Effects of Dual-Dose Clopidogrel, Clopidogrel Combined with Tongxinluo Capsule, and Ticagrelor on Patients with Coronary Heart Disease and CYP2C19*2 Gene Mutation After Percutaneous Coronary Interventions (PCI)

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Background: In recent years, genetic factors have attracted research interest as important predisposing factors for cardiovascular susceptibility. This study aimed to investigate the influences of dual-dose clopidogrel, clopidogrel combined with tongxinluo, and ticagrelor on the platelet activity and MACE events of patients with CYP2C19*2 gene function deficiency and poor clopidogrel response after PCI.

Material/Methods: We selected 458 patients with coronary heart disease undergoing PCI, and the genotype of CYP2C19*2 was detected by TaqMan real-time PCR. We finally enrolled 212 patients and divided them into 4 groups: a standard anti-platelet group of 46 patients, a clopidogrel double-dose group of 50 cases, a clopidogrel combined with tongxinluo group of 59 cases, and a ticagrelor group of 57. The platelet inhibition rate was detected by TEG. We analyzed and compared differences in platelet activity and the occurrence of MACE events in these 4 groups at different follow-up times.

Results: The results showed that inhibition of platelet aggregation was better in the double-dose clopidogrel group, the clopidogrel combined with tongxinluo group, and the ticagrelor group than in the regular-dose clopidogrel group, and ticagrelor was the best. We also found that the total incidence of MACE was much lower in the double-dose clopidogrel group, the clopidogrel combined with tongxinluo group, and the ticagrelor group, while the incidence of hemorrhage in the ticagrelor group was higher.

Conclusions: Adjusting the dose or combining with other drugs improves the efficacy of anti-platelet therapy and reduces the incidence of ischemic events after PCI.

MeSH Keywords: Antigens, Human Platelet • Cardiovascular Agents • Coronary Disease

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**Background**

Clopidogrel, which can inhibit adenosine diphosphate (ADP)-induced platelet aggregation, has been applied to the prevention of atherothrombotic events for over 15 years [1]. The variability in clopidogrel response is associated with environmental and genetic factors [2]. Although clopidogrel has been widely used in patients with ischemic stroke, decreased metabolic activation of clopidogrel still occurs because of genetic variations in CYP2C19. The stent thrombosis risk in patients carrying a CYP2C19 loss-of-function allele appears to be similarly elevated across stable and unstable patient populations, regardless of elective or urgent PCI, or treatment with bare-metal or drug-eluting stents [3,4]. Many factors can influence the response of patients to clopidogrel therapy, including age, smoking, diabetes, drug interactions, and genetics [5,6].

As a pro-drug, clopidogrel requires metabolic activation by the hepatic cytochrome (CYP) P450 system, which includes CYP2C19, CYP2C9, CYP2B6, CYP3A4, and CYP3A5 [2]. Specifically, the polymorphisms of CYP2C19 are thought to play an important role in this poor clopidogrel response [7]. Individuals carrying at least 1 loss-of-function allele (either *2 or *3) of the CYP2C19 gene demonstrate reduced active clopidogrel metabolites and suppressed anti-platelet activity [8]. The 2009 Japanese Guidelines for the Management of Anticoagulant and Anti-Platelet Therapy in Cardiovascular Disease recommend the use of anti-platelet agents for preventing reinfarction following percutaneous coronary intervention (PCI) in patients with acute coronary syndrome (ACS) [9]. The presence of at least 1 reduced-function CYP2C19 allele was associated with increased risk of major adverse cardiovascular events (MACE), particularly stent thrombosis, in clopidogrel-treated patients [10]. For this reason, CYP2C19 genetic testing has been proposed before starting clopidogrel therapy to identify patients likely to show reduced anti-platelet activity [11].

Ticagrelor (formerly developed as AZD6140) is a first-in-class anti-platelet agent chemically known as a cyclopentyl triazolopyrimidine, with distinguishing properties from that of the pyridines like ticlopidine, clopidogrel, or prasugrel. Ticagrelor, a novel oral P2Y12 antagonist that does not undergo bio-transformation to active metabolites, has many favorable pharmacological dynamic characteristics, including rapid onset of action and reversibility, and consistent inhibition of platelet function [12]. Some studies have shown a higher prevalence of on-treatment platelet re-activity, but with a similar thrombotic event rate after PCI in East Asian patients compared with white patients [13].

Tongxinluo in capsule form is a compound prescription formulated according to the meridian theory of traditional Chinese medicine and approved in 1996 by the State Food and Drug Administration of China for treatment of angina pectoris and ischemic stroke [13]. Pharmacological research has revealed that treatment with tongxinluo capsule has a variety of therapeutic effects, such as improvement of endothelial cell function, lipid lowering, anti-inflammation, anti-apoptosis, and enhancement of angiogenesis [14].

Our study was conducted to evaluate the influences of dual-dose clopidogrel, clopidogrel combined with tongxinluo, and ticagrelor on patients with CYP2C19 gene polymorphisms after PCI at 3, 6, and 12 months.

**Material and Methods**

**Study design and patient population**

Overall, 458 patients with cardiovascular disease who received PCI treatment from June 2012 to June 2014 at Department of Cardiology, Hebei Provincial People ‘s Hospital were selected. Patients were included in the study according to the following criteria: (1) age >18 years; (2) diagnosis of coronary artery disease by coronary angiography in parallel PCI; (3) carry at least 1 CYP2C19 *2 allele by genotyping assays; (4) PCI patients were treated with clopidogrel for anti-platelet therapy; (5) patients without other cardiovascular disease. Exclusion criteria were: (1) recent history of severe organ damage or surgery; (2) upper gastrointestinal ulcer or bleeding; (3) currently suffering from congenital heart disease, cardiomyopathy, myocarditis, peripheral vascular disease, or and infective endocarditis; (4) liver and kidney function was severely abnormal; (5) blood coagulation disorders; (6) cancer patients; (7) history of allergies to aspirin and clopidogrel; (8) took tongxinluo or ticagrelor before enrolment; (9) received warfarin sodium, propanolol, cimetidine, any kind of antibiotics or anti-fungal drugs, antidepressants, benzodiazepines, or anti-psychotics.

Patients were given aspirin 300 mg for the first time after admission, followed by 100 mg/day and clopidogrel 75 mg/day for routine treatment. Among the 458 patients with coronary artery disease who received successful PCI, 231 patients had at least 1 CYP2C19*2 allele. According to the inclusion and exclusion criteria and the patients, 212 patients were eventually selected and divided into the standard anti-platelet group (regular-dose clopidogrel group) (RCG) of 46 patients (75 mg/day, 12 months), the double-dose clopidogrel group (DCG) of 50 cases (150 mg/day for 1 month, then 75 mg/day), the clopidogrel combined with tongxinluo group (CTG) of 59 cases (0.26 g/tablet, 4×3/day, 3 months), and the ticagrelor group (TG) of 57 cases (90 mg×2/day, 12 months). The differences in platelet activity and the occurrence of MACE events in the 4 groups of patients at different follow-up times were analyzed and compared.
Results

Clinicopathological characteristics of patients

The patients with CYP2C19*1*2 or CYP2C19*2*2 were enrolled, and were divided into 4 groups: (1) regular-dose clopidogrel group; (2) double-dose clopidogrel group, (3) tongxinluo capsule combined with regular anti-platelet therapy group, and (4) ticagrelor group. The baseline information and clinicopathological characteristics of the 4 groups of patients are summarized in Table 1. There were no significant differences in age, sex, BMI, smoking status, hypertension, diabetes, dyslipidemia, number of stentings, platelet count, or ejection fraction among the 4 groups (P>0.05). During follow-up study, the drug use among patients had no significant differences (P>0.05).

The platelet inhibition rate in the 4 groups of patients varied during follow-up

At 3 days after PCI, the platelet inhibition rates of the double-dose clopidogrel group, the clopidogrel combined with tongxinluo group, and the ticagrelor group were lower than that of the standard anti-platelet therapy group. The 4 groups of patients had different platelet inhibition rates during the follow-up study. Using the repeated measurement of variance analysis, we found that the platelet inhibition rates were different at different time points (F=106.588, P<0.01) (Table 2), and there was interaction between time and grouping. The inhibition rates of platelets were analyzed at different time points. The results showed that the platelet inhibition rate of the double-dose clopidogrel group, the tongxinluo group, and the ticagrelor group was higher than that of the standard anti-platelet group at 1 month after the operation (P<0.05). After 6 months, the platelet inhibition rate in the ticagrelor group was higher than in the other 3 groups (P<0.05); however, the double-dose clopidogrel group and combined with tongxinluo group were lower than the standard anti-platelet group (P<0.05). At 12 months after surgery, statistical results were similar to those at 6 months, and the ticagrelor group had the highest platelet inhibition rate. However, we found that although the platelet inhibition rate of patients in the ticagrelor group was always at a high level, the platelet inhibition rate was still reduced with prolonged administration (Figure 1). The combined with tongxinluo group achieved the maximum platelet inhibition rate at 3 months, while the double-dose clopidogrel group and ticagrelor group reached the maximum in the first month.

Occurrence of MACE in patients from the 4 groups at 12-month follow-up

After 12 months of follow-up, the incidence of adverse cardiovascular events in the double-dose clopidogrel group, the combined tongxinluo group, and the ticagrelor group was
### Table 1. Comparison of baseline data between patients from 3 groups with different metabolic syndrome.

| General features                  | Regular dose of Clopidogrel group (n=46) | Double dose of Clopidogrel group (n=50) | Combined with Tongxinluo group (n=59) | Ticagrelor group (n=57) | F    | P    |
|-----------------------------------|-----------------------------------------|----------------------------------------|--------------------------------------|-------------------------|------|------|
| Age                               | 59.61±9.58                             | 59.84±10.01                            | 58.92±10.83                          | 60.79±9.18              | 0.348| 0.790|
| Gender                            | 29                                      | 31                                      | 35                                    | 34                      | 0.213| 0.975|
| Smoking status                    | 15                                      | 19                                      | 21                                    | 20                      | 0.308| 0.959|
| Hypertension                      | 25                                      | 28                                      | 34                                    | 32                      | 0.113| 0.990|
| Diabetes                          | 11                                      | 15                                      | 19                                    | 18                      | 1.013| 0.807|
| Dyslipidemia                      | 11                                      | 13                                      | 18                                    | 16                      | 0.630| 0.889|
| BMI (kg/m²)                       | 25.31±3.27                             | 26.16±2.94                             | 25.75±3.19                           | 25.83±3.57              | 0.441| 0.648|
| Number of diseased blood vessels | 1.68±0.58                              | 1.61±0.64                              | 1.66±0.72                            | 1.67±0.65               | 0.281| 0.839|
| Number of stenting                | 1.44±0.68                              | 1.47±0.79                              | 1.51±0.75                            | 1.46±0.72               | 0.361| 0.781|
| Platelet count                    | 210.57±56.43                           | 214.02±55.46                           | 209.79±58.59                         | 212.63±56.49            | 0.061| 0.980|
| Ejection fraction (%)             | 61.84±9.21                             | 59.94±10.68                            | 60.92±11.06                          | 61.02±9.48              | 0.529| 0.663|
| ACEI/ARB                          | 33                                      | 36                                      | 41                                    | 39                      | 0.230| 0.973|
| CCB                               | 13                                      | 15                                      | 21                                    | 18                      | 0.732| 0.866|
| PPI                               | 41                                      | 44                                      | 53                                    | 51                      | 0.103| 0.991|
| β-blocker                         | 30                                      | 35                                      | 42                                    | 38                      | 0.566| 0.904|

### Table 2. The change of platelet inhibition rate of patients from 4 groups during follow-up.

|                      | 3 days (%) | 1 month (%) | 3 month (%) | 6 month (%) | 12 month (%) |
|----------------------|------------|-------------|-------------|-------------|--------------|
| Regular dose of Clopidogrel group (n=46) | 50.16±11.21 | 48.15±10.35 | 48.27±11.45 | 47.58±11.34 | 46.76±10.61 |
| Double dose of Clopidogrel group (n=50) | 31.02±12.04* | 67.02±10.04** | 31.96±11.69* | 32.24±10.31* | 31.23±9.29* |
| Clopidogrel combined with Tongxinluo group (n=59) | 29.66±11.67* | 58.67±13.71** | 64.74±19.37** | 31.81±11.02* | 30.97±10.43* |
| Ticagrelor group (n=57) | 30.84±10.87* | 71.86±20.87** | 69.03±19.25** | 62.86±16.94** | 54.76±15.64** |

* P<0.05 compared with regular dose of clopidogrel group; ** P<0.05 compared with double dose of clopidogrel group; & P<0.05 compared with Clopidogrel combined with Tongxinluo group.
significantly lower than that in the standard anti-platelet group (Figure 2, Table 3), but single occurrence of adverse cardiovascular events such as MACE composite end point, cardiac death, non-fatal myocardial infarction, and TVR were not significantly different in any group (Figure 3, Table 3).

Occurrence of bleeding events in patients from the 4 groups after 12-month follow-up

All patients were followed up for 12 months, and we found that all groups of patients had mild bleeding, but no major bleeding occurred. The incidence of bleeding in the ticagrelor group was higher than in the other 3 group, and the difference was statistically significant (Table 4).

Table 3. Occurrence of MACE in patients from 4 groups after followed for 12 months.

| End event                  | Regular dose of Clopidogrel group (n=46) | Double dose of Clopidogrel group (n=50) | Combined with Tongxinluo group (n=59) | Ticagrelor group (n=57) | F    | P    |
|----------------------------|-----------------------------------------|----------------------------------------|--------------------------------------|------------------------|------|------|
| In-stent restenosis/thrombosis | 2                                       | 0                                      | 1                                    | 1                      | 2.264 | 0.537 |
| TVR                        | 2                                       | 1                                      | 0                                    | 0                      | 3.618 | 0.095 |
| UA                         | 4                                       | 2                                      | 1                                    | 1                      | 3.732 | 0.285 |
| Non-fatal myocardial infarction | 2                                       | 1                                      | 1                                    | 0                      | 2.526 | 0.415 |
| Cardiac death              | 1                                       | 0                                      | 0                                    | 0                      | 3.122 | 0.217 |
| MACE composite endpoint    | 2                                       | 0                                      | 1                                    | 0                      | 3.287 | 0.207 |
| Total adverse cardiovascular events | 14                                      | 4*                                     | 6*                                  | 3*                    | 14.339 | 0.002 |

* P<0.01 compared with regular dose of Clopidogrel group.

Discussion

In this study, patients after intervention received 1 of 4 therapeutic regimens: the standard anti-platelet group, clopidogrel double-dose group, combined with tongxinluo group, and ticagrelor group. After a 12-month follow-up, we observed the platelet aggregation rate of patients at 1, 3, 6, and 12 months postoperatively. The results showed that the platelet inhibition rates of the clopidogrel double-dose group, combined with tongxinluo group, and ticagrelor group were all higher than in the standard anti-platelet group after using the adjustment program. This suggests that patients in the clopidogrel double-dose group, combined with tongxinluo group, and ticagrelor group had lower odds.

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of adverse cardiovascular events than those in the standard anti-platelet group. Total MACE events were lower in the standard anti-platelet group than in the other 3 groups. However, any of these regimens can cause mild bleeding.

Von Beckerath et al. [15] showed that clopidogrel 75 mg/day at a maintenance dose of 150 mg/day was more effective in inhibiting ADP-induced platelet proliferation after PCI. Aradi et al. [16] administered clopidogrel 150 mg/day and 75 mg/day for 30 days and found that 150 mg/day of clopidogrel can effectively reduce the stability of patients with stable angina pectoris ischemic and thrombotic events. Tongxinluo capsule is a Chinese herbal compound preparation. Studies have shown that dual anti-platelet combined with the Naoxintong not only inhibits platelet aggregation and significantly reduces intracoronary microthrombosis, but also reduces the risk of bleeding. Ticagrelor is an ADP receptor antagonist that reversibly binds to the ADP receptor. Wallentin et al. [17] compared the efficacy and safety of ticagrelor and clopidogrel, and found that the incidence of combined end points was significantly lower in the tegrellozin group than in the clopidogrel group, whereas there was no significant difference between the 2 groups in total bleeding events. Gurbel et al. [18] found that in clopidogrel nonresponders, in the switch to ticagrelor, the platelet aggregation rate was significantly reduced, but when switching from ticagrelor to clopidogrel the platelet aggregation rate was significantly higher. This study is consistent with some previous non-Chinese studies, suggesting that patients with clopidogrel resistance can choose from among double-dose clopidogrel, clopidogrel combined with tongxinluo, or replacement of ticagrelor to relieve clopidogrel resistance.

The main components of tongxinluo capsule are ginseng, Quanjie, centipede, leech, soil insects, cicada skin, borneol, red peony, and other drugs. Modern pharmacological research found that tongxinluo capsules contains leeches, Quanjie, centipede, Eupolyphaga, and other insects. Its effects include hirudin effect, inhibition of extracellular matrix synthesis and secretion of inflammatory cells, and inflammatory cell aggregation. Its lumbrokinase-like effect can directly dissolve fibrin, but can also to activate plasminogen as plasmin, and thus the indirect dissolution of fibrin, with anti-platelet aggregation and anti-thrombotic effect. Ginseng stems and leaves, reed head, and fruit saponins can promote myocardial DNA synthesis in neonatal rats, and saponins promote prostaglandin I2 synthesis and inhibition of thromboxane formation. Red peony expands the coronary artery, inhibits platelet aggregation and smooth muscle hyperplasia, lowers blood lipids and lipid peroxidation, and protects vascular endothelial cells. Borneol can increase the plasma concentration of other drugs, promote gastrointestinal absorption, dilate the coronary artery, and slow the heart rate, thereby relieving angina. In addition, tongxinluo capsules can increase the expression of thrombin III, tissue plasminogen activator (t-PA), fibrinogen (FIB), and plasminogen activator inhibitor (Pal-1), thus regulating the balance of coagulation and fibrinolysis in patients with stent implantation, decreases the levels of ET-1, and increase NO and calcitonin gene-related peptide (CGRP). Studies have confirmed that tongxinluo capsules can reduce the incidence of clopidogrel resistance in ACS patients, but whether CYP2C19 loss-of-allele variants can slow

Table 4. Occurrence of bleeding events in patients from 4 groups after followed for 12 months.

|                      | Regular dose of Clopidogrel group (n=46) | Double dose of Clopidogrel group (n=50) | Clopidogrel combined with Tongxinluo group (n=59) | Ticagrelor group (n=57) | F     | P     |
|----------------------|-----------------------------------------|----------------------------------------|-------------------------------------------------|-------------------------|-------|-------|
| Bleeding events      | 1*                                      | 5*                                     | 4*                                              | 11*                     | 11.272| 0.008 |
| Severe bleeding      | 0                                       | 0                                      | 0                                               | 0                       | /     | /     |
| Mild bleeding        | 1*                                      | 5*                                     | 4*                                              | 11*                     | 11.272| 0.008 |

* P<0.01 compared with regular dose of Clopidogrel group; * P<0.01 compared with Clopidogrel combined with Tongxinluo group.
platelet function resistance, thereby reducing the incidence of cardiovascular events, needs further study and observation.

In addition to genetic factors, patient compliance, drug interactions, left ventricular function, increases in other platelet activation pathways, and increased ADP release all can contribute to the recurrence of adverse cardiovascular events in patients after PCI. Therefore, we should look for more patients with PCI after the recurrence of adverse cardiovascular events, and, through the early identification of CYP2C19*2 carriers, adjust the dose, combined with other anti-platelet drugs or replacement of ticagrelor to improve the efficacy of anti-platelet therapy and reduce the incidence of ischemic events after PCI.

Conclusions

Our data suggest that the effects of double-dose clopidogrel, clopidogrel combined with tongxinluo, and ticagrelor in inhibiting platelet aggregation was better than the regular-dose standard anti-platelet therapy, and ticagrelor was the best. The total incidence of MACE was much lower in the double-dose clopidogrel group, ticagrelor combined with tongxinluo group, and ticagrelor group, while the incidence of hemorrhage in the ticagrelor group was higher. Adjusting the dose or combining other drugs could improve the efficacy of anti-platelet therapy and reduce the incidence of ischemic events after PCI.

Disclosures

All authors declare no financial competing interests. All authors declare no non-financial competing interests.

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