

Poor Response to Aspirin on Platelet Function Assay and the Associated Factors in Patients with Ischemic Stroke

Jin Ok Kim, Tae-Hyung Kim, Jae Guk Kim, Sung-Yeon Sohn, Do-Hyung Kim and Soo Joo Lee*

Department of Neurology, Eulji University Hospital, Eulji University School of Medicine, Daejeon, Republic of Korea

*Corresponding Author: Soo Joo Lee, Department of Neurology, Eulji University Hospital, Eulji University School of Medicine, Daejeon, Republic of Korea.

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Abstract

Aim: Ischemic stroke may recur in many patients of previous ischemic events despite of taking antiplatelet drugs for secondary prevention of ischemic stroke. However, the proper laboratory test for monitoring antiplatelet therapy is not available in clinical situation. The rapid platelet function assay has been recently developed and used. The purposes of this study were to determine the frequency of aspirin resistance in patients with ischemic stroke and to identify the factors related with aspirin resistance using the platelet function test.

Methods: We consecutively enrolled 234 patients taking aspirin regularly for secondary prevention of ischemic stroke. Demographic data, vascular risk factors (hypertension, diabetes, hyperlipidemia), drug history, hemoglobin and platelet count were recorded. We used the VerifyNow®-Aspirin to measure the inhibition of platelet function by aspirin.platelet function test.

Results: Thirty patients (30/234, 12.8%) were identified as aspirin non-responders (ARU \geq 550) on the platelet function test. Metabolic syndrome was more often found in aspirin non-responders than in aspirin responders (66.7% vs. 40.7%, $p = 0.03$). There were no significant differences between aspirin responders and aspirin non-responders concerning age, gender, hypertension, diabetes mellitus, hyperlipidemia, obesity, hemoglobin, and platelet counts. The aspirin formula and duration of treatment were not significantly different between the two groups. However, the ineffective response was more often found in patients taking low-dose (100 mg/d) aspirin than in those taking the other dose (200 or 300 mg/d) aspirin (100 mg, 26.0%; 200 mg, 7.5%; 300 mg, 6.2%; $p = 0.02$).

Conclusion: Metabolic syndrome and low-dose aspirin may be associated with the aspirin resistance on the platelet function assay.

Keywords: Aspirin, Platelet

Abbreviations

ARU: Aspirin Reaction Unit; PFA: Platelet Function Analyzer; NSAID: Non-steroidal Anti-Inflammatory Drugs; BMI: Body Mass Index; COX: Cyclooxygenase; ATP-III: Adult Treatment Panel III; ADP: Adenosine Diphosphate; GPIIb-IIIa: Glycoprotein IIb/IIIa

Introduction

Because antiplatelet agents block the role of platelets and reduce the incidence of cerebral infarction and other thrombotic diseases, antiplatelet agents have been widely used together with anticoagulants to prevent or prevent recurrence. Previously known studies have shown that aspirin reduces myocardial infarction, stroke and death in patients with risk factors by about 25% compared with the control group [1]. Ischemic stroke may recur in many patients of previous ischemic events despite of taking antiplatelet drugs for secondary prevention of ischemic stroke. However, the proper laboratory test for monitoring antiplatelet therapy is not available in clinical situation.

The effect of platelet inhibition in platelet function tests was not constantly observed in all patients and the recurrence of thrombotic and atherogenic vascular diseases was frequently observed despite aspirin use that has been call aspirin resistance. Aspirin resistance has been reported to vary from 5% to 60% in the previous studies because of the different test methods and the lack of a standard for judging aspirin reactivity [2-4].

The most widely used platelet function tests are the conventional aggregometry (Born's method), which involves several steps. This test requires a large amount of blood, a long test time and a lot of cost in comparison with other test methods, and it requires the operator to operate the blood through several steps and the repetition rate of the test is low between the repeated tests. These disadvantages limit the direct application to the clinic [5].

On the other hand, the newly developed VerifyNow®-Aspirin method is based on turbidometry which measures the change of light transmittance by platelet aggregation reaction like the classic flocculation measuring method, and proceeds in a relatively simple order. First, 2 ml of venous blood is drawn into a vacuum tube filled with 3.2% sodium citrate. This tube was collected at room temperature and then bound to the prepared cartridge containing arachidonic acid as a platelet agonist between 30 and 240 minutes. When the platelet aggregation reaction occurred in the cartridge, the changed light transmittance and expressed as a value in units of an aspirin reaction unit

(ARU) [6]. This test has the advantage of being able to perform a point of care test by a non-specialist because the blood volume required for the test is small and the blood can be tested without any other treatment as compared to the coagulation measurement technique, and the test method is simple and the test time is short. VerifyNow®-Aspirin has a higher prevalence of 92% sensitivity and 85% specificity compared with conventional coagulation measuring methods [7].

There is no report of aspirin resistance, or platelet aggregation response, in patients with stroke using VerifyNow®-Aspirin, but studies of aspirin resistance in some cardiovascular patients have recently been reported [7,8].

In a comparative study using the conventional aggregometry (Born’s method), platelet function analyzer (PFA)-100 system and VerifyNow®-Aspirin to measure the inhibitory effect of aspirin on platelet aggregation, VerifyNow®-Aspirin inhibited platelet aggregation of aspirin and the reliability of the measurement was high [10]. These results suggest that this study may be useful in the evaluation of aspirin resistance in platelet function test using VerifyNow®-Aspirin. These results suggest that this study may be useful in the evaluation of aspirin resistance in platelet function test using VerifyNow®-Aspirin.

The purposes of this study were to determine the frequency of aspirin resistance in patients with ischemic stroke and to identify the factors related with aspirin resistance using the platelet function assay

Materials and Methods

Participants and data collection

We enrolled 234 patients with ischemic stroke or TIA, who had take aspirin for secondary prevention consecutively between November 2014 and August 2015. Patients had taken aspirin for at least 7 days. Exclusion criteria follow as; ingestion of clopidogrel, ticlopidine, dipyridamole, other anti-platelet drugs, NSAID, non-COX 2 specific NSAID, administration within 24 hours before enrollment of heparin or other anticoagulant, family or personal history of bleeding disorders, and platelet count < 150X10³/μL or > 450X10³/μL, hemoglobin < 8.0 g/dl. Demographic data, vascular risk factors (hypertension, diabetes, hyperlipidemia, obesity: BMI > 25), the presence of metabolic syndrome (defined as ATP-III guideline), drug history, hemoglobin and platelet counts were recorded.

Blood test

In the selected patients, 2 ml of venous blood was drawn into a vacuum tube filled with 3.2% sodium citrate using a needle of 22 gauge. Tube was gently tilted 3-5 times to allow citrate and blood to mix well. Platelet aggregation test was performed between 30 and 240 minutes after collection at room temperature. Platelet aggregation was measured using Ultegra VerifyNow®-Aspirin. The cartridges containing the platelet enhancer arachidonic acid were mounted on the machine and the tube containing the blood was gently tilted three to five times before binding to the cartridge. If the ARU was 550 or more, it was judged to be aspirin resistance. This criterion is the result of previous studies comparing the conventional measurement method [6].

Statistical analysis

The Mann-Whitney U-test, the Fisher’s exact test and the Kruskal-Wallis H test were used to compare the two groups. The two groups were divided into two groups: responder and non-responder (aspirin-resistant). A P-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS for window version.

Results

Thirty patients (30/234, 12.8%) were identified as aspirin non-responders (ARU ≥ 550) on the platelet function test. There were no significant differences between aspirin responders and aspirin non-responders concerning age, gender, hypertension, diabetes mellitus, hyperlipidemia, obesity, hemoglobin, and platelet counts. Metabolic syndrome was more often found in aspirin non-responders than in aspirin responders (66.7% vs. 40.7%, p = 0.03). The aspirin formula and duration of treatment were not significantly different between the two groups.

However, the ineffective response was more often found in patients taking low-dose (100 mg/d) aspirin than in those taking the other dose (200 or 300 mg/d) aspirin (100 mg, 26.0%; 200 mg, 7.5%; 300 mg, 6.2%; p = 0.02) (Table). Five (50%) of ten patients who had taken aspirin medication prior to ischemic events showed insufficient platelet inhibition on the blood test.

	Responder (n = 204)	Non-Responder (n = 30)
Age (years, mean ± SD)	64.5 ± 7.9*	64.5 ± 7.9*
Sex (male)	87 (42.6)	14 (46.6)
Hypertension	140 (68.6)	21 (70.0)
Diabetes mellitus	43 (21.1)	7 (23.3)
Hyperlipidemia	79 (38.7)	12 (40.0)
Obesity (BMI > 25)	82 (40.2)	13 (40.3)
Metabolic syndrome*	83 (40.7)	20 (66.7) ** p = 0.03
Hemoglobin (mg/dL, mean ± SD)	13.9 ± 1.6	13.4 ± 0.8
Platelet count (10 ³ /μL, mean ± SD)	267.3 ± 32.0	259.4 ± 52.2
Aspirin dose (mg/day)*		
100	54 (26.5)	19 (63.3) ** p = 0.02
200	74 (36.3)	6 (20)
300	76 (37.2)	5 (16.7)
Aspirin formula		
Enteric-coated tablet	110 (53.9)	16 (53.3)
Enteric-coated pellet	94 (46.1)	14 (46.7)
Duration of medication		
7 ~ 30 days	51 (25.0)	7 (23.3)
1 month ~ 1 year	88 (43.1)	14 (46.7)
> 1 year	65 (31.9)	9 (30.0)

Table 1: Comparison of between aspirin responder and non-responder.

*Data are expressed as mean ± standard deviation, ** Statistically significant by Fisher’s exact test.

Discussion

In this study, 30 out of 234 patients (12.8%) taking aspirin for secondary prevention of ischemic stroke had aspirin resistance in the platelet function test. The authors' study did not limit the time of aspirin administration. In other studies, aspirin resistance was assessed by platelet function test within the first month after aspirin administration. However, this study was changed to other medication during long-term administration. There is a possibility that many patients with good response to aspirin have been included. However, there is no statistically significant difference in the frequency of aspirin resistance between different dosing periods in this study, and the possibility of selection bias is low.

Previous studies on stroke patients showed a significant difference in the frequency of aspirin resistance (4.1 ~ 33.3%) according to the test method and the ratio in this study was similar to that of the study using classic measurement technique. Platelet function tests revealed aspirin resistance in 5 out of 10 patients with ischemic stroke recurrence. This is an example that suggests that this platelet function test is clinically useful because the resistance of platelet function test to aspirin is consistent with clinical resistance (recurrence of stroke).

We tried to find factors related to aspirin resistance but the number of patients with resistance was too small to identify a statistically significant difference. Other studies in other countries have shown that low compliance, insufficient capacity, formulation, high cholesterol, smoking, exercise, simultaneous administration of enteric-coated tablets, other nonsteroidal anti-inflammatory drugs, shortening of the platelet replacement cycle, platelets for ADP and collagen and GPIIb-IIIa receptor polymorphism. However, there are no related factors that have yet to be clarified [9-13].

The previous study of aspirin resistance before the patient's symptom recurrence through the blood test is rare because of the problems of the classical test method and the lack of certain criteria as mentioned above. A few previous studies have used a classical aggre-gometry, which were not constant due to the limitations mentioned above [2-4].

The purpose of this study is to confirm the frequency of aspirin resistance in Korean patients taking aspirin for the second prevention of ischemic stroke. There is still a small number of such data in Korea, and I think this result can attract interest in aspirin resistance in clinical practice. If more research is conducted in the future, I think we will be able to confirm that there is a difference between races compared to other foreign data. If clinical features and associated factors associated with aspirin resistance can be found, these patients should be reassessed by increasing aspirin dose or considering replacement with other antiplatelet agents (ticlopidine, clopidogrel, dipyridamole, etc.) [14-20]. It will be the basis for making guidelines. In the future, we can confirm the drug resistance by the same method for other platelet inhibiting drugs other than aspirin, and I think that the comparison between these drugs will be the foundation for further research. In addition, this study could be used as a basis for the usefulness of the above test method in clinical practice.

The important result of our study is that aspirin resistance was frequently observed in patients with metabolic syndrome. In another study, subjects with metabolic syndrome appeared to have increased baseline platelet reactivity and turnover and a lower antiplatelet response to aspirin compared with healthy subjects [21]. They had found that platelets from the subjects with metabolic syndrome had enhanced basal reactivity, as measured by P-selectin expression and flow-dependent Impact-R assay, as well as increased platelet turnover. In addition, the overall response to aspirin was significantly reduced in subjects with metabolic syndrome, as assessed by platelet aggregation in response to various agonists and the VerifyNow®-Aspirin assay. An important potential mechanism for the reduced response to aspirin is increased platelet turnover, leading to the release of younger reticulated platelets that are still able to form thromboxane, possibly through non-cyclooxygenase-1-dependent pathways [22]. Insulin resistance may also play a role in the reduced response to aspirin observed in subjects with metabolic syndrome. Metabolic syndrome is closely associated and predicts the subsequent development of type 2 diabetes mellitus. Both subjects with metabolic syndrome and patients with type 2 diabetes mellitus display platelet hyper-reactivity and reduced response to aspirin [23].

Our results showed that patients who received 100 mg aspirin daily had more platelet aggregation than patients who received 200 mg or 300 mg. Similar results have been shown in studies in patients with heart disease [8].

The use of low-dose versus intermediate- or high-dose aspirin is still controversial. In the recent Antithrombotic Trialists' Collaboration [1], which was a meta-analysis of 287 studies, high-dose aspirin had a similar proportional risk reduction as low-dose aspirin. Because high-dose aspirin was associated with more gastrointestinal side effects and bleeding, low-dose aspirin was generally preferred and recommended. However, laboratory tests showed that high-dose aspirin was associated with more complete platelet inhibition [12,24].

The limitations of this study are as follows. We did not confirm whether aspirin resistance in platelet function test was associated with clinical recurrence, and this should be followed by each patient. In addition, the results of platelet function tests may vary according to the timing of aspirin use in a patient, so it is better to interpret the results through repeated tests rather than to determine aspirin resistance with a single platelet function test [6]. In this study, we used 550 ARU compared to classic platelet aggregation as a reference for aspirin resistance, but other platelet function tests may help to improve the validity of the results [7]. Finally, this study was conducted in one

hospital. The number of patients was small. In order to compensate for these limitations, further studies on more patients from various research institutes will be needed.

Conclusion

In conclusion, aspirin resistance in ischemic stroke patients is not uncommon. Metabolic syndrome and low-dose aspirin may be associated with the aspirin resistance on the platelet function assay. The clinical usefulness of routine platelet function tests needs to be further study.

Conflict of Interest

None declared.

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