Serial Change in Platelet Activation Markers With Aspirin and Clopидогрел After Acute Ischemic Stroke

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Objectives: Antiplatelet drugs are widely used for secondary prevention after cerebral ischemia of noncardioembolic origin and different antiplatelet drugs exert different pharmacologic effects. This study investigated differences in platelet activation markers in patients taking either aspirin or clopidogrel after acute ischemic stroke.

Methods: A prospective randomized case-control study evaluated 70 patients with noncardioembolic stroke treated with either aspirin (100 mg/d) or clopidogrel (75 mg/d) after acute ischemic stroke. Platelet activation markers (CD62P, CD63, and CD40L) were measured by flow cytometry at different time points (<48 hours and days 7, 30, and 90 after stroke). The markers were also evaluated in 30 at-risk control subjects.

Results: Ischemic stroke patients had significantly increased circulating CD62P, CD63, and CD40L in the acute stage compared with the control group. Levels of CD62P, CD63, and CD40L were more significantly reduced in the clopidogrel group than in the aspirin group in the first week after stroke. Furthermore, differences in CD62P and CD63 levels were significant even at 1 month after stroke.

Conclusions: Patients treated with clopidogrel have lower platelet activity than those taking aspirin after acute ischemic stroke. The stronger effect of clopidogrel is notable 1 week after stroke and persists for at least 1 month. Further large-scale trials are warranted to clarify optimal treatment.

Key Words: ischemic stroke, platelet activation, aspirin, clopidogrel, flow cytometry

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latelets play a pivotal role in the pathogenesis of atherosclerosis and thrombus formation, which contribute to the development of an ischemic stroke, an acute coronary syndrome, and a peripheral arterial occlusion disease. Antiplatelet drugs are the most widely used drugs for secondary prevention after ischemic stroke of noncardioembolic origin. Clinical trials demonstrate that early antiplatelet treatment for patients after acute ischemic stroke is beneficial in reducing risk of early recurrence and preventing serious vascular events and mortality. Aspirin and other oral antiplatelet drugs are protective in patients who are at increased risk of occlusive vascular events. Adding a second antiplatelet drug to aspirin may produce additional benefits in some clinical circumstances. However, the risk of life-threatening or major bleeding may also be increased. More research is needed to clarify the efficacy of currently available antiplatelet drugs.

Aspirin inhibits platelet activation by inhibiting cyclooxygenase that leads to the formation of thromboxane A2, whereas clopidogrel reduces platelet activation via affecting low-affinity adenosine diphosphate (ADP) receptor activation processes, including adenylyl cyclase down-regulation, protein tyrosine phosphorylation, activation of the GPIIb- GPIIIa complex, fibrinogen binding, and platelet aggregation. Previous research has shown that aspirin reduces the relative risk of vascular events by 13% and stroke risk by 18% compared with placebo. In the Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events trial, administration of clopidogrel to patients with atherosclerotic vascular disease is more effective than aspirin in reducing the combined risk of ischemic stroke, myocardial infarction, and vascular death. Although the combination of aspirin and clopidogrel has shown to be a successful treatment strategy after coronary stenting and in unstable angina pectoris, it has no significant difference in reducing major vascular events that increase the risk of life-threatening or major bleeding in patients with recent ischemic stroke or transient ischemic attack.

Increased platelet activation is demonstrated in the acute and the convalescent phases after ischemic stroke by measuring the expression of surface markers (CD62P, CD63, and CD40L). CD62P, an α-granule glycoprotein, is rapidly translocated to the cell surface and mediates platelet-leukocyte interaction through a P-selectin glycoprotein ligand 1 on the leukocyte. CD63 is a lysosome-associated protein that is exposed to the surface of platelets after stronger stimulation, whereas CD40L is a homologue of tumor necrosis factor-α that serves in inflammatory processes and platelet aggregation.

Previous studies have shown that CD62P and CD63 expressions do not differ among patients treated with aspirin, clopidogrel, or both in the chronic phase of stroke. However, prospective data regarding the relationship between serial changes of platelet activity and various antiplatelet drugs after acute ischemic stroke are limited. As such, this prospective randomized case-control study aimed to test the difference in platelet activity between patients taking aspirin and those under clopidogrel after acute ischemic stroke by assessing the expressions of CD62P, CD63, and CD40L.

PATIENTS AND METHODS

Study Participants
From July 2007 to June 2008, consecutive patients with acute ischemic stroke admitted to the Chang Gung Memorial Hospital—Kaohsiung were examined. Acute ischemic stroke was defined as a sudden-onset loss of focal cerebral function
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Platelet Activity Under Aspirin or Clopidogrel

persisting for more than 24 hours. A stroke diagnosis was established based on clinical presentation, neurologic examination, and results of a brain magnetic resonance imaging with magnetic resonance angiography (MRA). Patients aged 18 to 85 years were included if they suffered acute noncardioembolic ischemic stroke and were divided into 2 major etiologic subtypes (ie, large artery disease and small artery disease) according to the Trial of ORG 10172 in Acute Stroke Treatment criteria.24

Large artery disease was diagnosed by both MRA and extracranial color-coded duplex sonography (ECCS) showing a stenosis of the brain-supplying arteries with a 50% diameter reduction and a typical morphology for atherosclerotic lesions. Small artery disease was diagnosed if neuroimaging showed ischemic lesions smaller than 1.5 cm with clinical symptoms consistent with typical lacunar syndromes (ie, pure motor stroke, pure sensory stroke, sensorimotor stroke, dysarthria–clumsy hand syndrome, and ataxic hemiparesis).

The exclusion criteria included (1) cardioembolic stroke; (2) evidences of fever after stroke or with a history of infection 1 week before stroke; (3) intracranial hemorrhage, a history of stroke, 10 were excluded because of fever or infection during the first week after the acute stroke; 1 week before stroke; (3) intracranial hemorrhage, a history of brain-supplying arteries with a 50% diameter reduction and a typical morphology for atherosclerotic lesions. Small artery disease was diagnosed if neuroimaging showed ischemic lesions smaller than 1.5 cm with clinical symptoms consistent with typical lacunar syndromes (ie, pure motor stroke, pure sensory stroke, sensorimotor stroke, dysarthria–clumsy hand syndrome, and ataxic hemiparesis).

The exclusion criteria included (1) cardioembolic stroke; (2) evidences of fever after stroke or with a history of infection 1 week before stroke; (3) intracranial hemorrhage, a history of recent surgery, or trauma within the preceding 3 months; (4) underlying neoplasm, hematologic disorders that affect platelet count or function, end-stage renal disease, liver cirrhosis, and congestive heart failure; and (5) comatose or considered unlikely to survive for more than 3 months.

Of 80 patients with acute noncardioembolic ischemic stroke, 10 were excluded because of fever during the first week of hospitalization (4 cases), end-stage renal disease (3 cases), and gastrointestinal bleeding in the acute stage of stroke (3 cases). Of the remaining 70 patients, 35 had small vessel disease and 35 had large vessel disease. They were randomized into 2 groups: 1 group took aspirin (100 mg/d) and the other took clopidogrel (75 mg/d). The present study enrolled only patients with acute ischemic stroke who were taking aspirin at 100 mg/d to exclude the dose-dependent effects of aspirin on stroke outcome. The therapeutic regimens were based on the American Heart Association/American Stroke Association guidelines for the prevention of ischemic stroke.25 The patients received the first dose of the antiplatelet drug orally within 24 hours after the acute stroke.

For the control group, 30 sex- and age-matched subjects without any of the previously mentioned exclusion criteria and with no clinical evidence of acute cerebral infarction within 1 year were included. The Institutional Review Committee on Human Research approved the study protocol, and all of the subjects provided informed consent.

Clinical Assessments

All of the subjects underwent complete neurologic examinations upon enrollment and during follow-up. Brain magnetic resonance imaging with MRA, ECCS, and transcranial color-coded sonography were performed on the patients with ischemic stroke. The control subjects underwent ECCS and transcranial color-coded sonography upon enrollment.

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS). The physical disability of stroke patients was evaluated using the modified Rankin Scale (MRS). Therapeutic outcomes were evaluated 3 months after stroke. A good outcome was defined as a 3-month MRS lower than 3 without any cardiovascular event, whereas poor outcome was any of the following end points: MRS of 3 at 3 months, any cardiovascular event, or death.26

Blood Sampling and Assessment of Platelet Activity

Blood samples were collected by venipuncture of forearm veins from acute stroke patients within 48 hours of stroke, on days 7 and 30, and 3 months after stroke. Blood samples were extracted from the control group on enrollment. Flow cytometry was done according to the modified protocol for flow cytometric characterization of platelet function as previously described.27 Briefly, sodium citrate containing blood was centrifuged for 15 minutes at 1500 rpm at room temperature. Platelet activity was assessed using the supernatant platelet-rich plasma, and platelet activation markers (CD62P, CD63, and CD40L) were determined. The samples were incubated with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control, n = 30</th>
<th>Aspirin, n = 35</th>
<th>Clopidogrel, n = 35</th>
<th>Total, n = 70</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), yr</td>
<td>66.5 (9.4)</td>
<td>67.9 (10.2)</td>
<td>66.1 (10.2)</td>
<td>67.3 (10.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Sex, male</td>
<td>18 (60.0%)</td>
<td>21</td>
<td>22</td>
<td>43 (61.4%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Hypertension</td>
<td>22 (73.3%)</td>
<td>25</td>
<td>30</td>
<td>55 (78.6%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (23.3%)</td>
<td>15</td>
<td>15</td>
<td>30 (42.9%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>15 (50%)</td>
<td>13</td>
<td>15</td>
<td>28 (40.0%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>0 (0%)</td>
<td>3</td>
<td>2</td>
<td>5 (7.1%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Current smoking</td>
<td>15 (50%)</td>
<td>15</td>
<td>13</td>
<td>28 (40.0%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Previous stroke history</td>
<td>6 (20%)</td>
<td>14</td>
<td>15</td>
<td>29 (41.4%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>19 (63.3%)</td>
<td>21</td>
<td>22</td>
<td>43 (61.4%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>14 (46.7%)</td>
<td>18</td>
<td>16</td>
<td>34 (48.6%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>13 (43.3%)</td>
<td>13</td>
<td>14</td>
<td>27 (38.6%)</td>
<td>0.66</td>
</tr>
<tr>
<td>Median NIHSS (IQR) scores on admission</td>
<td>—</td>
<td>5 (4.0, 6.0)</td>
<td>5 (3.0, 6.0)</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Stroke subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small vessel disease</td>
<td>—</td>
<td>24</td>
<td>24</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Large vessel disease</td>
<td>—</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The control versus the total patient groups.

IQR indicates interquartile range; NIHSS, National Institutes of Health Stroke Scale.

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TABLE 2. Laboratory Data of the Patients and the Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control, n = 30</th>
<th>Aspirin, n = 35</th>
<th>Clopidogrel, n = 35</th>
<th>Total, n = 70</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (x10^3/mL)</td>
<td>6.8 (0.5)</td>
<td>7.4 (0.4)</td>
<td>7.5 (0.5)</td>
<td>7.5 (0.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Red blood cell count (x10^6/mL)</td>
<td>4.6 (0.1)</td>
<td>4.5 (0.1)</td>
<td>4.4 (0.1)</td>
<td>4.5 (0.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>Platelet counts (x10^9/mL)</td>
<td>21.6 (1.3)</td>
<td>20.3 (1.0)</td>
<td>21.4 (1.1)</td>
<td>20.8 (0.7)</td>
<td>0.55</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>179.5 (5.4)</td>
<td>196.9 (8.6)</td>
<td>178.8 (9.4)</td>
<td>187.4 (6.2)</td>
<td>0.45</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>130.5 (12.4)</td>
<td>144.5 (14.8)</td>
<td>155.6 (19.1)</td>
<td>149.7 (11.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Activated partial thromboplastin, s</td>
<td>28.5 (1.0)</td>
<td>27.8 (0.4)</td>
<td>28.4 (0.6)</td>
<td>28.0 (0.3)</td>
<td>0.60</td>
</tr>
<tr>
<td>Prothrombin time, s</td>
<td>10.0 (0.4)</td>
<td>10.0 (0.1)</td>
<td>10.2 (0.1)</td>
<td>10.1 (0.1)</td>
<td>0.93</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>6.2 (0.2)</td>
<td>7.4 (0.4)</td>
<td>6.9 (0.3)</td>
<td>7.2 (0.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>CD62P, %</td>
<td>1.7 (0.2)</td>
<td>3.9 (0.5)</td>
<td>3.8 (0.5)</td>
<td>3.9 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD63, %</td>
<td>0.69 (0.10)</td>
<td>1.73 (0.25)</td>
<td>1.63 (0.22)</td>
<td>1.68 (0.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD40L, %</td>
<td>0.22 (0.03)</td>
<td>0.28 (0.04)</td>
<td>0.29 (0.03)</td>
<td>0.29 (0.04)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*The control versus the total patient groups.

saturating concentrations of phycoerythrin (PE)-labeled antibodies (Becton Dickinson Biosciences, California) against CD62P (clone AK-4), CD63 (clone H5C6), and CD40L (Clone TRAP1), with fluorescent isothiocyanate–labeled antibodies against CD61 (clone VI-PL2) for 30 minutes at room temperature in the dark.

As control experiments, platelets were incubated with PE-coupled un specific mouse IgG1 (Becton Dickinson Biosciences) with the same ratio and concentration of fluorochrome to protein as the specific IgG. After immunolabeling, the samples were analyzed by Coulter Epics XL flow cytometry (Beckman Coulter, Florida). Forward light scatter and expression of CD61 was used for platelet identification. Platelet-bound anti-CD62P, anti-CD63, and CD40L antibodies were determined by analyzing 10,000 platelets for PE-positive fluorescence.

The results were expressed as a percentage of antibody-positive platelets. Intra-assay variability based on repeated measurements of the same blood sample was low, with mean coefficients of variance of 7.5% (<48 hours), 7.3% (day 7), 6.9% (day 21), and 6.8% (day 90) for stroke patients and 6.0% in healthy control individuals.

Statistical Analysis

Data were presented as mean (SEM). Comparisons between the acute stroke and the control groups and among patients under different antiplatelet therapies were made by an independent t test. A χ² test or a Fisher exact test was used for comparison of proportions among groups. Repeated measures of analysis of variance were used to compare platelet activity at 4 different time points (<48 hours and on days 7, 30, and 90), whereas the Scheffe multiple comparison test was used to analyze the intraindividual course of parameters over time and to compare parameters of 2 different groups. Outcome was compared between the aspirin and the clopidogrel groups using the Mann-Whitney U test. A P < 0.05 was considered statistically significant. All statistical calculations were performed using the SAS software package, version 9.1 (2002; SAS Statistical Institute, Cary, NC).

RESULTS

Baseline Characteristics of the Patients and Control Subjects

Of the 70 patients with acute noncardioembolic ischemic stroke, gastrointestinal bleeding was noted in 1 patient taking aspirin and in another taking clopidogrel during follow-up. They discontinued their antiplatelet therapy. There were no recurrent stroke, cardiovascular events, and death during the follow-up period in both the aspirin and the clopidogrel groups. Demographic data of the patients and the control subjects were shown in Table 1. The age range of the stroke patients (35 aspirin and 35 clopidogrel) was 44 to 84 years (mean, 67.3 [10.4] years) and was 46 to 82 years (mean, 66.5 [9.4] years) for the control subjects (18 men and 12 women). There were no significant differences in sex, age, and underlying conditions between the 2 groups. Other
drugs that may affect these platelet markers (eg, statins, calcium channel blockers, and angiotensin II receptor blockers) were equal in each group. The median (interquartile range) NIHSS score on admission was also similar between the 2 groups.

Laboratory data and platelet activation markers were presented in Table 2. There were no significant differences among the groups in white blood cell, red blood cell, and platelet counts; serum triglyceride level; serum total cholesterol level; prothrombin time; and activated partial thromboplastin time. However, glycosylated hemoglobin (HbA1c) level and platelet counts were significantly higher in the stroke patients than in the controls (*P < 0.05). There was no significant difference between patients taking aspirin and those taking clopidogrel in laboratory data and platelet activation markers.

**Time Course of Platelet Activation Markers Under Aspirin and Clopidogrel**

Expressions of CD62P and CD63 in patients taking aspirin and clopidogrel were shown in Figures 1 and 2, respectively. The percentage of platelets expressing CD62P and CD63 was similar in both groups within the first day after stroke. However, platelet CD62P and CD63 levels were significantly lower in patients taking clopidogrel than in patients taking aspirin on days 7 and 30 after acute stroke (*P < 0.05). There were no more differences between the 2 groups on day 90 after stroke.

The percentage of platelet CD40L expression was not different between the 2 groups on the acute stage of ischemic stroke (Fig. 3). On day 7 after stroke, CD40L expression was significantly reduced in stroke patients under clopidogrel therapy (*P < 0.05) but was not different between the 2 groups on days 30 and 90 after stroke. Moreover, repeated analysis of variance with the Scheffe multiple comparison test demonstrated that the CD62P, the CD63, and the CD40L expressions between the 2 groups at 4 different time points (~24 hours and on days 7, 30, and 90) were significantly different (*P < 0.001).

**Therapeutic Outcome Under Antiplatelet Therapy**

The mean NIHSS total score on admission was 5.5 (4.4) for patients on clopidogrel therapy and 5.4 (2.2) for those on aspirin (*P = 0.21, Mann-Whitney U test). The therapeutic outcomes

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**TABLE 3.** Prospective Studies of Variable Platelet Functional Tests to Aspirin and Clopidogrel

<table>
<thead>
<tr>
<th>Study, yr</th>
<th>Subjects (n)</th>
<th>Dose, mg/d</th>
<th>Platelet Function Assay</th>
<th>Assay Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serebruany et al&lt;sup&gt;33&lt;/sup&gt;</td>
<td>Ischemic stroke within the last 3 mo (70)</td>
<td>ASA, 81; C, 75</td>
<td>Conventional aggregometry; PFA-100; Ultegra analyzer; flow cytometric assay (PECAM-1, P-selectin, PAC-1, vitronectin, and platelet-leukocyte microparticles)</td>
<td>C+ASA significantly inhibits ADP- and collagen-induced aggregation; CADP-CT prolongation; reduction of PAU with Ultegra; expression of PECAM-1 and PAC-1 when compared with the ASA group</td>
</tr>
<tr>
<td>Yip et al&lt;sup&gt;30&lt;/sup&gt;</td>
<td>Acute ischemic stroke (87)</td>
<td>ASA, not mentioned; C, not mentioned</td>
<td>Flow cytometric assay, CD62P</td>
<td>C &gt; ASA in suppressing CD62P expression on day 90 after stroke</td>
</tr>
<tr>
<td>Grau et al&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Prior ischemic stroke (31)</td>
<td>ASA, 100–300; change to C, 75; and ASA+C, 300/75 for 4 wk</td>
<td>Flow cytometry, CD62P and CD63; PFA-100, CEPI-CT and CADP-CT</td>
<td>CD62P and CD63 expressions were not different in both therapies. CEPI-CT, ASA+C; CADP-CT, ASA+ASA+C or ASA</td>
</tr>
<tr>
<td>Klinkhardt et al&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Prior atherosclerotic vascular disease and peripheral occlusions (44)</td>
<td>ASA, 100; C, 75; ASA+C, 100/75</td>
<td>Turbidimetric light transmittance, collagen and ADP; flow cytometric assay (CD62, PAC-1, and MAC-1) before (baseline) and after stimulation with TRAP or ADP; and soluble ICAM-1</td>
<td>Both at baseline and after stimulation, C and C+ASA exhibited significantly lower levels of CD62 expression and platelet-leukocyte aggregate when compared with ASA</td>
</tr>
<tr>
<td>Moshfegh et al&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Prior myocardial infarction (30)</td>
<td>ASA, 100; C, 75; ASA+C, 100/75</td>
<td>Optical aggregometry; flow cytometric assay (CD62P, CD63, and PAC-1) with various stimulators (ADP, collagen, and thrombin)</td>
<td>C and C+ASA in inhibition of ADP-mediated aggregation. C and C+ASA in inhibition of CD62P, CD63, and PAC-1 expressions after stimulation with ADP or thrombin</td>
</tr>
</tbody>
</table>

ASA indicates aspirin therapy; C, clopidogrel therapy; CADP-CT, collagen/ADP closure times; CEPI-CT, collagen/epinephrine closure times; ICAM-1, intercellular adhesion molecule 1; MAC-1, macrophage antigen-1; PAU, platelet activation units; PECAM-1, platelet endothelial cell adhesion molecule 1; PFA-100, platelet function analyzer; PAC-1, procaspase activating compound-1, first procaspase activating compound-1; TRAP, thrombin receptor-activating peptide.
among the 70 cases in the 3-month follow-up period were determined by MRS as complete recovery (18.6%, 13/70), no significant disabilities despite symptoms (44.3%, 31/70), with slight disabilities (20.0%, 14/70), with moderate disabilities (10.0%, 7/70), and with moderately severe disabilities (7.1%, 5/70). The mean MRS score at 3 months after stroke was not significantly different between the 2 groups (clopidogrel vs aspirin, 1.4 [1.1] vs 1.6 [1.1], respectively; \( P = 0.34, \) Mann-Whitney \( U \) test).

**DISCUSSION**

Increased expressions of platelet activation markers (CD62P, CD63, and CD40L) during the acute phase after an atherosclerotic ischemic stroke and their subsequent decline over time as shown here are consistent with the result of a previous study that did not evaluate the effects of different anti-platelet drugs.\(^{7,8}\) The current study demonstrates that surface expressions of CD62P and CD63 on platelets are significantly reduced in patients on clopidogrel than in those on aspirin at 1 week to 1 month after stroke. There was a significant decrease in the CD40L level in patients on clopidogrel compared with those taking aspirin at 1 week after stroke, but this decrease disappeared at 1 month.

Prospective studies of variable platelet functional tests to aspirin and clopidogrel are summarized in Table 3. Data on CD62P and CD63 expressions as measured by flow cytometry is controversial\(^ {23,30,32} \) and may be due to different study subjects, antiplatelet dosages, and study methods. However, clopidogrel combined with aspirin shows synergistic inhibitory effects after stimulation with collagen and thrombin compared with monotherapy.\(^ {2,22} \) Data of the platelet aggregation test, either clopidogrel alone or clopidogrel plus aspirin, show significantly greater inhibition of platelet activity than aspirin alone in patients with recent ischemic stroke or myocardial infarction.\(^ {12,32,33} \)

For noncardioembolic ischemic stroke or transient ischemic attack patients, antiplatelet agents rather than oral anticoagulants are recommended to reduce the risk of recurrent stroke and other cardiovascular events.\(^ {25} \) Although aspirin and clopidogrel are both acceptable for initial therapy, ischemic stroke, myocardial infarction, or vascular death occurs in 8.7% fewer patients treated with clopidogrel compared with aspirin.\(^ {12} \) With similar baseline characteristics and stroke subtypes, the current study demonstrates that patients on clopidogrel therapy have lower platelet activity compared with those on aspirin therapy in the subacute (day 7) and the convalescent phases (day 30) after ischemic stroke. For the chronic phase (>3 months), the data here is consistent with a recent study that shows that CD62P and CD63 expressions do not differ between patients treated with aspirin, clopidogrel, or both.\(^ {17,23} \)

Other studies have concordantly shown that aspirin does not affect flow cytometry analysis of platelet activation (neither CD62P nor CD63).\(^ {34-36} \) whereas reduced CD62P expression is noted for clopidogrel.\(^ {37} \) This suggests that clopidogrel may exert stronger inhibition of platelet activity than aspirin in the acute and the subacute phases, when there are increased CD62P and CD63 expressions, but not in the chronic phase after stroke. Furthermore, a recent study has demonstrated that cytosolic Ca\(^ {++} \)-related platelet activity may be induced through the ADP and platelet-activating factor pathways but not through the thrombin or arachidonic acid pathways. The dysregulation of Ca\(^ {++} \) movement in platelets may persist up to 1 month after ischemic stroke.\(^ {38} \) It is therefore possible that the significant difference between clopidogrel and aspirin is detectable in the acute and the convalescent phases after stroke because clopidogrel is an ADP receptor-dependent antiplatelet drug.

The differential expression pattern of platelet markers over time after ischemic stroke may be related to the characteristics of each protein. CD62P (P-selectin), a glycoprotein localized on the α-granule membrane, is rapidly translocated to the cell surface after stimulation and mediates platelet adhesion to leukocytes through a P-selectin glycoprotein ligand on the leukocyte.\(^ {19} \) CD63 is a lysosome-associated protein that is exposed to the surface of platelets after platelet activation.\(^ {20} \) CD40L, a transmembrane homologue of tumor necrosis factor-α, is responsible for the activation of B cells, macrophages, and endothelial cells that serve in the inflammatory processes. The interaction of CD40L with CD40 induces matrix metalloproteinase expression in atheroma and eventually causes plaque instability.\(^ {22,39} \)

Most previous studies demonstrate increased CD62P and CD62P expressions in the acute, the subacute, and the convalescent phases after ischemic stroke.\(^ {17,23} \) whereas platelets xpress CD40L on the cell surface within a very short period after stroke and is cleaved to a soluble, inactive form.\(^ {18,22} \) These suggest that the temporary up-regulation of CD40L on platelets contributes to the destabilization of atheroma and results in acute ischemic stroke, whereas increased CD63 and CD62P expressions participate in the inflammatory process of atherosclerosis.\(^ {18} \)

The current study shows that CD40L expression on platelets declines rapidly over time after ischemic stroke but is no longer different between patients and controls at 1 month after stroke. As a result, CD62P and CD63, rather than CD40L, are used for the in vitro assessment of long-term effects on platelet activity. Recent clinical research further demonstrates that high CD62P and CD40L expressions on admission are strongly associated with poor clinical outcomes after acute ischemic stroke.\(^ {40,41} \) Therefore, stronger inhibition of platelets activity in the acute phases of ischemic stroke may be beneficial for clinical outcomes.

This study has several limitations. First, platelet activity detected in vitro by flow cytometry may not actually reflect in vivo platelet activity. Second, the expressions of platelet activation markers may be different in specific stroke subtypes; that is, large vessel infarction has stronger platelet activation than lacunar stroke. This study enrolled both small and large vessel infarctions, and the number of cases was equal in both therapeutic groups. Lastly, the follow-up period was short, and the study subject was small. A prolonged period in a large-scale study is needed to further evaluate the association between clinical outcomes and expressions of platelet activation markers in patients taking different antiplatelet drugs.

In summary, platelet activity is significantly suppressed in patients on clopidogrel than in those taking aspirin in the subacute and the convalescent phases of noncardioembolic ischemic stroke. A prospective trial that will evaluate the relationship between platelet activation markers and outcomes in stroke patients under different antiplatelet therapies and that will clarify optimal treatment is warranted.

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**REFERENCES**


