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Effects of different CYP2C19 genotypes on prognosis of patients complicated with atrial fibrillation taking clopidogrel after PCI

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Abstract. The effects of different cytochrome P450 2C19 (CYP2C19) genotypes on the prognosis of clopidogrel resistance in patients complicated with atrial fibrillation taking clopidogrel after percutaneous coronary intervention (PCI) were investigated. Eighty patients who were complicated with atrial fibrillation and treated with clopidogrel antiplatelet therapy after PCI in Meizhou Hospital Affiliated to Zhongshan University from September 2015 to January 2017 were selected, and divided into two groups according to the CYP2C19 genotype: extensive metabolism (EM) group and poor metabolism (PM) group. The related risk factors of clopidogrel resistance were determined, and the platelet aggregation rate and clopidogrel resistance rate were compared between the two groups during treatment. Non-fatal myocardial infarction and serious life-threatening complications in the two groups were observed. The increased total cholesterol level and the history of smoking and drinking were the independent risk factors of atrial fibrillation after PCI. The platelet aggregation rates in the EM group at 1, 3 and 12 months after medication were significantly lower than those in the PM group in the same period ($P<0.05$). The clopidogrel resistance rates in EM group before medication and at 1, 3 and 12 months after medication were higher than those in PM group in the same period ($P<0.05$). The onset time of non-fatal myocardial infarction in EM group was earlier than that in PM group ($P<0.05$), the infarct area was larger than that in PM group ($P<0.05$), and the left ventricular ejection fraction (EF) after onset was lower than that in PM group ($P<0.05$). In conclusion, the increased total cholesterol level and the history of smoking and drinking are the independent risk factors of clopidogrel resistance in patients complicated with atrial fibrillation after PCI. The incidence rates of cardiac complications are increased significantly in patients with PM CYP2C19 genotype.

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Introduction

Clopidogrel is a drug used for restraining the function of platelets, and is widely used in thrombosis-related diseases in clinical practice, such as coronary heart disease and cerebral infarction (1). After percutaneous coronary intervention (PCI), clopidogrel should be used routinely to prevent thrombus, and it is recommended as the first-line drug for acute coronary syndrome by Guidelines for Treatment of Cardiovascular Diseases in China (2). Previous findings confirmed that (3), approximately 4-44% patients applying clopidogrel for a long time have no response to clopidogrel treatment or cannot achieve the desired clinical antiplatelet effect, which is clinically known as clopidogrel resistance.

The incidence rates of clinical cardiovascular events in patients with clopidogrel resistance are significantly increased, leading to poor prognosis (4). Patients complicated with atrial fibrillation after PCI are often treated with antiplatelet therapy via clopidogrel to prevent stent thrombosis, thereby enhancing the revascularization effect, and preventing myocardial ischemia, thromboembolism and other serious complications (5). Although some patients apply clopidogrel in antiplatelet therapy in clinical practice, myocardial ischemia and even thromboembolism still occur, suggesting that it may be related to the cytochrome P450 2C19 (CYP2C19) genotype (6).

To improve the treatment effect of clopidogrel on patients complicated with atrial fibrillation after PCI, the prognoses of patients with different CYP2C19 genotype after applying clopidogrel were investigated in this study.

Materials and methods

General data. Eighty patients who were complicated with atrial fibrillation and treated with clopidogrel antiplatelet therapy within one year after PCI in Meizhou Hospital Affiliated to Zhongshan University (Meizhou, China) from September 2015 to January 2017 were selected. All the patients were diagnosed via clinical manifestations and past medical history, and signed the informed consent before enrollment. This study was approved by the Ethics Committee of Meizhou Hospital Affiliated to Zhongshan University. The CYP2C19 genotypes were detected

Table I. Univariate analyses of measurement data (mean ± SD).

Parameters	With atrial fibrillation	Without atrial fibrillation	t-test	P-value
Age (years)	65.7±2.1	66.0±2.1	0.639	0.525
Body mass index (kg/m^2)	25.6±0.3	25.7±0.3	1.491	0.140
Blood platelet count (x10^9/l)	205.6±12.1	206.5±12.0	0.334	0.739
Hemoglobin level (g/l)	138.6±2.5	138.7±2.5	0.179	0.858
Total cholesterol level (mmom/l)	4.2±0.2	3.7±0.2	11.180	<0.001
Number of lesions at the onset (pcs)	2.1±0.2	1.5±0.1	16.971	<0.001
Length of thrombus at the onset (mm)	25.6±2.3	16.5±1.5	20.960	<0.001

Table II. Univariate analyses of enumeration data (n).

Characteristics	With atrial fibrillation	Without atrial fibrillation	χ^2 test	P-value
Sex			0.065	0.799
Male	30	29		
Female	10	11		
Smoking history			15.622	<0.001
Yes	20	3		
No	20	37		
Drinking history			18.119	<0.001
Yes	20	2		
No	20	38		
Hypertension history			2.813	0.094
Yes	11	5		
No	29	35		

before enrollment. Subjects were divided into two groups according to the CYP2C19 genotype: extensive metabolism (EM) group (CYP2C19-1) and poor metabolism (PM) group (CYP2C19-2 and CYP2C19-3). The EM group comprised 30 males and 10 females aged 40-70 years, with an average of 65.7±2.1 years. In terms of the New York Heart Association (NYHA) cardiac function grading at enrollment, there were 32 cases of grade II and below, and 8 cases of grade III and above. The PM group was comprised of 29 males and 11 females aged 40-70 years, with an average of 66.0±2.1 years. In terms of the NYHA cardiac function grading at enrollment, there were 31 cases of grade II and below, and 9 cases of grade III and above. There were no statistically significant differences in the sex, age and NYHA cardiac function grading at enrollment between the two groups (P>0.05).

Instruments and reagents and genotype detection method. DNA extraction kit (Qiagen), CYP2C19 hybridization developing kit (BaiO, Shanghai, China), GTR22-1 high-speed freezing centrifuge (Beijing Shidai Beili Co., Ltd., Beijing, China), BaiOBE2.0 bio-chip reader (BaiO), DYY-6C electrophoresis apparatus (Beijing Liuyi Instrument Plant, Beijing, China) and experimental primers (Sangon Biotech, Shanghai, China) were used. CYP2C19 genotypes of all the subjects were hybridized using an e-Hyb full-automatic hybridization instrument and read using BaiOBE 2.0 biochip reader.

Observation indexes. After operation, all subjects regularly took 75 mg clopidogrel (national medicine permission no. J20080090, Sanofi Pharmaceutical Co., Ltd.) orally to maintain the treatment. The univariate and multivariate analyses were performed for the sex, age, body mass index, blood platelet count, hemoglobin level, total cholesterol level, number of lesions at the onset, length of thrombus at the onset, history of smoking, drinking and hypertension of patients. The related risk factors and independent risk factors of clopidogrel resistance were determined, and the platelet aggregation rate and clopidogrel resistance rate were compared between the two groups during treatment. Finally, the non-fatal myocardial infarction and serious life-threatening complications in both groups were observed.

Evaluation criteria. Determination of platelet aggregation rate: Before and at 1, 3 and 12 months after application of antiplatelet drugs, the platelet aggregation rates in patients were determined using the Annoron measuring equipment (Beijing, China). Adenosine diphosphate (ADP) was used as the inducer, and all operations were performed by laboratory physicians with more than 5 years of experience in strict accordance with the instructions. Clopidogrel resistance was detected via light turbidimetry using 5 μmol/l ADP as the inducer. The difference between the actual platelet aggregation rate and the maximum platelet aggregation rate after application of clopidogrel of <10% indicated clopidogrel resistance; the difference between 10 and 29% indicated clopidogrel semi-resistance; the difference of >30% indicated normal response to clopidogrel.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 21.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Measurement data were presented as mean ± standard deviation (mean ± SD). The t-test was used for the comparison of means between the two groups, and the Chi-square test was used for the comparison of rates between the two groups. Univariate analyses were first performed for the sex, age, body mass index, blood platelet count, hemoglobin level, total cholesterol level, number of lesions at the onset, length of thrombus at the onset, the history of smoking, drinking and hypertension of patients. Non-conditional multivariate logistic regression analyses were then performed for

Table III. Multivariable logistic regression analyses of clopidogrel resistance.

Characteristics	β	SE	W	P-value	Odds ratio (OR)	95% confidence interval (CI)
Total cholesterol level (mmom/l)	1.873	0.834	5.050	0.025	6.503	1.272-33.273
Number of lesions at the onset (pcs)	0.786	0.613	1.645	0.201	2.193	0.661-7.729
Length of thrombus at the onset (mm)	0.035	0.044	0.601	0.439	1.035	0.951-1.127
Smoking history	1.835	0.777	5.595	0.018	0.159	0.035-0.731
Drinking history	1.738	0.707	6.086	0.041	0.553	1.505-36.589

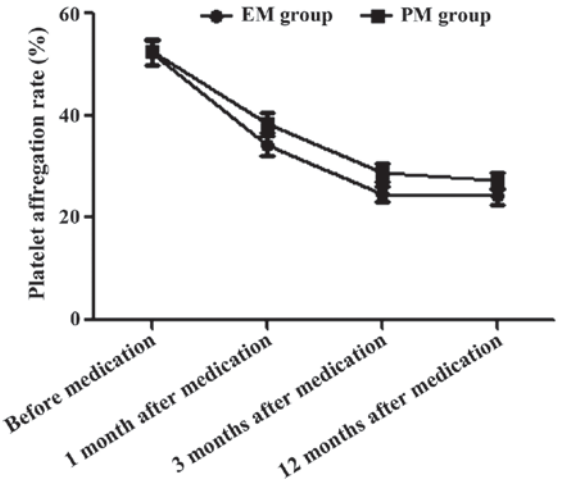

Figure 1. Comparison of platelet aggregation rate between the two groups during treatment. The platelet aggregation rates in EM group at 1, 3 and 12 months after medication are significantly lower than those in PM group in the same period (P<0.05).

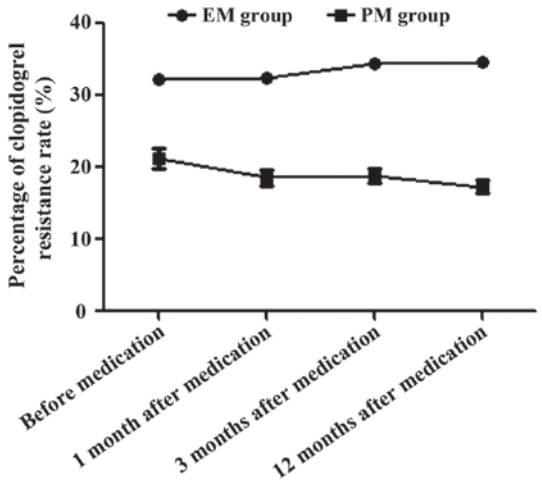

Figure 2. Comparison of clopidogrel resistance rate between the two groups during treatment. The clopidogrel resistance rates in EM group before medication and at 1, 3 and 12 months after medication are significantly higher than those in PM group in the same period (P<0.05).

items with statistical significance. P<0.05 was considered to indicate a statistically significant difference.

Results

Univariate analyses of clopidogrel resistance. Univariate analyses showed that the increased total cholesterol level, higher number of lesions at the onset, larger length of thrombus at the onset, and the history of smoking and drinking were the related risk factors of clopidogrel resistance in patients complicated with atrial fibrillation after PCI (Tables I and II).

Multivariable logistic regression analyses of clopidogrel resistance. Multivariable logistic regression analyses with atrial fibrillation as a dependent variable showed that the increased total cholesterol level and the history of smoking and drinking were the independent risk factors of atrial fibrillation after PCI (Table III).

Comparison of platelet aggregation rate between the two groups during treatment. Before medication and at 1, 3 and 12 months after medication, the platelet aggregation rates in EM group were 52.3±2.5, 34.2±1.9, 24.5±1.5 and 24.3±1.6%, respectively; the rates in PM group were 52.4±2.5, 38.6±2.1, 28.9±1.7 and 27.3±1.6%, respectively. The platelet aggregation rate before application of antiplatelet drugs had no statistically significant difference between the two groups (t=0.179, P>0.05); the platelet aggregation rates in EM group at 1, 3 and 12 months after medication were significantly lower than those in PM group in the same period (t=9.826, 12.274 and 8.386, P<0.05) (Fig. 1).

Table IV. Comparison of non-fatal myocardial infarction between the two groups [n (%)].

Group	Onset time (month)	Infarct area (%)	EF% after onset (%)
EM group	5.9±0.5	15.6±1.3	40.1±2.1
PM group	9.1±1.1	10.5±1.8	45.6±2.5
t-test	16.750	14.527	10.654
P-value	<0.001	<0.001	<0.001

Comparison of clopidogrel resistance rate between the two groups during treatment. Before medication and at 1, 3 and 12 months after medication, the clopidogrel resistance rates in EM group were 32.3±0.5, 32.4±0.5, 34.5±0.5 and 34.6±0.6%, respectively; the rates in PM group were 21.3±1.4, 18.6±1.1, 18.9±1.0 and 17.3±0.9%, respectively. The clopidogrel resistance rate before application of antiplatelet drugs

Table V. Comparison of serious life-threatening complications between two groups [n (%)].

Group	Angina relapse	Stent thrombosis	Left heart failure	Cardiac death	Cerebral hemorrhage	Total incidence rate
EM group	8	1	5	1	1	16 (40.0%)
PM group	1	1	0	0	0	2 (5.0%)
χ^2 test			-			12.115
P-value			-			0.001

had no statistically significant difference between the two groups (t=46.797, P>0.05); the clopidogrel resistance rates in EM group before medication and at 1, 3 and 12 months after medication were significantly higher than those in PM group in the same period (t=72.233, 88.247 and 101.154, P<0.05) (Fig. 2).

Comparison of non-fatal myocardial infarction between two groups. The onset time of non-fatal myocardial infarction in EM group was earlier than that in PM group (P<0.05), the infarct area was larger than that in PM group (P<0.05), and the left ventricular ejection fraction (EF) after onset was lower than that in PM group (P<0.05) (Table IV).

Comparison of serious life-threatening complications between the two groups. The total proportion of angina relapse, stent thrombosis, left heart failure, cardiac death and cerebral hemorrhage in EM group was obviously lower than that in PM group (P<0.05) (Table V).

Discussion

Patients complicated with atrial fibrillation after PCI require continuous anticoagulation and antiplatelet therapy, among which clopidogrel is the most commonly-used anti-platelet drug (7). The research suggests that CYP2C19 gene polymorphism is closely associated with the pharmacokinetics of clopidogrel, and the risk of relapses of severe cardiovascular events in patients with clopidogrel resistance is significantly higher than that in patients with normal susceptibility (8). It is suggested that the usage amount of clopidogrel be increased for patients with clopidogrel resistance (9). CYP2C19-1, CYP2C19-2 and CYP2C19-3 are three major clopidogrel genotypes in Chinese population, the first one of which belongs to the EM type, namely the clopidogrel-sensitive type, and the last two of which belong to the PM type, namely the high-risk clopidogrel-resistant type (10).

In this study, the related factors of atrial fibrillation in subjects with different CYP2C19 phenotypes taking clopidogrel after PCI were analyzed, and it was found that the increased total cholesterol level, higher number of lesions at the onset, larger length of thrombus at the onset, and the history of smoking and drinking were the related risk factors of atrial fibrillation after PCI, and the increased total cholesterol level and the history of smoking and drinking were the independent risk factors of atrial fibrillation after PCI. In addition, the comparisons of platelet aggregation rate and clopidogrel resistance rate between the two groups during treatment showed that the platelet aggregation rates in EM group at 1, 3 and 12 months after medication were significantly lower than those in PM group in the same period, and the clopidogrel resistance rates in EM group before medication and at 1, 3 and 12 months after medication were significantly higher than those in PM group in the same period, suggesting that the platelet aggregation rate in EM group was affected and the clopidogrel resistance rate was higher. At the same time, the comparison of non-fatal myocardial infarction between the two groups revealed that the onset time of non-fatal myocardial infarction in EM group was earlier than that in PM group, the infarct area was larger than that in PM group, and the EF% after onset was lower than that in PM group. Finally, the comparisons of serious life-threatening complications between the two groups showed that the total proportion of angina relapse, stent thrombosis, left heart failure, cardiac death and cerebral hemorrhage in EM group was obviously lower than that in PM group, indicating that the risk of fatal complications in EM group was obviously lower than that in PM group.

Clopidogrel metabolizes mainly through the cytochrome P450 enzyme system in liver (11), producing active metabolites, thus inhibiting the platelet aggregation and achieving an anti-platelet effect (12). Previous findigs have confirmed that (13) CYP2C19 gene polymorphism is a major factor affecting the anti-platelet effect of clopidogrel. CYP2C19-2 and CYP2C19-3 are PM subtypes of CYP2C19 (14), which are mainly expressed in Asian populations (15) with higher mutation rates (16); thus, the incidence rate of severe cardiovascular complications is increased significantly in PM subjects (17). The long-term application of clopidogrel in patients complicated with atrial fibrillation after PCI should be paid attention to (18); the anti-platelet therapy regimen should be adjusted in time (19) to avoid stent thrombosis-induced complications (20).

In conclusion, the increased total cholesterol level and the history of smoking and drinking are the independent risk factors of clopidogrel resistance in patients complicated with atrial fibrillation after PCI. The incidence rates of fatal and non-fatal cardiac complications are increased significantly in patients with PM CYP2C19 genotype, and attention is required to this in clinical practice.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QZ and ZZ collected the general information of patients. BL, ZL and PZ were responsible for observation index analysis. ZY and XH contributed to the conception and design of the study. HW, WC and JH analysed the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The patients were diagnosed via clinical manifestations and past medical history, and signed the informed consent before enrollment. This study was approved by the Ethics Committee of Meizhou Hospital Affiliated to Zhongshan University (Meizhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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