Lower Platelet Aggregation Is a Risk Factor for Dual Antiplatelet Therapy-Associated Bleeding: A Preliminary Retrospective Study with Genotype Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCD 1 **Dongdong Yuan**
BCD 2 **Xiangfen Shi**
BF 1 **Liping Guo**
BF 1 **Gaobiao Wang**
B 1 **Yujie Zhao**
B 1 **Yuling Yang**
B 1 **Hanjuan Zhang**
B 1 **Qiong Huang**
AE 1 **Yiqiang Yuan**

1 Department of Cardiology, The 7th People's Hospital of Zhengzhou, Zhengzhou, Henan, P.R. China
2 Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, P.R. China

Corresponding Author: Yiqing Yuan, e-mail: y19847816@163.com
Source of support: Departmental sources

Background: The purpose of this study was to investigate factors influencing bleeding in patients with acute coronary syndrome (ACS) who are on aspirin and ticagrelor as dual antiplatelet therapy.

Material/Methods: This retrospective case-control study included 50 patients with ACS (25 with reported bleeding events and 25 without) on aspirin and ticagrelor. Adenosine diphosphate (ADP)- and arachidonic acid (ACA)-induced platelet aggregation rates were measured using light transmission aggregometry. Single-nucleotide polymorphisms (SNPs) in *PEAR1*, *GP1BA*, and *GSTP1* were genotyped.

Results: ACA-induced platelet aggregation rates were obviously lower in patients with bleeding events than in those without (13.28±8.46% *vs.* 24.93±9.89%, P<0.001). No significant differences in ADP-induced platelet aggregation rates were observed between the 2 groups (16.17±9.74% *vs.* 16.88±12.69%, P>0.05). Among those with bleeding events and among controls, 70% and 80% had an ACA-induced platelet aggregation rate of 0–18% and 18–50%, respectively. Mutation rates of *rs6065* in *GP1BA* and rs1695, rs4891, and rs8191439 in *GSTP1* also differed significantly between the 2 groups.

Conclusions: Lower ACA-induced platelet aggregation rates are associated with increased risk of bleeding in patients with ACS who are on aspirin and ticagrelor. An ACA-induced platelet aggregation rate of 18% may be considered the cutoff point for identifying high risk of aspirin-associated bleeding events in patients with ACS. SNP genotyping may also help predict the risk of bleeding in patients with ACS.

MeSH Keywords: Acute Coronary Syndrome • Genotype • Hemorrhage • Platelet Aggregation

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/923758
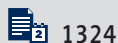
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Background

Patients with acute coronary syndrome (ACS) often need percutaneous coronary intervention, such as stenting [1]. However, the procedure may cause injury to the arterial endothelium and platelet activation. Platelet activation and aggregation increase the risk of coronary thrombosis and recurrent ACS. Therefore, antiplatelet therapy is necessary in ACS patients after interventional treatment to reduce the risk of ischemic events without increasing the risk of bleeding. Recent guidelines from the European Society of Cardiology and the European Association for Cardio-Thoracic Surgery recommend aspirin and ticagrelor as post-procedural dual antiplatelet therapy in patients with ACS [2,3].

Ticagrelor is an oral, direct, reversibly binding, P2Y12 receptor antagonist [4]. The P2Y12 receptor is the predominant receptor involved in the adenosine diphosphate (ADP)-stimulated activation of the glycoprotein IIb/IIIa receptor. Activation of the glycoprotein IIb/IIIa receptor results in enhanced platelet degranulation and thromboxane production, and prolonged platelet aggregation. Aspirin (acetylsalicylic acid) irreversibly inhibits prostaglandin H synthase (cyclooxygenase-1) in platelets, and thereby blocks the formation of thromboxane A_2, which has a potent pro-aggregant effect on platelets. However, associated bleeding events can lead to patient distress and premature discontinuation of the recommended therapy.

Platelet function can be evaluated by measuring platelet aggregation, and platelet aggregation rates reflect bleeding risk in patients with ACS who are on ticagrelor. Platelet endothelial aggregation receptor-1 (PEAR1) is crucial in platelet aggregation and function. Further, single-nucleotide polymorphisms (SNPs) in *PEAR1* are associated with varied responses to aspirin [5]. In addition, SNPs in genes for glycoprotein Ib platelet subunit alpha (GP1BA) [6] and glutathione S-transferase pi 1 (GSTP1) [7] have been previously shown to be correlated with aspirin-associated bleeding. However, there is still a lack of clinical data on the association between bleeding risks and SNPs in these genes in ACS patients on aspirin and ticagrelor.

The purpose of our study was to investigate the potential association between bleeding and platelet aggregation and SNPs in *PEAR1*, *GP1BA*, and *GSTP1* in patients with ACS on aspirin and ticagrelor.

Material and Methods

Patient selection

Our study was approved by the Ethics Committee of our hospital. This retrospective case-control study included 50 age- and platelet count-matched patients with ACS. The inclusion criteria were: diagnosed with ACS and went through percutaneous coronary intervention; age >18 years; and used aspirin and ticagrelor as post-procedural dual antiplatelet therapy. Post-procedural dual antiplatelet therapy comprised 100 mg aspirin once daily and 90 mg ticagrelor twice daily. Patients were subdivided into the case group (25 patients with reported bleeding events) and the control group (25 patients without reported bleeding events). Bleeding was defined as skin petechiae, nosebleed, hematuria, black or tarry stools, mucosal hematomas, and gum bleeding. Patients with active infections, renal or liver dysfunction, uncontrolled hypertension, history of allergy to the investigated drugs, and history of bleeding were excluded.

Platelet aggregation assay

Platelet aggregation rates were measured using light transmission aggregometry [8]. Platelet aggregation was induced with either 10 μmol/L ADP or 0.7 mmol/L arachidonic acid (ACA). Blood was tested within 2 h of collection.

Genotyping assay

Venous blood from each patient was collected, and 2 ml of the sample was used for genotyping. Genomic DNA was isolated for genotyping of SNPs in *PEAR1*, *GP1BA*, and *GSTP1* (rs3737224, rs12137505, rs11264581, rs57731889, rs77235035, rs1952294, rs735953, rs12407843, rs6067, rs2243093, rs570515282, rs748102207, rs6065, rs4147581, rs1695, rs4891, rs762803, rs1871042, rs117928, and rs8191439).

Statistical analysis

Continuous data are expressed as means and standard deviations. Platelet aggregation rates were expressed as percentages. Comparisons were made using the *t* test or chi-square test. All statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL). *P*<0.05 was considered statistically significant.

Results

There were no obvious differences between the demographic data of the patients in the case and control groups (Table 1). ACA-induced platelet aggregation rates were obviously lower in the case group than in the control group (13.28±8.46% *vs.* 24.93±9.89%, respectively; P<0.001) (Table 2). However, there were no significant differences in the ADP-induced platelet aggregation rates between the 2 groups (16.17±9.74% *vs.*16.88±12.69%, respectively; P>0.05). In the case group, 70% of patients had an ACA-induced platelet aggregation rate of 0–18% (Table 3). In the control group, 80% of patients had an

Table 1. Patients' demographic data.

	Bleeding patients (n=25)	Non-bleeding patients (n=25)	P
Age (year)	64.00±10.76	59.59±12.98	0.227
Platelet (10^9/L)	273.35±122.6	224.05±85.65	0.135
Total cholesterol (mmol/L)	3.42±1.09	4.03±1.2	0.123
Prothrombin time (s)	9.75±0.91	10.06±1.00	0.346
Body mass index (kg/m²)	24.88±3.32	25.06±2.89	0.853
Alanine transaminase (IU/L)	41.77±30.47	41.0±23.13	0.925
Aspartate transaminase (IU/L)	35.36±29.00	31.64±20.06	0.623
Activated partial thromboplastin time (s)	35.12±11.38	29.33±7.46	0.081

Table 2. Comparison of the platelet aggregation rates of the patients with bleeding events and those without bleeding events.

	Bleeding patients (n=25)	Non-bleeding patients (n=25)	P
ACA-induced platelet aggregation (%)	13.28±8.46	24.93±9.89	<0.001
ADP-induced platelet aggregation (%)	16.17±9.74	16.88±12.69	0.829
ACA+ADP-induced platelet aggregation (%)	30.70±17.79	47.28±14.84	0.003

ACA – arachidonic acid; ADP – adenosine diphosphate.

Table 3. Distribution of arachidonic acid-induced platelet aggregation rates in patients with reported bleeding events.

ACA-induced platelet aggregation (%)	Patients (n=25)	%
0–5	5	25%
0–10	9	35%
0–15	15	60%
0–18	18	70%
0–25	23	90%
0–30	25	100%

ACA – arachidonic acid.

ACA-induced platelet aggregation rate of 18–50% (Table 4). A comparison of SNPs in *PEAR1*, *GP1BA*, and *GSTP1* is shown in Table 5. Mutation rates in the *GP1BA SNP* rs6065 (CT) and *GSTP1 SNPs* rs1695 (AG), *rs4891* (TC), and rs8191439 (GA) differed significantly between the 2 groups.

Table 4. Distribution of arachidonic acid-induced platelet aggregation rates in patients without reported bleeding events.

ACA-induced platelet aggregation (%)	Patients (n=25)	%
50–30	7	30
50–25	11	45
50–20	15	60
50–18	20	80
50–5	25	100

ACA – arachidonic acid.

Discussion

Our study demonstrated that the ACA-induced platelet aggregation rate was significantly lower in patients with reported bleeding events than in those without. However, no significant differences were found in the ADP-induced platelet aggregation rates between the 2 groups. The mutation rates of the *GP1BA* SNP rs6065 (CT) and *GSTP1* SNPs rs1695 (AG), rs4891 (TC), and rs8191439 (GA) also differed significantly between the 2 groups.

Table 5. Comparison of single-nucleotide polymorphisms between patients with and without reported bleeding events.

	Bleeding patients (n=25)	Non-bleeding patients (n=25)	P
PEAR1 SNPs			
rs3737224 (CT) (%)	52.38	47.22	0.707
rs12137505 (AG) (%)	71.43	75.00	0.768
rs11264581 (GA) (%)	52.38	69.44	0.198
rs57731889 (CT) (%)	47.62	69.44	0.103
rs77235035 (CA) (%)	66.67	55.56	0.409
rs1952294 (TC) (%)	100.00	100.00	/
rs735953 (TC) (%)	66.67	58.33	0.533
rs12407843 (GA) (%)	57.14	50.00	0.602
GP1BA SNPs			
rs6067 (AG) (%)	47.62	44.44	0.816
rs2243093 (TC) (%)	47.62	47.22	0.977
rs570515282 (AC) (%)	4.76	0.00	0.187
rs748102207 (CT) (%)	0.00	5.56	0.272
rs6065 (CT) (%)	0.00	16.67	0.048
GSTP1 SNPs			
rs4147581 (CG) (%)	95.24	97.22	0.695
rs1695 (AG) (%)	57.14	30.56	0.048
rs4891 (TC) (%)	57.14	30.56	0.048
rs762803 (CA) (%)	52.38	30.55	0.103
rs1871042 (CT) (%)	52.38	30.55	0.103
rs117928 (TC) (%)	9.52	0.00	0.059
rs8191439 (GA) (%)	19.05	0.00	0.007

Ticagrelor is a potent platelet inhibitor that binds reversibly to P2Y12, an ADP receptor. ADP-induced platelet aggregation is a good indicator of ticagrelor pharmacodynamics [9], which may explain the lack of a significant difference in the ADP-induced platelet aggregation rate observed between the 2 groups. However, the variation in the pharmacodynamics of aspirin is greater than the variation in that of ticagrelor, which is correlated with aspirin metabolism-associated SNPs such as *PEAR1* and *GP1BA* SNPs [10]. ACA-induced platelet aggregation is typically an indicator of aspirin pharmacodynamics [11,12]. The significant difference in the ACA-induced platelet aggregation rate between the patients with and without bleeding events suggests that aspirin is the determinant cause of bleeding when used in combination with ticagrelor.

We also found that 70% of the patients with reported bleeding had an ACA-induced platelet aggregation rate of 0–18% and that 80% of the non-bleeding patients had an ACA-induced

platelet aggregation rate of 18–50%, which is similar to previous findings [13]. We speculate that an ACA-induced platelet aggregation rate of 18% is a satisfactory cutoff for identifying patients with ACS with a high risk of aspirin-associated bleeding. This finding may provide a useful biomarker for assessing the risk of aspirin-associated bleeding in ACS patients.

We found that the *GP1BA* SNP rs6065 (CT) and *GSTP1* SNPs rs1695 (AG), rs4891 (TC), and rs8191439 (GA) were associated with bleeding events in the patients with ACS. Both *GP1BA* and *GSTP1* are associated with aspirin-associated bleeding. As per the 2016 American College of Cardiology/American Heart Association (ACC/AHA) guidelines, the recommended aspirin dose for dual antiplatelet therapy is 81 mg (75–100 mg) [13]. However, in China, a 100-mg dose of aspirin is more commonly used. Because East Asians have lower height and weight than whites, this high dose of aspirin may lead to an increased risk of bleeding in Chinese patients. The PCI-CURE study and

the CURRENT-OASIS study have suggested that variations in the dosage of aspirin do not significantly affect cardiovascular events; however, a lower aspirin dose is associated with a lower risk of bleeding [14]. Considering these results as well as the results of our study, we suggest that it could be beneficial in Chinese or East Asian patients to lower the dose of aspirin to 75 mg. Further investigation is also needed to gain more evidence concerning this suggestion. Using a genotyping assay to evaluate *GP1BA* and *GSTP1* SNPs may also help predict aspirin-associated bleeding risk.

Our study had some limitations. First, platelet function and the SNPs were tested after bleeding events occurred, which may not support causative relationships. Second, our sample size was small, and the study was conducted at a single hospital, which may limit the generalization of our findings.

Conclusions

Low ACA-induced platelet aggregation rates were associated with an increased risk of bleeding in patients with ACS on aspirin and ticagrelor dual antiplatelet therapy. An ACA-induced platelet aggregation rate of 18% might be considered a cutoff for identifying high risk of aspirin-associated bleeding in patients with ACS. Genotyping the *GP1BA* SNP rs6065 (CT) and *GSTP1* SNPs rs1695 (AG), rs4891 (TC), and rs8191439 (GA) may also help predict the risk of bleeding in these patients.

Conflicts of interest

None.

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