Evaluation of Clopidogrel Resistance in Ischemic Stroke Patients

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Abstract

Objective Clopidogrel has potent antiplatelet effects, but recent interest has focused on clopidogrel resistance, in which platelet function is not inhibited despite taking the drug. This study evaluated clopidogrel resistance in ischemic stroke patients.

Methods After taking oral clopidogrel 75 mg/day for ≥1 week, platelet aggregometry was performed by turbidimetry in all patients, and by a screen filtration pressure method using whole blood in 37 patients. Using turbidimetry, resistance was defined as platelet maximum aggregation rate ≥34% with aggregation-inducing agent ADP 1 μmol/L, or ≥66% with ADP 4 μmol/L. Using the screen filtration pressure method, resistance was defined as a minimum concentration of ≤3 μmol/L ADP to induce secondary aggregation of platelets.

Patients This study was conducted in 72 patients (52 men, 20 women; mean age, 69 ± 8 years; range, 50-84 years) with non-cardiogenic ischemic cerebrovascular disease.

Results Based on turbidimetry, the rate of clopidogrel resistance was 8.3% with ADP 1 μmol/L and 18.1% with 4 μmol/L. Based on the screen filtration pressure, the rate of clopidogrel resistance was 8.1%. The differences between turbidimetry and screen filtration pressure methods, regarding the measurement of the presence of resistance in the same patient, were observed.

Conclusion Clopidogrel resistance varies greatly depending on the method of measuring platelet aggregation and the definition of resistance. Rates of 8-18% were obtained using our methods and criteria.

Key words: clopidogrel resistance, ischemic stroke, platelet aggregation


Introduction

Clopidogrel, a thienopyridine compound, is a prodrug that is absorbed in the gastrointestinal tract. About 80% is degraded by gastrointestinal enzymes, and about 20% reaches the liver from the portal system, where it is metabolized by cytochrome P450 in two steps (CYP1A2, 2B6, 2C19; then CYP3A4/A5, 2B6, 2C9, 2C19) to form the active metabolite. This active metabolite specifically binds to platelet ADP receptor P2Y12 and irreversibly inhibits platelet function to exhibit antiplatelet effects. Clopidogrel is thus widely used to prevent recurrence of ischemic strokes. However, clopidogrel resistance, in which platelet aggregation is not inhibited despite taking clopidogrel, has recently been identified as a problem (1, 2).

For clopidogrel resistance, methods of measuring platelet aggregation differ, cut-off values for resistance differ, and no standardized definition has been established, so reported rates have varied widely from 5% to 44% (3-7). In addition, most patients studied have had ischemic heart disease, whereas clopidogrel resistance in ischemic stroke has not been adequately investigated.

This study evaluated clopidogrel resistance in patients with non-cardiogenic ischemic stroke using turbidimetry and a screen filtration pressure (SFP) method (WBA-Neo; ISK Corp., Tokyo, Japan) using whole blood.

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Patients

This study was conducted in 72 patients (52 men, 20 women; mean age, 69 ± 8 years; range, 50-84 years) with non-cardiovascular ischemic cerebrovascular disease. Clinical type was atherothrombotic stroke in 42 patients, lacunar infarction in 24, transient ischemic attack in 5, and cerebrovascular dissection in 1. Time interval from stroke onset to platelet aggregometry ranged from 7 to 62 days (mean 14 days). All patients received oral clopidogrel at 75 mg/day for at least one week.

Methods

Platelet aggregation was measured by turbidimetry in all 72 patients, and by the SFP method using whole blood in 37 patients. For turbidimetry, the serum and platelet-rich plasma (PRP) were separated, then after adding the clotting agent ADP (1 or 4 μmol/L), platelet aggregation was measured by a photoadsorption method to determine the platelet maximum aggregation rate (MAR). For the SFP method, peripheral venous blood samples were drawn using a 21 G needle and added to a test tube containing a final concentration of 0.313% sodium citrate. Whole blood samples were kept at room temperature for 1 hour, and thereafter platelet aggregation activity in response to adenosine diphosphate was measured by the SFP method. Aggregation reactions were initiated by adding 4 different concentrations of adenosine diphosphate (1, 2, 4, or 8 μmol/L) to whole blood in test tubes, while constantly stirring at 37°C. Five minutes after stimulation, the absorbing pressure was measured through a microsieve with 30×30-μm holes. The estimated adenosine diphosphate concentration resulting in 50% filter blockage by platelet aggregation was calculated and defined as the platelet aggregation threshold index (PATI). For the SFP method, centrifugation is unnecessary, whole blood can be used and reacted with ADP, and the concentration threshold for 50% MAR can be calculated. For the SFP method, using a method applying the ED50 for a dose-response curve, clopidogrel resistance was defined as a minimum concentration ≤3 μmol/L of the aggregation-inducing agent ADP to induce secondary aggregation of platelets according to the data of Imiya and Matsuo (8) and Kondo et al (9). Imiya and Matsuo (8) measured platelet aggregation with ADP using the SFP method in 34 healthy males and 54 healthy females and reported that the PATI was approximately 1.5 μmol/L for both males and females. In addition, Kondo et al (9) measured platelet aggregation with ADP using the SFP method in 25 non-stroke patients and reported a PATI of 2.08 ± 1.67 μmol/L. Thus, for platelet aggregation with ADP in healthy subjects, the PATI is about 1.5-2 μmol/L. For turbidimetry, the criteria for clopidogrel resistance (i.e., low responders) was determined by the receiver-operating-characteristic (ROC) curve with the reference of data of SFP methods. Data were analyzed using JMP Ver 7.0 for Windows. Cut-off values of maximum aggregation rate for predicting clopidogrel resistance according to ROC curve were 34.0% in ADP 1 μmol/L (sensitivity,100%; specificity 0%; AUC 1.0), and 66.0% in ADP 4 μmol/L (sensitivity, 85%; specificity 66%; AUC 0.77), respectively. This study was approved by the ethics committee at Saitama Medical University.

Results

Figure 1 shows the results of platelet aggregation by turbidimetry in patients taking clopidogrel. The rate of clopidogrel resistance was 8.3% with ADP 1 μmol/L and 18.1% with ADP 4 μmol/L. Figure 2 depicts the results of platelet aggregation by the SFP method in patients taking clopidogrel. The rate of clopidogrel resistance was 8.1%.

Figure 3, 4 compare turbidimetry and the SFP method in the same cases. Figure 3 compares ADP 1 μmol/L with the SFP method, and Fig. 4 compares ADP 4 μmol/L with the SFP method. Differences in the assessment of resistance were clearly apparent between turbidimetry and the SFP method (Fig. 3, 4). Comparisons of non-resistant patients with the 7 resistant patients with ADP 4 μmol/L by turbidimetry, and with the 3 resistant patients by SFP, showed no significant differences in age, gender, treatment duration, risk factors, or other medications. Thus, the underlying factors associated with resistance remain unclear.

Discussion

Most reports to date of clopidogrel resistance have discussed patients who underwent cardiac catheterization for myocardial infarction, with clopidogrel used for post-treatment therapy. However, methods of measuring platelet aggregation have differed, cut-off values have varied, and uniform criteria for resistance have not been applied.

Angiolillo et al (7) administered clopidogrel 300 mg to 48 patients for post-coronary artery stent placement for myocardial infarction and used turbidimetry to determine clopidogrel resistance. Defining platelet aggregation inhibition ≤40% as resistance, 44% of patients showed resistance following administration of ADP 6 μmol/L. In 746 patients for post-coronary artery stent placement for myocardial infarction, Gori et al (4) administered clopidogrel 600 mg and measured platelet aggregation by turbidimetry. Following ADP 10 μmol/L, platelet MAR ≥70% was defined as resistance, with 6% showing resistance. Lau et al (6) examined 32 patients post-coronary artery stent placement and 35 healthy adults, using turbidimetry. Following ADP 20 μmol/L, platelet aggregation inhibition ≤10% was defined as resistance. Their results showed resistance in 22% of post-coronary artery stent placement, and in 16% of healthy adults.

Djukanovic et al (3) compared aspirin + ticlopidine (15 patients) with aspirin + clopidogrel (17 patients). They measured the phosphorylation of vasodilator-stimulated...
phosphoprotein (VASP), a platelet protein that is cAMP-dependently phosphorylated, by flow cytometry. VASP ≥ 50% was defined as resistance, and the rate of resistance was 29.4%. Lee et al (5) administered clopidogrel to 387 patients for post-coronary artery stent placement for myocardial infarction, as a 300-mg loading dose and 75-mg maintenance dose. Platelet function was evaluated with VerifyNow P2Y12 Assay (Accumetrics Inc., San Dieg, CA) using whole blood, revealing a 28.9% rate of resistance. Thus, methods for measuring platelet aggregation and resistance

**Figure 1.** Evaluation of the effects of clopidogrel on platelet aggregation using turbidimetry. The rate of resistance was 8.3% with ADP 1 μmol/L and 18.1% with ADP 4 μmol/L.

**Figure 2.** Evaluation of the effects of clopidogrel on platelet aggregation using the SFP method. The rate of resistance was 8.1%.
threshold values have not been standardized, and results have varied widely between reports.

The present investigation of clopidogrel resistance in patients with ischemic stroke showed resistance rates of 8.3-18.1% for turbidimetry using PRP and 8.1% for the SFP method using whole blood. These rates were lower than the rate of clopidogrel resistance in patients with ischemic heart disease. Although this difference may be due to differences in the underlying disease, it was thought to be due to differences in cut-off value and the method of measuring platelet aggregation. In addition, differences between the turbidimetry and SFP methods were observed in reference to whether
resistance existed in the same patient, indicating that results are greatly affected by the measurement method and whether PRP or whole blood is used. In the future, the ongoing debate regarding clopidogrel resistance (low responders) will require the standardization of assay methods and cut-off values.

The factors causing clopidogrel resistance have not been completely elucidated. Factors known to be involved in resistance include: genetic factors (CYP, GPIa, P2Y12, and GPIIIa polymorphisms); cellular factors (shortened platelet turnover time, increased metabolism of the reduced form of CYP3A4, exposure to increased ADP, up-regulation of the P2Y12/P2Y1 pathway); and clinical factors (poor drug compliance, inadequate dosing, poor drug absorption, drug interactions mediated via CYP3A4, acute coronary syndrome, diabetes, and increased body mass index) (10).

The present study did not evaluate relationships between clopidogrel resistance and gene polymorphisms, nor the relationships between resistance in patients and stroke recurrence. These are issues warranting further investigation.

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

References

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