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Comparison of the antiplatelet effect of clopidogrel benzene sulfonate and clopidogrel hydrogen sulfate in stable coronary heart disease

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ABSTRACT. Clopidogrel hydrogen sulfate (CHS) is a thienopyridine, which can be used to prevent cardiovascular complications alone or in combination with acetyl salicylic acid as an important antiplatelet agent. Clopidogrel benzene sulfonate (CB) is a special clopidogrel salt that can be used as a conventional drug for antiplatelet effects, but the mechanism is still unknown. This study aimed to compare the antiplatelet effects of CHS and CB in stable coronary artery disease patients. Stable coronary artery disease patients (N = 119) were randomly divided into two groups receiving CHS (N = 67) or CB (N = 52). The patients were administered the drugs (600 mg dosage) and monitored for 12 to 14 h to detect antiplatelet effects. Antiplatelet response was evaluated by the P2Y12 response unit (PRU) and P2Y12 suppression percentage. In addition, all patients' CYP2C19*2, CYP2C19*3, and CYP3A5 polymorphisms were studied. Similar clinical manifestations were observed in the two groups. No obvious difference was detected in the platelet levels of patients given CHS or CB. The antiplatelet response (PRU and P2Y12 evaluation) of the patients using CHS and CB was not significantly different. In the two groups, the CYP2C19*2 polymorphic

heterozygote number and antiplatelet response were similar. CB and CHS presented similar antiplatelet effects in stable coronary artery disease patients, and there was no difference in the CYP2C19*2 heterozygous polymorphism.

Key words: Clopidogrel hydrogen sulfate; Clopidogrel benzene sulfonate; Platelet response

INTRODUCTION

Antiplatelet therapy is a basic treatment for coronary artery disease. Clopidogrel and aspirin are the most common platelet inhibitors used in coronary heart disease treatment. Clopidogrel is a precursor drug with no activity. It becomes active when irreversibly combined with platelet receptor P2Y12 after oxidation in the liver, suppressing platelet aggregation. The clinical safety and efficacy of clopidogrel used alone or in combination with aspirin in coronary heart disease patients has been confirmed in numerous randomized trials using clopidogrel hydrogen sulfate (CHS) (Yusuf et al., 2001; Steinhubl et al., 2002; Mehta et al., 2010).

Recently, clopidogrel has been used as the main drug for coronary heart disease treatment because of its low cost (Wang et al., 2006; Gurbel and Tantry, 2007; Baumgärtel et al., 2012). The most commonly used form of the drug is clopidogrel benzene sulfonate (CB). However, there is still a lack of information about the platelet response of coronary artery disease patients to these new preparations (Furman et al., 1998; Matetzky et al., 2004; Geisler et al., 2006). Even if the pharmacokinetics and pharmacodynamics of CB performance are the same, a possible platelet suppression discrepancy caused by using different salts may result in atherosclerosis.

This study aimed to determine whether the same loading dose of CB resulted in a similar platelet response as CHS in stable coronary artery disease patients. We also investigated the influence of the drugs on three kinds of polymorphism (CYP3A5*3, CYP2C19*2, and CYP2C19*3). The polymorphism studies showed that their existence has a strong association with low reactivity to clopidogrel treatment (Suh et al., 2006; Mega et al., 2009; Momary et al., 2010; Park et al., 2012; Laine et al., 2013).

MATERIAL AND METHODS

Patients

This study was a monocentral prospective randomized trial. Ninety-one patients with coronary angiography clinical indication who received clopidogrel treatment for the first time were enrolled between May 2012 and April 2014. If coronary angiography showed that the patient had coronary artery lesions at 30-70% and no coronary artery reconstruction plan, the patients randomly received 600 mg CB or CHS. The drugs were administered 4-8 h after coronary angiography. The drugs were administered under the supervision of a nurse to avoid compliance problems. The exclusion criteria were as follows: left ventricular ejection fraction <30%, recent acute coronary syndrome (<1 month), platelet count <100 x 10^3/dL, and spontaneous bleeding history. Blood samples were collected at 12-14 h after

drug administration. The P2Y12 platelet response was tested by a VerifyNow experiment. All patients provided written informed consent, and this study was approved by the Institutional Review Board of People's Hospital of Zhangqiu.

Platelet response evaluation

The VerifyNow test was used to evaluate the platelet response to CB and CHS. VerifyNow is a rapid test for platelet function. Special ink was used for P2Y12 (clopidogrel) and aspirin.

VerifyNow P2Y12 was designed to measure drug effects on P2Y12 recipients directly. Prostaglandin (PGE_1) increased free cyclic adenosine monophosphate concentration instead of adenosine diphosphate, and the results are reported in P2Y12 response units (PRUs). The P2Y12 inhibition rate was calculated using the thrombin receptor activation peptide. The formula was (1 - PRU / baseline) x 100 (Malinin et al., 2007; Michelson, 2009).

Polymorphism evaluation

CYP3A5*3 (rs776746), CYP2C19*2 (rs4244285), and CYP2C19*3 (rs4986893) polymorphisms were tested. Peripheral blood DNA was extracted using a DNA extraction kit (cat# 158422, QIAGEN).

Quantitative polymerase chain reaction (PCR) allele identification was applied for genotyping. Genome template DNA (10 ng) was dissolved in 5 L solution for each amplification reaction. The genotyping probe test preparation was as follows (final concentration 20X): 2X genotyping probe mixture (No. 4371355, Applied Biosystems), free DNA, and free RNA solvent.

Real-time PCR amplification was performed on a 7900HT system (Applied Biosystems). The cycling conditions consisted of 10 min at 95°C followed by 15 s at 92°C and 1.5 s at 60°C. Amplification product detection and genotyping analysis were performed using the SDS2.4 software.

For CYP2C19*2 and CYP2C19*3 polymorphisms, one allele loss of function was considered moderate clopidogrel metabolism (e.g., *1/*2), while a two allele loss of function was deemed a lack of clopidogrel metabolism (e.g., *2/*2) (Furman et al., 1998). For the CYP3A5*3 polymorphism, only a two allele loss of function (e.g., *3/*3) was considered "no CYP3A5 expression", and the *1/*1 and *1/*3 genotypes were defined as "CYP3A5 expression" (Suh et al., 2006).

Randomization

A random plan was produced by a statistical analysis system (SAS). The standard consecutive number method was applied and the letter was sealed in an envelope to ensure the allocation concealment.

Statistical analysis

The GraphPad Prism software version 5.0 was used for the statistical analysis. Continuous data are reported as means and standard deviation (± SD), while classified variables

are reported as frequency and percentage. PRU distribution normality was confirmed by the Kolmogorov-Smirnov test. The Student *t*-test or the Wilcoxon test were used to compare continuous variables. The chi-square test was performed to test the difference between classified variables. A sample size calculation showed that 90 subjects were needed to test the difference in 40 units at a bilateral 5% level of 90%. $P < 0.05$ was considered significant.

RESULTS

No significant differences in clinical features and laboratory characteristics were observed between the two groups (Table 1). The variables in Table 1 were suitable for all patients, while the ejection fraction was only applicable to 26 CHS patients and 24 CB patients.

Table 1. Clinical and laboratory characteristics between the groups.

	CHS (N = 67)	CB (N = 52)	P value
Age (years)	65 ± 10	62 ± 7	0.85
Gender (male, %)	71	76	0.41
Body mass index	28.9 ± 4	27.8 ± 5	0.89
Hypertension	51	71	0.3
Coronary artery disease family history (%)	11	18	0.36
β-receptor inhibitor (%)	38	19	0.34
Nitrate (%)	3	4	1
Aspirin (%)	32	30	0.62
Ejection fraction (%)	56.1 ± 6.7	55.8 ± 5.2	0.92
Platelet (k/μL)	204 ± 58.5	198 ± 22.8	0.31
Cholesterol (mg/dL)	202 ± 41	199 ± 31	0.75
Glomerular filtration rate	87.3 ± 35	88.6 ± 28	0.68

Antiplatelet activity

In the whole group, the median time between antiplatelet therapy administration and venous blood collection was 12 h (IQR 12-13 h). It was the same between CHS patients [median 12 h (IQR 12-13 h)] and CB patients [median 12 h (IQR 12-13 h)] ($P = 0.727$).

The PRU value was widely distributed and could indicate a heterologous antiplatelet effect (Figure 1). No statistically significant difference in baseline platelet activity was found between CHS and CB patients (245 ± 34 versus 244 ± 48, $P = 0.617$). For the clopidogrel response, the mean PRU values were 187 ± 44 and 213 ± 27 in CHS and CB groups, respectively ($P = 0.521$). The P2Y12 inhibition percentages were 22 ± 23 and 27 ± 19% in CHS and CB groups, respectively ($P = 0.946$).

Nineteen patients in the CHS group and 16 patients in the CB received long-term aspirin treatment. Their PRU value (179 ± 51 versus 162 ± 64, respectively, $P = 0.491$) and inhibition percentage (22 ± 16 versus 27 ± 22, respectively, $P = 0.326$) were similar to patients in the CHS and CB groups.

Polymorphism

All three types of polymorphisms existed in 119 patients (67 in the CHS group and 52 in the CB group). The results are listed in Table 2.

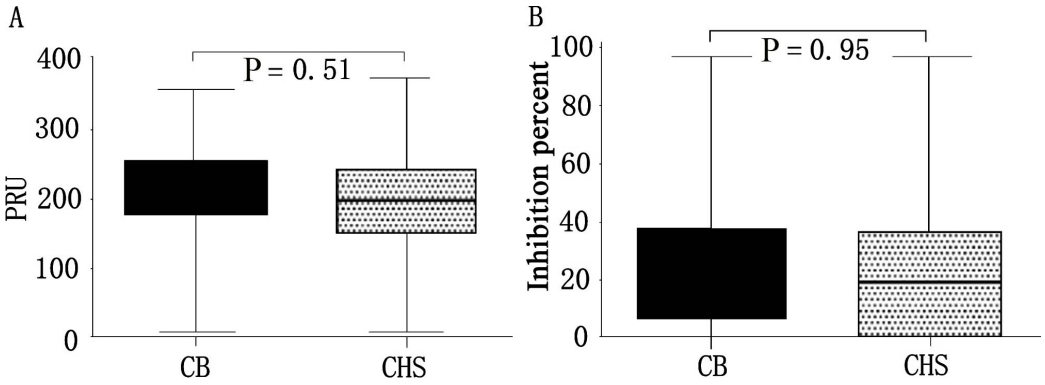

Figure 1. PRU (**A**) and inhibition percentage (**B**) comparison between CHS and CB group.

Table 2. CYP2C19 and CYP3A5 genotype morbidity.

Genotype	Total patients (N = 117)	CHS patients (N = 67)	CB patients (N = 52)
CYP2C19*2			
CYP2C19*1/*1	88 (75%)	56 (84%)	36 (70%)
CYP2C19*1/*2	28 (24%)	9 (14%)	16 (30%)
CYP2C19*2/*2	1 (1%)	2 (2%)	0
CYP2C19*3			
CYP2C19*1/*1	115 (98%)	66 (99%)	51 (98%)
CYP2C19*1/*3	1 (1%)	1 (1%)	0
CYP2C19*3/*3	1 (1%)	0	1 (2%)
CYP3A5*3			
CYP3A5*1/*1	95 (89%)	59 (88%)	47 (90%)
CYP3A5*1/*3	10 (9%)	7 (11%)	4 (8%)
CYP3A5*3/*3	2 (2%)	1 (1%)	1 (2%)

Twelve patients in the CHS group (17%) and 14 patients in the CB group (26%) showed CYP2C19*2 polymorphism, the moderate clopidogrel metabolism heterozygote phenotype. In these patients, the PRU (241 ± 52 versus 215 ± 38, respectively, P = 0.592) and inhibition percentage (18 ± 21 versus 15 ± 12, respectively, P = 0.593) were not significantly different. One patient in the CHS group lacked clopidogrel metabolism ability (Table 2).

Six CYP3A5*3 polymorphism heterozygotes appeared in all groups, while the CHS group had only one homozygote. There were no significant differences in PRU (251 ± 85 versus 211 ± 56, respectively, P = 0.564) and inhibition percentage (17 ± 28 versus 31 ± 21, respectively, P = 0.457).

DISCUSSION AND CONCLUSION

Our study showed that CHS and CB have the same antiplatelet effects in stable coronary artery disease patients. To our knowledge, this is the first study on the impact of different kinds of clopidogrel salts on platelet response polymorphism. We found that in patients with CYP2C19*2 polymorphism, different clopidogrel salts resulted in no differences in platelet response.

In recent years, a variety of clopidogrel salts has been applied in the clinic. In 2009, the European Medicines Agency (EMA) approved numerous clopidogrel drugs. In May 2012,

the Food and Drug Administration (FDA) approved the use of general clopidogrel salt for secondary prevention. CB was the most common type. Theoretically, different kinds of salt may produce different effects clinically. Salt is converted to drug molecules with ionization characteristic that improve their physical and chemical properties. Formulating different drug salts can change the solubility and pharmacokinetic properties. In addition, the salt form is more stable than the free form. Different types of salts have different clinical efficacies, such as metoprolol salt (MERIT-HF Study Group, 1999; Poole-Wilson et al., 2003). Therefore, a study comparing two different kinds of clopidogrel drugs for treatment of coronary artery disease is necessary, considering the polytropy of platelet responses to clopidogrel treatment and thrombosis time caused by the clopidogrel response.

There are very few clinical studies that use CB and most of them are conducted on healthy volunteers and are retrospective or not random. Neubauer et al. (2009) and others investigated 21 healthy volunteers' platelet responses to CB and CHS. Patients were randomly assigned one of the two drugs and received another after 21 days of the metabolic cycle. The whole blood coagulation method and flow cytometry were used to evaluate the platelet aggregation after treatment. No differences were found between the two salts. Jeong et al. (2010) analyzed CHS and CB in 20 coronary artery disease patients with the non-random method and found a similar ADP-induced platelet aggregation. Borsiczky et al. (2012) retrospectively studied 150 coronary heart disease patients who received CHS therapy, including 94 patients changed to CB, and the ADP-induced platelet aggregation in these patients was almost the same.

In another experiment, Tsoumani et al. (2012a,b) studied 96 ACS patients treated with CB or CHS and tested VASP platelet aggregation. There was no difference between the variability of clopidogrel response at a few days and 1 month after treatment. The three kinds of polymorphisms observed in our study were similar to those found in European and Greek populations (Arvanitidis et al., 2007). In addition, there was no significant difference in the clopidogrel antiplatelet effect on CYP2C19*2 gene deletion heterozygote patients. Comprehensive analysis showed that because platelet reaction decreased, CYP2C19*2 carriers exhibited a higher cardiovascular occurrence rate (heterozygous hazard ratio (HR) = 1.55, 95% confidence interval (CI) = 1.11-2.17; homozygous HR = 1.76, 95%CI = 1.24-2.50) and higher risk of stent thrombosis after clopidogrel treatment (heterozygous HR = 2.67, 95%CI = 1.69-4.22; homozygous HR = 3.97, 95%CI = 1.75-9.02). We know that polymorphism can lead to antiplatelet response that is unapparent after clopidogrel treatment, but there were no differences between clopidogrel salts.

Stable coronary artery disease subjects cannot receive vascular remodeling. Platelet activity increases in stable coronary artery disease patients are caused by environmental changes, which make platelets more sensitive to stimulation. Stable coronary artery disease patients maintain circulating degranulation platelets, circulating monocyte platelet aggregation, platelet reactivity elevation, and the tendency to form monocyte platelets (Furman et al., 1998).

Our study had some limitations. First, we did not compare the two different salts' pharmacodynamics after long-term treatment. However, since a 600 mg dose of clopidogrel can achieve the maximum antiplatelet response, we believe that this dosage was sufficient for determining differences between the different salts. Second, we did not select a clinical end point. A large number of patients was needed to determine the potential end point differences in low risk patients. Finally, the study sample size was relatively small, and we failed to determine the potential PRU differences between the three polymorphisms. However, thus far, this is the largest randomized study on stable coronary artery disease.

The antiplatelet activity of CHS and CB on stable coronary artery disease patients was similar.

Conflicts of interest

The authors declare no conflict of interest.

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