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# Cilostazol Reduces PAC-1 Expression on Platelets in Ischemic Stroke

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**Background and Purpose** Cilostazol, a phosphodiesterase III inhibitor, is known to be a useful antiplatelet agent that inhibits the progression of atherosclerosis in ischemic stroke. This study investigated the effects of combining cilostazol with aspirin on the expressions of P-selectin and PAC-1 on activated platelets in acute ischemic stroke.

**Methods** We analyzed 70 patients with acute ischemic stroke (<72 hrs of an ischemic event). The daily intake was 100 mg of aspirin in 37 patients and 100 mg of aspirin plus 200 mg of cilostazol in 33 patients. The expressions of P-selectin and PAC-1 on activated platelets were measured on the day of admission and 5 days later. We also evaluated the clinical progression using the National Institutes of Health Stroke Scale (NIHSS) at the same times.

**Results** After 5 days the extent of PAC-1 expression on activated platelets was significantly lower for combined aspirin and cilostazol treatment (61.0±19.3%, $p$=0.008; mean±standard deviation) than the baseline level (70.9±12.9%), but did not differ between aspirin alone (66.0±19.0%) and baseline (70.1±15.7%). The expression of P-selectin did not differ between combined aspirin and cilostazol treatment and baseline. The clinical progression did not differ between the two groups, as indicated by the absence of significant changes on the NIHSS in the acute period.

**Conclusions** This study found that the combined regimen of aspirin and cilostazol exerts the beneficial effect of reducing PAC-1 activity on activated platelets in acute ischemic stroke. However, the clinical outcome of this regimen was no better than that of the aspirin-only regimen. Therefore, further detailed studies of the possible clinical benefits of cilostazol in acute ischemic stroke are needed.
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**Key Words** cilostazol, acute ischemic stroke.

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## Introduction

Platelet activation has been considered an important pathophysiologic process in acute ischemic stroke,[1] which has led to antiplatelet agents being a very important therapeutic tool in curbing its progression.[2,3] Although there has been considerable debate about the optimal medical therapy to apply in acute ischemic stroke, aspirin is still the most useful therapeutic regimen for preventing its progression.[4,5] The beneficial effect of preventing the progression of intracranial stenosis was recently found to be greater for a combined aspirin and cilostazol regimen than for aspirin alone.[6] However, there are few data on the changes in platelet activation induced by cilostazol in acute ischemic stroke.

In this study, we retrospectively analyzed the cilostazol-induced changes in platelet activation in acute ischemic stroke.

## Methods

We evaluated 440 patients with acute ischemic stroke (within 72 hrs of an ischemic event) from November 2005 to December 2006. Platelet function assays were applied to 111 of the patients, and we selected 83 of these patients who had been prescribed aspirin alone ($n$=47) or aspirin plus cilostazol ($n$=36). To improve the quality of our data, we excluded 13 patients due to the presence of cardioembolism ($n$=4), infection ($n$=4), and malignancy ($n$=5). We retrospectively investigated the types of ischemic stroke,[7] vascular risk fac-

 

tors, and changes in scores on the National Institutes of Health Stroke Scale (NIHSS) and the Modified Rankin Scale (mRS) at 30 days after ischemic stroke onset, and laboratory findings including complete blood counts, a lipid battery, and C-reactive protein (CRP).

### Measurement of platelet activation

Peripheral venous blood was taken at two time points: before prescribing the medication and after 5 days of using it. The samples were anticoagulated with 3.2% sodium citrate. All patients or their relatives gave informed consent.

The citrated whole blood samples were diluted sixfold in 30 mL of HEPES buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 20 mmol/L HEPES, 1 mg/mL bovine serum albumin, and 3.3 mmol/L $NaH_2PO_4$; pH 7.4). Platelets were activated by adding adenosine diphosphate at a final concentration of 100 $\mu$M. The platelet population was detected using phycoerythrin (PE)-conjugated anti-CD42a (Pharmingen). Five milliliters of fluorescein isothiocyanate anti-P-selectin (Pharmingen) or PAC-1 monoclonal antibody (Pharmingen) was used for surface staining to detect the activated platelets. CD42a is present on both resting and activating platelets, while P-selectin and PAC-1 were expressed only on activated platelets. After 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 platelet-specific CD42a antigen was identified in 99% of the platelet population. After identifying positive fluorescence for the anti-CD42a monoclonal antibody, the single-platelet population was differentiated from microaggregates according to the degree of forward scatter. Microparticles were identified by positive fluorescence for PE-conjugated anti-CD42a and forward scatter below a size threshold of 0.5 mm. Antibody binding was expressed as the percentage of antibody-positive platelets. We calculated the differences between unstimulated and stimulated platelets for each antibody.

## Results

We finally reviewed the results of the platelet function assay in 70 patients with acute ischemic stroke (aspirin alone in 37 patients, and combined aspirin and cilostazol in 33 patients). Patient characteristics, risk factors, and laboratory findings taken on the admission day are listed in Table 1. None of these parameters differed significantly between the two groups.

The expressions of P-selectin and PAC-1 on activated platelets did not decrease during the observation period in the aspirin-only group. However, the expression of PAC-1 was significantly down-regulated after 7 days compared to the baseline in the aspirin plus cilostazol group (Figs. 1 and 2). CRP did not decrease significantly in either group during the

**Table 1.** Comparison of clinical and laboratory findings between aspirin alone and aspirin plus cilostazol

| | Aspirin alone | Aspirin plus cilostazol | *p* |
|---|---|---|---|
| Number | 37 | 33 | |
| Men | 25 | 22 | 0.938 |
| Age (yrs) | 62.4±10.7 | 63.2±12.2 | 0.696 |
| Hypertension | 30 (81.1) | 22 (66.7) | 0.244 |
| Diabetes mellitus | 12 (32.4) | 12 (36.4) | 0.8 |
| Smoking | 18 (48.6) | 16 (48.5) | 0.899 |
| Myocardial infarction | 4 | 2 | 0.503 |
| Old cerebral infarction | 2 | 2 | 0.884 |
| NIHSS score at baseline | 5.2±3.6 | 5.8±4.6 | 0.136 |
| TOAST | | | |
| Atherosclerosis | 24 (64.9) | 25 (75.8) | 0.362 |
| Small-vessel disease | 13 | 8 | |
| mRS score at baseline | 2.1±1.0 | 2.2±1.1 | 0.849 |
| White blood cell count (×1,000/$\mu$L) | 8.5±2.2 | 7.9±2.0 | 0.185 |
| Platelet count (×1,000/$\mu$L) | 248.6±69.2 | 270.6±65.6 | 0.12 |
| Glucose (mg%) | 175.6±81.2 | 173.7±110.4 | 0.913 |
| Total cholesterol (mg%) | 205.7±40.9 | 214.1±77.2 | 0.335 |
| LDL cholesterol (mg%) | 109.4±37.2 | 118.6±70.7 | 0.497 |
| Triglyceride (mg%) | 124.4±81.7 | 144.5±111.7 | 0.292 |
| C-reactive protein (mg%) | 0.42±1.00 | 0.60±1.83 | 0.59 |

Data are number (percentage) or mean±standard deviation values.
NIHSS: National Institutes of Health Stroke Scale, mRS: modified Rankin Scale, LDL: low-density lipoprotein, TOAST: Trial of ORG 10172 in Acute Stroke Treatment

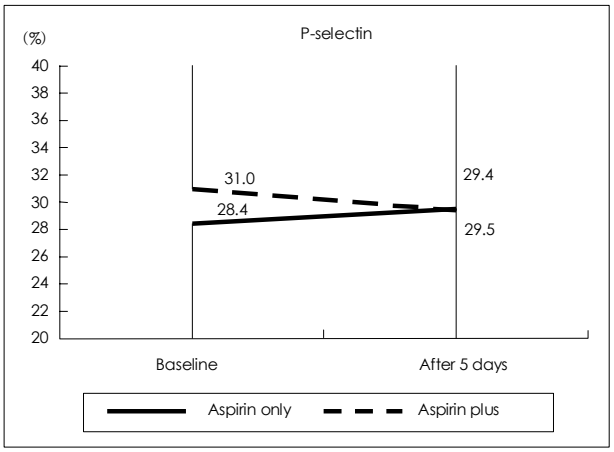


**Fig. 1.** Changes in 50 mean platelet P-selectin expression. Vertical bars indicate standard deviations.

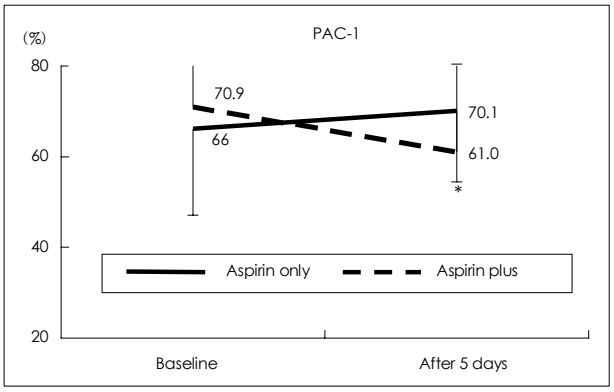


**Fig. 2.** Changes in mean platelet PAC-1 expression. *$p$<0.05 versus baseline, vertical bars are standard deviations.

observation period. The clinical improvement as measured by the NIHSS score did not differ significantly between the two groups (Fig. 3).

## Discussion

Preventing early recurrence is the most important issue in the management of acute ischemic stroke.[8,9] The higher recurrence rate in atherosclerotic ischemic stroke than in other types of ischemic stroke[9] has been attributed to the progression of platelet activation, which has led to aspirin being a very important therapeutic strategy in acute ischemic stroke.[2] However, the effects of aspirin might be too weak to curb platelet activation in acute ischemic stroke,[10] and there have been several trials of the effects of combined antiplatelet agents on preventing the recurrence of ischemic events in ischemic stroke.[11,12] In contrast to several successful trials using combined antiplatelet agents in coronary syndrome,[13] many trials have failed to show the superiority of using two antiplatelet agents compared to using aspirin alone in ischemic stroke.[11,14]

Cilostazol is a type of phosphodiesterase inhibitor that both

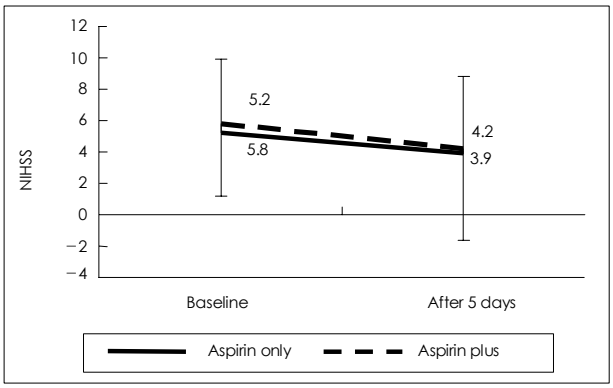


**Fig. 3.** Changes in mean NIHSS (National Institutes of Health Stroke Scale) scores. Vertical bars are standard deviations.

reduces platelet aggregation and enhances vascular dilatation.[15] Several recent studies found that the progression of atherosclerosis was less for aspirin plus cilostazol than for aspirin alone,[6,16] particularly in intracranial atherothrombosis in transcranial Doppler and magnetic resonance angiography. These findings suggest that cilostazol provides advantages in curbing platelet activation in ischemic stroke.[6] However, there are few data supporting this hypothesis.

This study evaluated whether aspirin plus cilostazol could reduce the expressions of P-selectin and PAC-1 on activated platelets in acute ischemic stroke. During platelet activation, the glycoprotein IIb/IIIa (GP IIb/IIIa) receptor changes into the activated form and connects with another activated platelets via fibrinogen-bridging platelet aggregation.[17] PAC-1 is a monoclonal antibody for detecting the activated GPIIb/IIIa receptor on activated platelets.[18] In the present study, aspirin plus cilostazol significantly decreased the expression of PAC-1 on activated platelets in acute ischemic stroke, whereas aspirin alone had no effect on PAC-1 expression. Although many previous studies have demonstrated that aspirin can efficiently block platelet aggregation within 30 min[19] the reported positive results appeared only under certain experimental conditions. Several stuies actually found that aspirin alone did not significantly regulate platelet activation or aggregation in atherothrombosis.[20,21] This could be due to platelet activation being enhanced by several other pathways in atherothrombosis, such as inflammatory reactions, free radical formation, and oxidized low-density lipoprotein.[22] Therefore, the blocking of only one of several platelet activation pathways might not regulate platelet aggregation in atherothrombosis.

It has previously been shown that aspirin plus cilostazol regulates PAC-1 expression on activated platelets in chronic atherothrombosis.[23] However, to the best of our knowledge, the present study is the first to show the usefulness of cilostazol in reducing PAC-1 expression in ischemic stroke.

We found that P-selectin expression on activated platelets was not changed by cilostazol in acute ischemic stroke. P-selectin is a transmembrane protein of the alpha-granules that becomes expressed on activated platelets.[17] Thereafter it interacts with P-selectin glycoprotein ligand 1 (PSGL-1) on leukocytes, which then secrets cytokines and chemokines to aggravate the inflammatory processes, eventually increasing the atherosclerotic mass. However, few studies have shown that it is efficiently regulated by antiplatelet agents at usual doses in ischemic stroke. We considered that the expression of P-selectin on activated platelets would not be easily regulated by antiplatelet medication at usual doses. In line with our results, the Plavix Use for Treatment stroke trial[24] showed little changes in P-selectin expression on activated platelets in ischemic stroke when using the usual doses of aspirin alone or aspirin plus clopidogrel.

We initially hypothesized that aspirin plus cilostazol curbs the inflammatory processes in acute ischemic stroke, but found that this was not the case. It is known that platelet activation reciprocally interacts with inflammatory processes in atherothrombosis.[25] Among several platelet activations, the expression of P-selectin on activated platelets might be intimately related with recruitment of inflammatory cells in atherogenic vessels. We therefore suggest that the small changes in the CRP level are due to the inability to reduce P-selectin expression on activated platelets for combined aspirin and cilostazol treatment.

This study was subject to some limitations. First, it had a retrospective design, and there might have been an inhomogeneous distribution in the study population. Second, the study population was too small to predict the clinical benefit of cilostazol in acute ischemic stroke. However, together the results from this study show the beneficial effect of aspirin plus cilostazol in regulating platelet activation in acute ischemic stroke patients.

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