Dual Antiplatelet Therapy Clopidogrel with Low-dose Cilostazol Intensifies Platelet Inhibition in Patients with Ischemic Stroke

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Abstract

Objective  We previously reported that the antiplatelet action is intensified with combined use of clopidogrel and cilostazol in ischemic stroke patients using the VerifyNow P2Y12 Assay. In this study, the relationship between the cilostazol dose and the platelet function achieved with combination therapy was investigated.

Methods  The subjects included 231 patients with noncardiogenic ischemic stroke treated at our hospital (18 patients treated with a combination of clopidogrel (75 mg) and cilostazol (100 mg), 52 patients treated with a combination of clopidogrel (75 mg) and cilostazol (200 mg), 126 patients treated with clopidogrel (75 mg) alone and 35 patients treated with cilostazol (200 mg) alone). The platelet function achieved with 20 μM of adenosine diphosphate was measured using the VerifyNow P2Y12 Assay. Clopidogrel resistance was defined as P2Y12 Reaction Units (PRU) >230 and/or % inhibition <20%.

Results  The PRU was >230 in 32 patients (25.4%) receiving clopidogrel alone, one patient (5.6%) receiving combination therapy with cilostazol (100 mg) and one patient (1.9%) receiving combination therapy with cilostazol (200 mg). The rate of PRU >230 was significantly lower in both of the cilostazol combination groups than in the clopidogrel alone group. The percent inhibition was <20% in 41 patients (32.5%) receiving clopidogrel alone, one patient (5.6%) receiving a combination with cilostazol (100 mg) and one patient (1.9%) receiving a combination with cilostazol (200 mg). The rate of % inhibition <20% was significantly lower in both of the cilostazol combination groups than in the clopidogrel alone group.

Conclusion  Clopidogrel resistance was clearly decreased with combination clopidogrel (75 mg) and low-dose (100 mg) cilostazol therapy. The use of combination therapy with clopidogrel and low-dose cilostazol may be one means of overcoming clopidogrel resistance.

Key words: clopidogrel resistance, cilostazol, ischemic stroke, VerifyNow P2Y12 Assay, dual antiplatelet therapy


Introduction

There are individual differences in the response to clopidogrel, which is affected by the presence or absence of drug metabolizing enzyme CYP2C19 gene polymorphism (1-5). We previously measured the platelet function ability of ischemic stroke patients using the VerifyNow P2Y12 Assay (Accumetrics Inc., San Diego, CA, USA) and reported that clopidogrel resistance was present in 29% of the patients (6). We also reported that the antiplatelet action is strengthened with combined use of clopidogrel and cilostazol (6). However, we did not investigate the dose of cilostazol administered in combination therapy with clopidogrel and cilostazol. In this study, a greater number of patients receiving combination therapy with clopidogrel and cilostazol

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Received for publication December 18, 2012; Accepted for publication January 22, 2013

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was included and the relationship between the dose of cilostazol used in combination therapy and the platelet function was therefore investigated.

**Materials and Methods**

The subjects consisted of 231 noncardiogenic ischemic stroke patients treated at our hospital between October 2009 and September 2012 (174 men, 57 women, mean age 67.0±9.5 years, 161 patients with atherothrombotic infarction, 51 patients with lacunar infarction and 19 patients with transient ischemic attack). The treatment included combined clopidogrel (75 mg) and cilostazol (100 mg) in 18 patients (16 men, two women, mean age 69.1±8.2 years), combined clopidogrel (75 mg) and cilostazol (200 mg) in 52 patients (38 men, 14 women, mean age 66.9±7.8 years), clopidogrel (75 mg) alone in 126 patients (95 men, 31 women, mean age 66.8±9.6 years) and cilostazol (200 mg) alone in 35 patients (25 men, 10 women, mean age 66.6±11.9 years). The recommended dose of cilostazol in Japan is 200 mg. The reason for selecting clopidogrel at a dose of 75 mg and cilostazol at a dose of 100 mg was that, in seven of 18 patients, headaches and tachycardia occurred with the administration of 200 mg of cilostazol. The dose was therefore reduced to 100 mg. In the 11 other patients, a dose of 100 mg was selected by the primary physician out of concern for headaches or tachycardia. All patients received an antiplatelet agent in a given oral dose for seven days or more, and the number of days from onset until measurement of the platelet function was ≥14 days. The clinical stroke type and risk factors in each patient group are shown in the Table. The cilostazol combination groups exhibited a higher frequency of atherothrombotic infarction and a lower frequency of lacunar infarction than the clopidogrel alone group or the cilostazol alone group. The frequencies of risk factors were not clearly different between the two groups receiving combination cilostazol. The frequencies of diabetes, hypertension and dyslipidemia were lower in the cilostazol alone group than in the other three groups.

Blood (1.8 mL) was collected into a vacuum blood collection tube containing 0.2 mL of 3.2% sodium citrate using a 21-G blood collection needle. The platelet function was measured with 20 μM adenosine diphosphate (ADP) using the VerifyNow P2Y12 Assay between 10 minutes and four hours after collection. The platelet function was then compared among the clopidogrel (75 mg) and cilostazol (100 mg) combination group, the clopidogrel (75 mg) and cilostazol (200 mg) combination group, the clopidogrel (75 mg) alone group and the cilostazol (200 mg) alone group. Next, the rate of clopidogrel resistance was compared among the clopidogrel (75 mg) and cilostazol (100 mg) combination group, the clopidogrel (75 mg) and cilostazol (200 mg) combination group and the clopidogrel (75 mg) alone group. With reference to previous reports, clopidogrel resistance was defined in this study as P2Y12 Reaction Units (PRU) > 230 (7-9) and/or % inhibition <20% (10-12).

**VerifyNow P2Y12 Assay**

The VerifyNow P2Y12 Assay is a system to measure P2Y12 receptor inhibition of platelets with whole blood specimens. This system measures the platelet function based on the fibrinogen binding capacity of activated platelets. There are reaction chambers with ADP 20 μM + prostaglandin E1 22 nM and isothrombin receptor activating peptide (iso-TRAP) as platelet activating substances in the measurement cartridge. Fibrinogen is placed into each chamber. The fibrinogen aggregates in whole blood are measured in proportion to the number of glycoprotein (GP) IIb/IIIa receptors on activated platelets. Changes in platelet activation are detected by monitoring changes in the light transmittance produced by aggregate formation. The extent of aggregation is expressed by the PRU and % inhibition. The PRU is the amount of aggregation due specifically to ADP in platelet P2Y12 receptors, calculated from the speed and extent of platelet aggregation in the reaction chamber containing ADP. The percent inhibition is the percent change from the baseline aggregation ability, calculated from the PRU results and baseline results (BASE). BASE is an independently measured value based on the speed and extent of platelet aggregation due to thrombin receptors, especially protease-activated receptor-1, 4 (PAR-1, 4) receptors. To activate platelets, iso-TRAP and PAR-4 activating peptide (PAR-4 AP) are incorporated into the reaction chamber for BASE measurement. The percent inhibition is obtained from the following formula: % inhibition=100×(BASE-PRU)/BASE.
Fig. 1 shows the PRU results. The PRU was significantly lower in the three groups that received clopidogrel than in the cilostazol alone group (p<0.001). In addition, the PRU was significantly lower in the clopidogrel (75 mg) and cilostazol (200 mg) combination group than in the clopidogrel (75 mg) alone group (p<0.001); however, there were no significant differences compared with that observed in the clopidogrel (75 mg) and cilostazol (100 mg) combination group.

Fig. 2 shows the % inhibition results. The percent inhibition was significantly higher in the three groups that received clopidogrel than in the cilostazol alone group. The rate of % inhibition <20% was significantly lower in both groups with combination cilostazol than in the clopidogrel alone group.

Statistical analysis

The IBM SPSS Statistics 20 software program (IBM SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. A one-way analysis of variance and Tukey’s honestly significant difference test were used in the comparisons of PRU and % inhibition. The results are expressed as the mean ± SD. A one-tailed Fisher’s exact test was used to compare the rate of clopidogrel resistance. p<0.05 was taken to be significant.

Ethical approval

This study was approved by the ethics committee of Saitama Medical University.

Results

Fig. 1 shows the PRU results. The PRU was 141±62 in the clopidogrel (75 mg) and cilostazol (100 mg) combination group, 102±74 in the clopidogrel (75 mg) and cilostazol (200 mg) combination group, 174±89 in the clopidogrel (75 mg) alone group and 249±57 in the cilostazol (200 mg) alone group. The PRU was significantly lower in the three groups receiving clopidogrel than in the cilostazol alone group (p<0.001). The PRU was also significantly lower in the clopidogrel (75 mg) and cilostazol (200 mg) combination group than in the clopidogrel (75 mg) alone group (p<0.001); however, it was not significantly different from that observed in the clopidogrel (75 mg) and cilostazol (100 mg) combination group.

Fig. 2 shows the % inhibition results. The percent inhibition was 49.2±21.6% in the clopidogrel (75 mg) and cilostazol (100 mg) combination group, 63.9±25.2% in the clopidogrel (75 mg) and cilostazol (200 mg) combination group, 37.4±28.0% in the clopidogrel (75 mg) alone group and 12.7±11.8% in the cilostazol (200 mg) alone group. The percent inhibition was significantly higher in the three groups that received clopidogrel than in the cilostazol alone group (p<0.001). The percent inhibition was also significantly higher in the clopidogrel (75 mg) and cilostazol (200 mg) combination group than in the clopidogrel (75 mg) alone group (p<0.001); however, it was not significantly different from that observed in the clopidogrel (75 mg) and cilostazol (100 mg) combination group.

Fig. 3 shows the rate of PRU >230 in the three groups that received clopidogrel. The PRU was >230 in 1/18 patients (5.6%) in the clopidogrel (75 mg) and cilostazol (100 mg) combination group, 1/52 patients (1.9%) in the clopidogrel (75 mg) and cilostazol (200 mg) combination group and 32/126 patients (25.4%) in the clopidogrel (75 mg) alone group. The rate of PRU >230 was significantly lower in both groups with combination cilostazol than in the clopidogrel alone group.

Fig. 4 shows the rate of % inhibition <20% in the three groups that received clopidogrel. The rate was 1/18 patients (5.6%) in the clopidogrel (75 mg) and cilostazol (100 mg) combination group, 1/52 patients (1.9%) in the clopidogrel (75 mg) and cilostazol (200 mg) combination group and 41/126 patients (32.5%) in the clopidogrel (75 mg) alone group. The rate of % inhibition <20% was significantly lower in both groups with combination cilostazol than in the clopidogrel alone group.
good antiplatelet action is obtained in platelet aggregation during coronary artery stent placement for myocardial infarction, a phenomenon that has yet to be determined in Japanese patients. Second, this study was not a randomized trial.

Now P2Y12 Assay has yet to be determined in Japanese patients. Second, this study was not a randomized trial. First, the number of subjects was small. In the future, large-scale trials are needed to evaluate the effects of 100 mg of cilostazol in combination treatment.

There are very few reports on the use of combined clopidogrel and cilostazol therapy in patients with cerebral ischemia. We presented the first such report, with ischemic stroke patients as subjects (6). Next, Haraguchi et al. (19) measured the platelet function in patients who had undergone endovascular treatment (coil embolization for brain aneurysm, carotid artery stenting) and reported that the antiplatelet action was strengthened with combined clopidogrel and cilostazol. They found no significant differences between the cilostazol 100 mg and 200 mg combination groups. Yama
gami et al. (20) reported decreased incidences of in-stent stenosis and new stenosis within treated vessels with combination cilostazol in patients who underwent carotid artery stenting. Therefore, the combined use of clopidogrel and cilostazol has also attracted attention in recent years from a clinical viewpoint.

The mechanism underlying the strengthened antiplatelet action observed with the concurrent use of clopidogrel and cilostazol, as described in our previous report, is thought to involve an increase in cyclic adenosine monophosphate (cAMP) within platelets as a result of inhibition of P2Y12 receptors by clopidogrel and inhibition of phosphodiesterase 3 (PDE III) by cilostazol (6).

This study is associated with several limitations. First, the cutoff value to define clopidogrel resistance using the VerifyNow P2Y12 Assay has yet to be determined in Japanese patients. Second, this study was not a randomized trial. Third, the number of subjects was small. In the future, large-scale trials are needed to confirm the clinical effectiveness of combined clopidogrel and cilostazol treatment.
The authors state that they have no Conflict of Interest (COI).

clincial trials are needed to confirm the effects of combination therapy with clopidogrel and low-dose cilostazol in the prevention of ischemic stroke events. Finally, CYP2C19 gene polymorphism has been shown to contribute to clopidogrel resistance; however, gene polymorphism was not investigated in the present study. Nevertheless, the present results showed that clopidogrel resistance is clearly reduced with the combined use of clopidogrel and cilostazol, and even a low dose of 100 mg of cilostazol seems to be sufficient for use in combination therapy. Since low-dose cilostazol leads to a decrease in adverse effects such as headaches and tachycardia, the use of combination therapy with clopidogrel and low-dose cilostazol may be one means of overcoming clopidogrel resistance in the future.

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References