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□ ORIGINAL ARTICLE □

Clopidogrel Resistance and the Effect of Combination Cilostazol in Patients with Ischemic Stroke or Carotid Artery Stenting Using the VerifyNow P2Y12 Assay

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Abstract

Objective The inhibitory response to clopidogrel considerably varies among individuals and clopidogrel resistance is a risk factor for thrombotic events in patients with cardiovascular disease. Based on the platelet aggregation evaluated by the VerifyNow P2Y12 Assay, the present study investigated clopidogrel resistance and the effect of cilostazol addition.

Methods We measured the ability of 20 μM ADP to aggregate platelets using the VerifyNow P2Y12 Assay. Clopidogrel resistance was defined as % inhibition of <20% in this assay.

Patients We examined 77 patients (53 men and 24 women, aged 65.8±9.9 years) with ischemic stroke or carotid artery stenting who received clopidogrel (75 mg) for >7 days at our hospital between October 2009 and March 2010. For 62 patients (42 men and 20 women, aged 65.3±9.9 years) 75 mg clopidogrel alone was administered (clopidogrel only group); the other 15 patients (11 men and 4 women, aged 67.9±9.9 years) received 75 mg of clopidogrel plus 100 or 200 mg of cilostazol (cilostazol combination group).

Results Clopidogrel resistance was identified in 18 (29%) of the 62 patients in the clopidogrel only group. The percent inhibition was significantly higher in the cilostazol combination group than in the clopidogrel only group (41.7±28.0% vs. 64.9±22.7%, p=0.005). None of the patients in the cilostazol combination group had % inhibition of <20%.

Conclusion Clopidogrel resistance developed in 29% of patients given clopidogrel alone. The addition of cilostazol to clopidogrel may have intensified platelet inhibition.

Key words: clopidogrel resistance, cilostazol, ischemic stroke, platelet aggregation

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Introduction

Clopidogrel is a thienopyridine compound antagonist of adenosine diphosphate (ADP) receptor subtype P2Y12. It is a prodrug that first exhibits antiplatelet action after becoming activated through metabolism in the liver. Recently, much attention has been paid to clopidogrel resistance (1-6), however, there are few reports of clopidogrel resistance in ischemic stroke patients. Using a turbidimetric method with a low dose of ADP (1-4 μM) and screen filtration pressure, we determined that the rate of clopidogrel resistance in patients with ischemic stroke is 8-18% (7).

Cilostazol is an inhibitor of phoshodiesterase 3 (PDEIII) and leads to an increase in intraplatelet cyclic adenosine monophosphate (cAMP) levels. It was reported that cilostazol seems to be non-inferior, and may even be superior to aspirin for the prevention of stroke after an ischemic stroke; further, it was associated with fewer hemorrhagic events in the second Cilostazol Stroke Prevention Study (CSPS 2) (8). Cilostazol is commonly used for ischemic stroke in Japan.

In this study, clopidogrel resistance and the effect of the combination of cilostazol and clopidogrel in patients taking oral clopidogrel were examined using the VerifyNow P2Y12

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Table 1. Baseline Clinical and Laboratory Characteristics

Variables	Clopidogrel only group (n=62)	Cilostazol combination group (n=15)	p value
Age (years)	65.3±9.9	67.9±9.9	0.589
Male	42	11	0.765
BMI (kg/m^2)	23.6±3.0	23.0±2.6	0.738
Smoking	29	8	0.766
Diabetes mellitus	17	4	1.0
HbA1c (%)	5.5±0.9	5.9±1.5	0.554
Hypertension	42	13	0.208
Hyperlipidemia	44	9	0.535
TC (mg/dL)	187.2±37.4	181.3±42.4	0.439
LDL-C (mg/dL)	111.3±32.6	108.3±33.3	0.487
HDL-C (mg/dL)	47.2±19.4	48.8±14.0	0.510
TG (mg/dL)	144.2±77.9	122.9±46.2	0.545
History of CAS	11	5	0.284
History of ischemic stroke	51	10	0.284
Progressive symptoms during hospitalization	1	7	<0.001
Concomitant medications			
ARBs	28	8	0.388
Statins	26	9	0.166
PPIs	37	7	0.265

BMI; body mass index, HbA1c; hemoglobin A1c, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides, CAS; carotid artery stenting, ARB; angiotensin II receptor blocker, PPI; proton pump inhibitor

Assay (Accumetrics Inc., San Diego, CA).

Subjects and Methods

The subjects were 77 patients (53 males, 24 females; mean age 65.8±9.9 years) with ischemic stroke or carotid artery stenting who were in the outpatient clinic of our hospital between October 2009 and March 2010. These patients had taken oral clopidogrel (75 mg) for 1 week or more (7-307 days, mean 53 days) and gave informed consent to participate in the study. Overall, 55 patients had cerebral infarction, 6 had transient cerebral ischemic attack, and 16 had carotid artery stenting. Sixty-two patients (42 males, 20 females; mean age 65.3±9.9 years) were taking oral clopidogrel alone (clopidogrel only group), and 15 (11 males, 4 females; mean age 67.9±9.9 years) were taking combination cilostazol and clopidogrel (cilostazol combination group). The dose of cilostazol was 100 mg in 4 patients and 200 mg in 11 patients. Baseline clinical and laboratory characteristics of the two groups are shown in Table 1. There were no significant differences between baseline clinical and laboratory characteristics of the two groups except for progressive symptoms during hospitalization.

Patients' blood (1.8 mL) was collected in vacuum collection tubes containing 0.2 mL of 3.2% sodium citrate using blood collection needles of 21 gauge or larger. Ten minutes to 4 hours after blood collection, platelet adhesiveness was measured with 20 μM ADP using the VerifyNow P2Y12 Assay. Platelet aggregation was then compared between the group taking clopidogrel (75 mg) alone and the cilostazol combination group (100 or 200 mg).

The VerifyNow P2Y12 Assay used in this study is an instrument to measure P2Y12 receptor inhibition of platelets in whole blood samples. This instrument measures platelet function based on fibrinogen binding capacity of activated platelets. The measurement cartridge has reaction chambers for ADP 20 μM + prostaglandin E1 22 nM and iso-thrombin receptor activating peptide (iso-TRAP) as platelet-activating substances. Fibrinogen is put into each chamber. Fibrinogen aggregates in whole blood in proportion to the number of glycoprotein (GP) IIb/IIIa receptors on activated platelets, and changes in platelet activation are seen by monitoring changes in light transmittance that result from the production of clumps. The degree of aggregation is expressed as P2Y12 Reaction Units (PRU) and % inhibition. PRU is the amount of aggregation specifically from ADP in platelet P2Y12 receptors, and it is calculated from the speed and level of platelet aggregation in the reaction chambers containing ADP. The percent inhibition is the percent change from baseline aggregation, calculated from the results for PRU and baseline (BASE). BASE is an independent value measured using the speed and level of platelet aggregation from protease-activated receptor-1,4 (PAR-1,4) receptors in particular. To activate the platelets, iso-TRAP and PAR-4 activating peptide (PAR-4 AP) were inserted into the reaction chambers for BASE measurement. The percent inhibition was obtained from the following equation: % inhibition = 100× (BASE-PRU)/BASE. With reference to previous reports, clopidogrel resistance was defined in this study as a % inhibition <20% (9, 10).

Statistical analysis was done using SPSS (version 12.0, SPSS Inc., Chicago, IL), and $p<0.05$ was taken to indicate statistical significance.

This study was approved by the ethics committee at Sai-

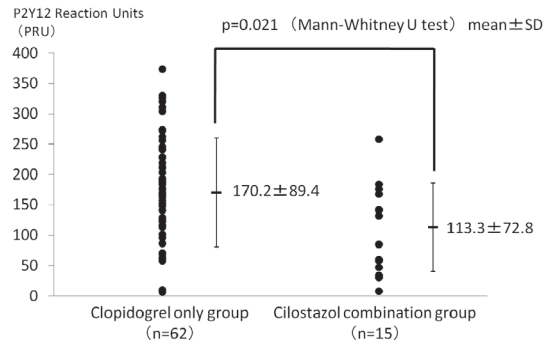

Figure 1. **Results of P2Y12 Reaction Units (PRU). PRU was significantly lower in the combination cilostazol group than in the clopidogrel only group.**

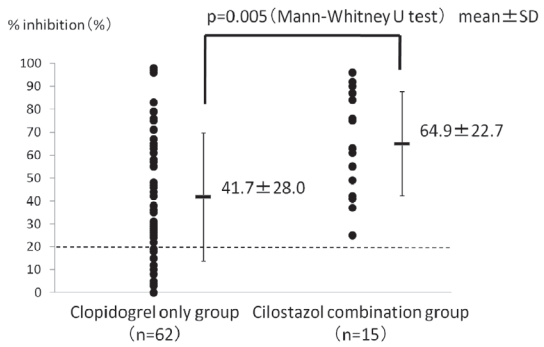

Figure 2. **Results for the % inhibition. The percent inhibition was significantly higher in the combination cilostazol group than in the clopidogrel only group. Patients with a % inhibition <20% accounted for 18/62 patients (29%) in the clopidogrel only group, whereas not a single patient in the combination cilostazol group had a % inhibition <20%.**

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Results

The results for PRU are shown in Fig. 1. PRU was significantly lower in the cilostazol combination group (170.2± 89.4) than in the clopidogrel only group (113.3±72.8; p= 0.021). The results for the % inhibition are shown in Fig. 2. The percent inhibition was significantly higher in the cilostazol combination group (64.9%±22.7%) than in the clopidogrel only group (41.7%±28.0%; p=0.005). In addition, whereas clopidogrel resistance was seen in 18 (29%) of 62 patients in the clopidogrel only group, not a single patient in the cilostazol combination group had a % inhibition of <20%.

Discussion

The majority of clopidogrel resistance studies using the VerifyNow P2Y12 Assay measured platelet aggregation in ischemic heart patients who had undergone stent placement and were taking aspirin and clopidogrel after the procedure. To our knowledge, this is the first report with ischemic stroke patients using the VerifyNow P2Y12 Assay.

The definition of clopidogrel resistance in the various reports differed, since the cut-off value of the VerifyNow P2Y12 Assay is not determined. Godino et al reported that the rate of clopidogrel resistance defined as a % inhibition of ≤15% was 21% (11). Shim et al reported that the rate of clopidogrel resistance defined as a % inhibition <20% was 40% (9). Malinin et al reported that 21% of the participants had % inhibition <30% (12). Lee et al reported that the rate of clopidogrel resistance defined as a % inhibition <40% was 42.9% (13). The rate of clopidogrel resistance using 20 μM ADP and <20% inhibition was 29% in the present study, which was higher than our previous findings (8-18%) using turbidimetry, 1-4 μM ADP and screen filtration pressure (7). The frequency of clopidogrel resistance was dependent upon the cut-off point or type of measurement method used. In recent years, it is a matter of dispute whether the presence or absence of gene polymorphism in drug-metabolizing enzyme CYP2C19 plays a role in clopidogrel resistance and activation or not (14, 15). An increase in cardiovascular events in clopidogrel resistant patients has also been shown. In the present study, we could not examine the relatioionship between clopidogrel resistance and gene polymorphism. Whether or not there is an increase of ischemic stroke events in clopidogrel resistant patients is under investigation.

Cilostazol has properties of not only inhibition of platelet function but also improvement of endothelial cell function or antisclerotic activity. Combination antiplatelet therapy, aspirin + cilostazol or clopidogrel + cilostazol, is often used for high risk patients of ischemic stroke. However, there are few reports which have examined the effect of combination antiplatelet therapy in patients of ischemic stroke. In recent years, there have been increasing reports suggesting that, in cardiovascular patients who have undergone coronary artery stenting due to myocardial infarction, a better antiplatelet effect is obtained in platelet aggregation tests using ADP in groups that receive aspirin + clopidogrel + cilostazol (triple antiplatelet therapy) than in groups that receive aspirin + clopidogrel (standard dual antiplatelet therapy) (9, 10, 16-18). Shim et al. measured platelet aggregation using the VerifyNow P2Y12 Assay in 186 patients receiving dual antiplatelet therapy with aspirin and clopidogrel, and in 193 patients receiving triple antiplatelet therapy with aspirin + clopidogrel + cilostazol following coronary artery stent placement (9). They found clopidogrel resistance in 74 patients (40%) in the dual antiplatelet therapy group and in 19 patients (9.8%) in the triple antiplatelet therapy group. Similar to these reports, in the present study, a stronger antiplatelet effect was observed with combination cilostazol than with clopidogrel alone, which would seem to confirm that the antiplatelet effect might have been enhanced with combination cilostazol even in ischemic stroke patients.

The mechanism by which the antiplatelet action of clopi-

dogrel is enhanced with combination cilostazol is thought to be as follows. When clopidogrel blocks the binding of ADP to P2Y12 receptors on platelets, adenylyl cyclase is increased, and synthesis of cAMP is induced. The cAMP-dependent protein kinase (PKA) that is activated by cAMP is inactivated by phosphorylation of vasodilator-stimulated phosphoprotein (VASP), which has a platelet-activating effect, and platelet aggregation is inhibited. Cilostazol enhances cAMP within platelets by blocking PDE III. Therefore, since both clopidogrel and cilostazol augment cAMP in the signal transduction pathway from P2Y12 receptors, the combined use of the 2 drugs strengthens the ADP aggregation inhibition effect (10, 19).

A few limitations of this study need to be addressed. First, patients were divided into a clopidogrel only group and a combination cilostazol group in this study, but in the future it will be necessary to measure platelet aggregation with additional cilostazol in the same patients. Secondly, we did not check the platelet activity with any method other than the VerifyNow P2Y12 Assay. But, the VerifyNow P2Y12 Assay has been already authorized and approved by the Food and Drug Administration in USA for analyzing clopidogrel resistance. Therefore, this method can analyze the drug-specific responsiveness of platelets. Thirdly, not only the platelet aggregation of combining clopidogrel and cilostazol but also the frequency of bleeding complications and cerebrovascular event must be investigated clinically.

In conclusion, 18 (29%) of 62 patients in the clopidogrel only group were thought to have clopidogrel resistance. The antiplatelet effect might have been enhanced with combination cilostazol in ischemic stroke patients.

The authors state that they have no Conflict of Interest (COI).

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