expression of all the patients after the 12-weeks use and the 12-weeks discontinuance of simvastatin. The platelet P-selectin expression was significant reduced after treatment of simvastatin 20 mg for 12 weeks ($p < 0.001$). However, the effect of statin to reduce platelet P-selectin expression disappeared after 12 weeks of cessation of statin. In addition, the P-selectin changes induced by statin were independent of the changes of the LDL cholesterol ($r = -0.311$, $p = 0.386$). This study demonstrated that the use of statin might be a helpful add-on therapy to regulate the platelet related thrombogenesis in atherosclerotic ischemic stroke.**

Key Words. **platelet, thrombosis, statin**

Introduction

Platelet activation is a major pathophysiological process in atherothrombosis [1,2]. During the atherothrombosis, several structural changes begin on the surface of platelets. The expression of P-selectin on activated platelets interacts with P-selectin glycoprotein ligand (PSGL)-1 on leukocytes, which then secretes monocyte chemotactic protein (MCP)-1 to accumulate monocytes, eventually leading to build-up the atherosclerotic mass [3,4]. Platelet P-selectin expression was related with severity of ischemic stroke [5] and did not decrease in the chronic atherosclerotic ischemic stroke [6]. Therefore, it is logical that platelet P-selectin expression may be an important factor to progress the ongoing process of atherothrombosis.

The potent lipid lowering agent, HMG-co-A reductase inhibitor, has pleuripotential effects to prevent the progression of atherothrombosis [7–10]. Recently, it has been reported that statin could modulate P-selectin expression of platelet in hypercholesterolemia [11]. However, there has been little attempt to investigate the effect of statin to regulate the platelet P-selectin expression in atherosclerotic ischemic stroke.

In this study, we investigated the effects of simvastatin to reduce platelet P-selectin expression in patients with atherosclerotic ischemic stroke.

Subjects and Methods

In the prospective study, we included 32 patients (28 men, 4 women; mean age 59.8 ± 9.6 years) with atherosclerotic ischemic stroke according to the TOAST classification [12] at the minimum 90 days after the onset of their ischemic stroke. Exclusion criteria were previous statin therapy (<90 days), low LDL cholesterol level (<100 mg/dl), advanced hepatic and renal dysfunction, inflammatory diseases, systemic infection, or malignancy. Patients characteristics, including risk factors, and laboratory data taken at baseline are shown in Table 1.

Simvastatin 20 mg daily was prescribed for 12 weeks. During the observation period, we used the identical anti-platelet medication (clopidogrel 75 mg plus aspirin 100 mg) for all patients. All patients gave informed consent for the study.

Blood sampling

Peripheral venous blood was taken at 3 time points; before taking simvastatin as the baseline, after 12 weeks of simvastatin use and after 12 weeks of statin discontinuance. The samples were anticoagulated with 3.2% sodium citrate.

Laboratory findings

Hematological (leukocytes, hemoglobin, and platelet counts), lipid profiles (total serum cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride),

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Table 1. *Baseline clinical and laboratory characteristics in 32 patients with atherosclerotic ischemic stroke*

	Atherosclerotic ischemic stroke ($n = 32$)
Age (years)	59.8 ± 9.6
Men	28 (87.5%)
Hypertension	21 (65.6%)
Diabetes mellitus	10 (31.3%)
HbA1C	7.5 ± 1.2
Old MI	6 (18.8%)
Smoking	13 (40.64)
Leukocytes ($X10^3/\mu l$)	6.7 ± 1.3
Platelets ($X10^3/\mu l$)	249.4 ± 49.0
C-reactive protein (mg/l)	0.58 ± 1.0
Total cholesterol (mg%)	223.0 ± 31.6
LDL-cholesterol (mg%)	145.9 ± 29.3
Triglyceride (mg%)	159.1 ± 74.9

Data are expressed as mean ± SD.
MI: myocardial ischemia.

plasma C-reactive protein level by using particle-enhanced immunonephelometry (LX-2200, EIKEN) were measured for all subjects at 3 times: before statin use, after 12 weeks simvastatin use, and after 12 weeks of statin discontinuance.

Preparation of whole blood for flow cytometry

The citrated whole blood samples were diluted at 6 folds in 30 μl of HEPES buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 20 mmol/L HEPES, 1 mg/mL bovine serum albumin, 3.3 mmol/L NaH_2PO_4, pH7.4). The population of platelets was detected by using phycoerythrin (PE) conjugated anti-CD42a (Pharmingen). 5 μL fluorescein isothiocyanate (FITC) conjugated anti-P-selectin (Pharmingen) monoclonal antibody were used for surface staining to detect the activated platelets. CD42a present on both resting and activating platelets, while P-selectin is expressed only upon activated platelets. After careful mixing and incubation for 15 min at room temperature, 2.5 mL of HEPES buffer, containing 0.2% formaldehyde was added.

The stained platelets were analyzed by FACscan (EPICS XL, Coulter Electronics). The assay that we used showed reproducible results and has been verified in other clinical studies [13–15]. The recognition of platelet population was found to be ≥99% for platelets-specific CD42a antigen. After identification through positive fluorescence for anti-CD42a monoclonal antibody, the single platelet population was differentiated from the microaggregates, according to their degree of forward scatter. Microparticles were identified by positive fluorescence for CD42a-PE and forward scatter below a size threshold below 0.5 μm. Platelet expression of P-selectin was assayed by selecting the single platelet population. The extent of antibody binding was expressed as the mean fluorescence intensity (MFI) of total platelet population and was used as a quantitative measure for glycoprotein surface expression.

Statistical analysis

All data were presented as mean ± SD. Statistically analysis was performed by using the SAS program. Continuous variables were compared with t-test. Correlation between changes of LDL cholesterol and changes of platelet P-selectin expression during observation period was examined by using Pearson correlations. $P < 0.05$ was considered statistically significant.

Results

The changes of laboratory findings by statin

Before the initiation of simvastatin medication, the mean serum LDL concentration was 145.9 ± 29.3 mg% (range; 100–220 mg%). Among the patients, 9 patients (28.1%) had normal LDL level (<130 mg%). After taking simvastatin 20 mg for 12 weeks, the serum concentration of LDL cholesterol was significantly decreased (92.3 ± 31.2 mg%, $p < 0.001$) compared with the value at the baseline. In addition, plasma level of C-reactive protein was significantly reduced ($p < 0.01$) after using statin, and then increased, again, after withdrawal of it approximating the baseline (Table 2).

The change of platelet P-selectin expression by statin

After taking simvastatin 20 mg for 12 weeks, the platelet P-selectin expression (86.7 ± 8.3, $p < 0.001$)

Table 2. *Changes of laboratory findings according to the medication of statin*

	Before the use of statin	After the use of statin	After discontinuance of statin
Serum total cholesterol (mg%)	223.0 ± 31.6	162.6 ± 34.6*	217.9 ± 34.5**
Serum LDL cholesterol (mg%)	145.9 ± 29.3	92.3 ± 31.2*	140.6 ± 36.0**
Serum triglyceride (mg%)	159.1 ± 74.9	155.7 ± 68.7	174.1 ± 51.7
Serum C-reactive protein (mg/l)	0.58 ± 1.0	0.11 ± 0.2*	0.36 ± 0.3**

* $p < 0.05$ versus before the use of statin.
** $p < 0.05$ versus after the use of statin.

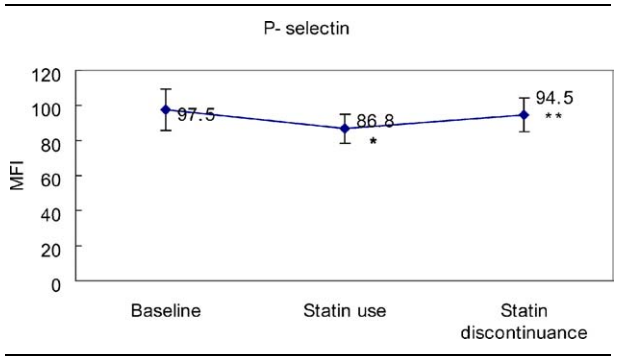

Fig. 1. *The change of platelet P-selectin expression according to the Prescription of simvastatin (20 mg). *$p < 0.05$ versus baseline; **$p < 0.05$ versus statin use; MFI: mean fluorescence intensity.*

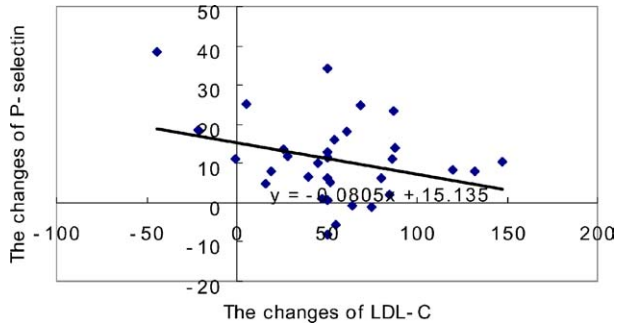

Fig. 2. *The correlation between the change of LDL cholesterol and the changes of platelet P-selectin expression during observation period. There was no significant correlationship ($r = -0.311$, $p = 0.386$) between the changes of LDL cholesterol and the changes of platelet P-selectin expression during observation period.*

was significantly decreased compared with that at the baseline (97.5 ± 11.9). However, after the following 12 weeks of simvstatin cessation, the platelet P-selectin expression returned to the baseline levels (Fig. 1). The relationship between the changes in LDL cholesterol and platelet P-selectin expression was showen in Figure 2. The changes of LDL cholesterol were unrelated with that of platelet P-selectin expression ($r = -0.311$, $p = 0.386$).

Regarding the safety of medication of simvastatin for 12 weeks, two patients experienced fatigability and 1 patient decreased libido. However, there was no serious complication of statin.

Discussion

It has been well known that platelet P-selectin expression is an established marker in acute and chronic atherothrombosis [16]. The upregulated expressions of P-selectin on platelets were found in acute ischemic stroke and more profound in atherosclerotic ischemic stroke than other subtypes [17]. Also, these increments of platelet P-selectin expression in atherosclerotic ischemic stroke were observed in chronic stage [18]. These findings suggested that platelet P-selectin expression might reveal the ongoing atherosclerotic changes rather than the effect of acute vascular events. In this study, the prescription of simvstatin 20 mg for 12 weeks significantly reduced platelet P-selectin expression, suggesting that statin has a beneficial anti-thrombogenic effect. Huhle et al. [11] also showed the similar findings in hypercholesterolemic patients (>160 mg% of LDL cholesterol level). However, 50% of our study population was below the 160 mg% of LDL cholesterol level and 28% below the 130 m%, suggesting that the prescription of statin may be also helpful to reduce the platelet activation in normocholemic and mild hypercholemic patients. In our results, these effect of statin to regulate platelet P-selectin expression were independent with the degree of LDL cholesterol reduction, which support the previous reports demonstrating statin modulated the elevated CRP, adhesion molecules, various thrombotic materials beyond lipid lowering mechanism [19,20]. Also, our results that the platelet P-selectin expression was significantly increased 12 weeks after withdrawal of statin prescription is similar to the previous report [21] that termination of statin treatment resulted aggravation of platelet activation immediately. However, we cannot exactly explain how statin reduce the platelet P-selectin expression in atherothrombosis. We hypothesize the following mechanisms. First, statin increases the concentration of nitric oxide [22], reduce the thromboxane formation [23], and changes the platelet membrane composition to decrease the platelet activation [24]. Second, the regulation effect of statin on inflammation may contribute to the downregulation of platelet activation as the inflammatory mediators such as cytokines induce the platelet hyperactivation [25], and the inflammatory processes of atherothrombosis are reciprocally affected by platelet activation [26]. The second hypothesis was drawn by our result that the prescription of statin significantly reduced the plasma level of C-reactive protein, which is known as an acute phase reactant and sensitive marker of systemic inflammation of atherothrombosis [27].

Our study had some limitation as follows; First, because men comprised the majority of the study population (87.5%), we can not definitely conclude the prescription of statin can similarly reduce the platelet activation in women. Second, our study does not show whether the prescription of statin can also reduce the platelet activation in ischemic stroke patients who did not meet the criteria for use of statin for secondary prevention. Finally, the effect of statin to reduce the platelet activation was not compared with that of anti-platelet intervention.

The inhibition of platelet P-selectin expression by statin has clinical significance to reduce recurrence of atherosclerotic ischemic stroke. It has been reported that the actions mediated by the contents from alpha granules of activated platelet cannot easily blocked by anti-platelet agents [28]. Therefore, it should be needed that the use of statin considered as a mandatory add-on therapeutic regimen to reduce the platelet activation in atherosclerotic ischemic stroke.

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