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Pharmacogenetics

Influence of *CYP2C19* loss-of-function variants on the metabolism of clopidogrel in patients from north-western China

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SUMMARY

What is known and objective: **Variation of the cytochrome P450 2C19 gene coding for the CYP2C19 enzyme has been reported to be associated with clopidogrel response variability. The activity of the CYP2C19 enzyme is genetically influenced by polymorphisms of its gene.**
Methods: **This study was conducted to assess the impact of *CYP2C19* polymorphism on the clopidogrel metabolism, indirectly selecting the plasma concentration ratios of clopidogrel to its inactive metabolite SR26334 as an evaluation index. Genotyping and plasma concentration results of 366 patients on clopidogrel maintenance therapy (75 mg daily dose) were analysed in this study. *CYP2C19* genotypes were determined by PCR-restriction fragment length polymorphism method.**
Results and Discussion: **As for *CYP2C19*, patients were classified into three metabolism genotype groups: EM (44·3%), IM (43·4%) and PM (12·3%). The mean plasma concentration ratio of clopidogrel to its inactive metabolite SR26334 for the entire sample was 0·507. The plasma concentration ratios of the 3 metabolism groups were significantly different ($P < 0·001$). The lowest plasma concentration ratio value was observed for PM patients.**
What is new and conclusion: **Polymorphism of *CYP2C19* was significantly associated with plasma concentration ratios of clopidogrel to its inactive metabolite SR26334. Clopidogrel metabolism was regulated by *CYP2C19*. The *2 and *3 allele carriage were independently associated with the antiplatelet effect of chronic clopidogrel therapy.**

INTRODUCTION

Clopidogrel, methyl (+)-(S)-2-(2-chlorophenyl)2-{6,7-dihy-drothiene[3,2-c]pyridine–5(4H)}-acetate (SR25990C, Fig. 1a), is an orally administered prodrug that is widely used for the prevention of recurrent ischaemic events in patients with acute coronary syndrome (ACS).[1] However, for 5–40% of patients receiving treatment with conventional doses of clopidogrel, variability of response has been associated with an increased risk of thrombotic recurrences or death.[2] The effects of clopidogrel are mediated by its active thiol metabolite; it requires a two-step sequential biotransformation for clopidogrel into its active metabolite by the hepatic cytochrome P450 (CYP) system, which is mainly located in the liver.[3]

Different CYP450 isoenzymes, including CYP2C19, CYP2B6, CYP3A4/CYP3A5, CYP1A2 and CYP2C9, are involved in the process of biotransformation of clopidogrel to its thiol metabolite.[4] Among the enzymes mediating this conversion, evidence is accumulating that the CYP2C19 contributes to 44·9% of the transformation of clopidogrel to 2-oxo-clopidogrel and about 20% to the formation of the active thiol metabolite from 2-oxo-clopidogrel. Therefore, CYP2C19 is dominant for both of the oxidative steps required for clopidogrel bioactivation.[5] The hepatic CYP2C19, as a member of the cytochrome P450 superfamily, is a monooxygenase enzyme localized to the endoplasmic reticulum, which plays a critical role in the metabolism of many drugs in current clinical use such as some proton pump inhibitors, mephenytoin, antidepressants, benzodiazepines and clopidogrel.[6]

The *CYP2C19* gene, which encodes the CYP2C19, is located on chromosome 10q24. This gene shows polymorphism with more than 25 known variant alleles. The wild-type *CYPC19**1 allele is the only completely functional form of the CYP2C19 enzyme.[7] In patients who have experienced ACS or stent (ST) surgery, or both, any loss-of-function *CYP2C19* alleles *2, *3, *4, *5 or *6 (which echo with gain-of-function genes, such as *CYP2C19**17) contribute to an increased risk of stent thrombosis and ischaemic events during treatment with clopidogrel.[8,9] Related research has demonstrated that carriers of *CYP2C19**2 or*3 would generate a smaller amount of the active metabolite of clopidogrel. Despite receiving preventative antiplatelet treatment with clopidogrel, patients with *CYP2C19**2 and *CYP2C19**3 show a reduced conversion of clopidogrel to its active metabolite and therefore may experience a subsequent atherothrombotic event.[10]

Because up to 85% of the absorbed drug is hydrolysed to the carboxylic acid derivative of clopidogrel (SR26334, Fig. 1b), which is its inactive metabolite, and the active metabolite is highly unstable and needs special collection method using tubes containing an atypical preservative, the exposure and pharmacokinetic parameters for clopidogrel have been indirectly determined by quantifying the SR26334 or by simultaneous quantification of clopidogrel and SR26334.[11] In the present study, a sensitive HPLC–MS/MS method[12,13] was used for the simultaneous measurement of clopidogrel and its inactive metabolite in human plasma. This method has been successfully applied to the therapeutic drug monitoring of clopidogrel in treated patients.[14]

To the best of our knowledge, the relationship between *CYP2C19* genotype and plasma concentration variation in the

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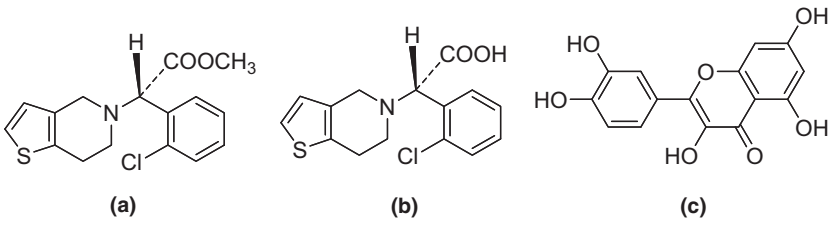

Fig. 1. Chemical structures of clopidogrel (a); its inactive metabolite SR26334 (b); and quercetin (c).

course of clopidogrel metabolism has not yet been studied on patients in north-western China. Based on the pharmacokinetics of clopidogrel metabolism,[15–17] we aimed to simultaneously determine the plasma concentrations of prototype drug clopidogrel and non-active metabolism SR26334 of subjects, who have different *CYP2C19* genotypes [extensive metabolizers (EM) *1/*1, intermediate metabolizers (IM) *1/*2 or *1/*3, and poor metabolizers (PM) *2/*2, *3/*3 or *2/*3]. Another objective of our study was to assess the influence of genetic polymorphisms of loss-of-function *CYP2C19* alleles on clopidogrel metabolism by comparing the plasma concentration ratio values of clopidogrel to SR26334 in ischaemic heart disease patients.

MATERIALS AND METHODS

IRB approval

The study protocol was approved by the Food and Drug Administration Research Involving Human Subjects Ethical Committee of First Affiliated Hospital of Xi'an Jiaotong University, Xi'an Jiaotong University, China. All patients signed written informed consents for the intervention, plasma concentration testing and genotype determination before the study.

Materials and reagents

Clopidogrel (99% purity by HPLC) and its carboxylic acid metabolite (CLPM; SR26334; purity: 99%) were purchased from Wuhan East Cogent Technology Co., Ltd. (Wuhan, China). Quercetin, used as internal standard (IS, Fig. 1c; purity: 99·3%), was obtained from Shanghai Yong Ye Biotechnology Co., Ltd. (Shanghai, China). HPLC grade of methanol and acetonitrile were supplied by Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Formic acid (98·0%) was from Guangdong Guanghua Chemical Factory Co., Ltd. (Guangdong, China). Ultrapure water was deionized in a TTL-30 purification system (Tongtai Science & Technology Development Co., Beijing, China). Drug-free heparinized human plasma and drug-containing plasma were provided by the Clinical Trial Center of First Affiliated Hospital of Xi'an Jiaotong University (Shaanxi, China). The plasma was stored at −20 °C until further use for analysis.

All stock solutions were prepared in methanol at a concentration of 0·1 mg/mL and stored at −20 °C. The intermediate solutions of clopidogrel and SR26334 were diluted from stock solutions with the incubation buffer. In addition, the IS stock solution was further diluted to 20 ng/mL in metabolite for routine use.

Study populations

The study subjects, recruited from August 2012 to June 2014, consisted of 366 patients with ischaemic heart disease who were pretreated with maintenance therapy of 75 mg clopidogrel (Plavix®; Hangzhou Sanofi-Aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China) as a daily dose for at least 7 days, and their age was 30 years or above. All patients were from five provinces (including Shanxi 153, Gansu 85, Qinghai 44, Ningxia 49 and Xinjiang 35 cases) in north-western China with three consecutive generations residing in China and nominating Chinese as their native language. Among the study subjects, patients were excluded from this study if they had renal or hepatic impairment. Patients suffering from bleeding disorders and other coagulopathies were also excluded.

Information on sex, age, body weight, height, diabetes mellitus, hypertension, alcohol drinking, smoking status, and levels of high-sensitivity C-reactive protein (hs-CRP), HDL-cholesterol, LDL-cholesterol and glycated haemoglobin were obtained from the clinical records of patients.

Blood sampling

For all patients, peripheral venous blood samples (10 mL) were collected using the sodium heparin tubes at hospital admission for DNA analysis, clopidogrel and SR26334 plasma concentration measurements. The blood samples were stored at −20 °C until analysis.

Genotyping

Genomic DNA was extracted from 200 μL of blood using a commercially available BaiO® DNA™ Blood Mini Kit (Shanghai, China) according to manufacturer's instructions. Specific primers were used for *CYP2C19* genotyping. Primers were obtained from Sangon Biotech (Shanghai, China). Genotyping was performed on the chip-based matrix-assisted laser desorption ionization time-of-flight mass spectrometer from MassARRAY Compact System (Sequenom, San Diego, CA, USA) in First Affiliated Hospital of Xi'an Jiaotong University. Analyses were carried out using an Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR was performed with an initial denaturation at 50 °C for 5 min, annealing at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The *CYP2C19* genetic polymorphism related to this study includes the EM (*1/*1), IM (*1/*2, *1/*3) and PM (*2/*2, *2/*3, *3/*3). Genotyping of *CYP2C19**2 (rs 4244285, 681G>A) and *CYP2C19**3 (rs 4986893, 636G>A) was performed using the PCR-based restriction fragment length polymorphism (RFLP) method according to the procedure as described previously.[18] The *CYP2C19* polymorphisms of randomly selected homozygous and heterozygous mutant samples were confirmed by sequencing analysis.

Determination of plasma concentrations

Pretreatment of blood samples. Briefly, 0·5 mL plasma was extracted from 5 ml blood sample. To 0·5 mL plasma sample, 2 mL acetonitrile containing 40 μL IS (quercetin at 20 ng/mL in methanol) was added for protein precipitation. Then, 10 μL supernatant was injected into the LC–MS/MS system. All prepared samples were kept at 4 °C until autosampler injection.

LC–MS/MS analysis. The plasma was analysed by a chromatograph with VI500 HPLC system (Shanghai WoWorkers Scientific Instrument Co., Ltd. Shanghai, China) which was coupled to an triple-quadrupole tandem mass spectrometer (API3200; AB SCIEX, Foster City, CA, USA) in ESI mode to generate positive molecular ions $[M+H]^+$. Clopidogrel, SR26334 and IS were separated on a C_{18} RP column (*Kromasil®*, 150 × 4·6 mm id, 5·0 μm) at a column temperature of 35 °C. The mobile phase was a mix of methanol (A) and ultrapure water (B) containing 0·05% formic acid. The gradient was as follows: 0·01 min, 90% B; 5 min, 40% B; 10 min, 0% B; 20 min: 0% B; and 20·01 min, return to initial conditions. Mobile phase flow was set to 1 mL/min, and injected volume was 10 μL for each sample.

The eluent from HPLC column was introduced directly to the MS, with the positive electrospray ionization mode used. Mass spectrometer parameters were as follows: optimized ion spray voltage 4500 V and optimized temperature 400 °C. Dry nitrogen (Z 99·5%) produced by N_2 generator F-DBS was used as desolvation gas, flow 10 L/min, temperature 300 °C. Nitrogen was also used as nebulizer gas (pressure 40 psi)and collision gas. The operating conditions, which were optimized by infusing 5 ng/mL standard solutions of the analytes at 20 L/min, were as follows: collision energy, declustering potential, and entrance potential was 23, 40 and 10 V for clopidogrel; 41, 41 and 9 V for SR26334; 23,166 and 10 V for IS, respectively. The collision cell exit potentials were all 10 V.

The mass transition used for clopidogrel, SR26334 and IS was m/z 322·1→212·1, 308·1→198·1 and 301·0→151·0, respectively, which was monitored by the multiple reaction monitoring (MRM) mode. All analytical data were acquired and processed by ANALYST software (version 1.5.1; Applied Biosystems, Foster City, CA, USA).

Calibration curves for clopidogrel were estimated by selecting the peak area ratio of clopidogrel to IS as a function of analyte concentration covering the range of 1–100 ng/mL. Correspondingly, the linear range of SR26334 was 20–2000 ng/mL. Calibration standards were prepared by serial dilution of the working solution in blank plasma resulting in three individual concentrations of 1, 50 and 100 ng/mL for clopidogrel and 20, 1000 and 2000 ng/mL for SR26334.

The validation parameters were selectivity, accuracy, precision, sensitivity, matrix effect, and stability for clopidogrel and SR26334 in human plasma. HPLC–MS/MS method validations were performed according to currently accepted United States Food and Drug Administration (US FDA) bioanalytical method validation procedures.[19]

Study design

A total of 366 subjects having clear *CYP2C19* genotypes were available for this study. Blood samples for quantification of plasma concentration were collected 8 h after the daily dose of 75 mg of clopidogrel. Each subject took a single dose of drug with 200 mL water and was asked to remain a seated position for 2 h after taking clopidogrel.

For the primary analyses, plasma concentration ratios of clopidogrel to SR26334 were determined. For secondary analyses, the impact of *CYP2C19* polymorphisms on plasma concentration values was assessed.

Statistical analysis

Statistical variables are expressed as mean ± standard deviation, number (percentages), or median with interquartile range (IQR), which were performed using STATISTICA Software (SPSS 18.0; SPSS Inc., Chicago, IL, USA). The chi-square test was used to compare categorical variables and to assess possible deviations of the genotype distribution from the Hardy–Weinberg equilibrium (HWE). The Gaussian distribution of continuous data was checked using Kolmogorov–Smirnov test. Gaussian-distributed continuous variables were compared across genotype groups by means of the unpaired two-tailed *t*-test or ANOVA for >2 groups, whereas continuous variables with a non-Gaussian distribution were compared by the Kruskal–Wallis test. Meanwhile, we applied a multiple linear regression model to evaluate the correlations between various genotypes and plasma concentration. The differences were considered to be significant when $P \leq 0{\cdot}05$.

RESULTS

Study population and genotyping for CYP2C19

Detailed characteristics of patients according to the *CYP2C19* genotypes are presented in Table 1. Demographic indicators and clinical variables were well balanced between the six treatment groups. Among the 366 patients having available genotyping results, 162 were EM, 159 were IM and 45 were PM. Thus, 204 patients were carriers of at least one *2 or *3 allele. The allele frequencies from these genotype distributions were as follows: 44·3% for the wild-type allele vs. 55·7% for the *CYP2C19* mutant allelic variant. The genotype frequencies were consistent with Hardy–Weinberg predictions ($P = 0{\cdot}65$).

Determination of plasma concentrations

The retention time of clopidogrel, SR26334 and quercetin was 11·08, 6·93 and 8·11 min, respectively, and no interfering peaks were observed at the elution times of these target peak. Representative chromatograms for the three analytes in drug-free human plasma, a standard plasma sample at LLOQ and a real plasma sample from a subject are shown in Fig. 2. The calibration curve was established in the range of 1–100 ng/mL for clopidogrel and 20–2000 ng/mL for SR26334; both exhibited good linearity with the correlation coefficient of 0·9999 for clopidogrel and 0·9998 for SR26334.

The detection of clopidogrel and SR26334 using MRM mode was highly selective with no interference from endogenous compounds of plasma samples. In the worked out conditions, LLOQ was 1 ng/mL for clopidogrel and 20 ng/mL for SR26334. Intraday and interday accuracy of the method, expressed as % RE, was ≤15·0% for clopidogrel and ≤6·8% for SR26334. Intraday and interday precision of the method, expressed as% RSD, was <15·0% for quality control samples and <20·0% for LLOQ of the all abovementioned analytes. No significant matrix effect was observed.

Table 1. Detailed characteristics in relation to *CYP2C19* genotypes

Characteristic	Entire cohort (n = 366)	*CYP2C19* polymorphism						P
		*1/*1 (n = 162)	*1/*2 (n = 120)	*1/*3 (n = 39)	*2/*2 (n = 24)	*2/*3 (n = 18)	*3/*3 (n = 3)	
Basic information								
Age, years	63·8 ± 9·5	63·9 ± 9·7	63·1 ± 9·2	62·8 ± 8·7	63·3 ± 9·5	64·2 ± 10·1	63·7 ± 9·6	0·432
Female, n (%)	87 (23·8)	37 (22·8)	28 (23·3)	10 (25·6)	6 (25)	5 (27·8)	1 (33·3)	0·273
Body mass index, kg/m²	25·6 ± 3·8	25·7 ± 4·1	25·2 ± 3·9	25·8 ± 3·5	25·5 ± 3·7	26·0 ± 3·3	25·4 ± 3·5	0·925
Height, cm	171 ± 43	170 ± 51	169 ± 44	172 ± 47	170 ± 49	170 ± 45	174 ± 41	0·523
Risk factors								
Diabetic, n (%)	75 (20·5)	30 (18·5)	27 (22·5)	9 (23·1)	5 (20·8)	3 (16·7)	1 (33·3)	0·459
Hypertensive, n (%)	237 (648)	105 (64·8)	78 (64·2)	25 (64·1)	16 (66·7)	11 (61·1)	2 (66·7)	0·355
Current smoker, n (%)	127 (34·7)	58 (35·8)	41 (34·2)	14 (35·8)	8 (33·3)	5 (27·8)	1 (33·3)	0·698
hs-CRP, mg/L	7·2 ± 1·3	6·8 ± 1·0	7·2 ± 1·2	6·9 ± 1·1	7·1 ± 1·1	7·7 ± 1·2	6·5 ± 0·9	0·253
HDL-cholesterol, mmol/L	0·99 ± 0·30	1·01 ± 0·41	1·08 ± 0·39	0·98 ± 0·35	1·12 ± 0·42	1·06 ± 0·33	1·03 ± 0·32	0·542
LDL-cholesterol, mmol/L	2·69 ± 0·78	2·73 ± 0·82	2·71 ± 0·79	2·66 ± 0·72	2·70 ± 0·81	2·72 ± 0·82	2·70 ± 0·86	0·571
Glycated haemoglobin (%)	6·7 ± 0·8	6·2 ± 0·6	6·5 ± 0·7	6·4 ± 0·7	6·6 ± 0·8	6·8 ± 0·9	6·5 ± 0·6	0·811

Stability tests showed that clopidogrel and SR26334 were stable at least 3 months in methanol stored at −20 °C, as demonstrated by % RSD in the range of 5·1–14·3%.

CYP2C19 genotype and plasma concentration ratios

In this study, the plasma concentration ratios of clopidogrel to SR26334 were calculated to evaluate the effect of *CYP2C19* genotypes on clopidogrel treatment; the comparison of results is presented in Fig. 3. The mean value of plasma concentration ratio in the study population was 0·507 (IQR 0·162–0·724). Plasma concentration ratio values across *CYP2C19* genotypes are shown in Table 2.

The plasma concentrations ratios of clopidogrel to SR26334 between different metabolic capacity groups identified according to the genotype had significant differences ($P < 0·001$). The lowest plasma concentration ratio value was observed for patients with PM genotype ($P < 0·001$ for PM patients vs. EM patients; $P < 0·001$ for PM patients vs. IM patients). This shows that most of the PM group were distributed in the range of low serum concentration ratio values.

Regression analyses

Nine variables were selected to demonstrate that *CYP2C19**2 or *CYP2C19**3 allele carriage was independently associated with plasma concentration ratio of clopidogrel to SR26334 (Table 3). Of all the independent variables considered, only the *CYP2C19* polymorphism was found to be associated with the plasma concentration ratio.

DISCUSSION

In this study, we employed the plasma concentration ratio values of clopidogrel to SR26334 to evaluate the influence of *CYP2C19* polymorphism on clopidogrel metabolism in north-western China patients. *CYP2C19* is a highly polymorphic gene with an important and broad role in drug metabolism and activation within the liver. The foremost finding of this study showed in reporting the impact of the *CYP2C19* genetic diversity on the biotransformation process of clopidogrel to its active metabolite in Chinese patients undergoing PCI. To the best of our knowledge, we found that loss-of-function *CYP2C19* genotypes (*2 or *3) have a significant impact on clopidogrel treatment, reducing the active metabolite formation and resulting in lower plasma concentration ratio values.

Clopidogrel itself does not have biological activities; it requires hepatic biotransformation to play its therapy function. The activity of enzymes involved in the metabolism of clopidogrel is important and can vary according to genetic polymorphisms.[20] Clopidogrel is metabolized into 15% active metabolite and 85% of inactive metabolite by P450 system. Previous studies conducted in different populations including Italian,[10] Japanese,[21] Iran,[22] Germany,[23] Korean[24] and French[25] have reported that among the patients and healthy cases treated with clopidogrel, carriers with at least one variant of *CYP2C19* alleles (2*, 3*) had a reduced clopidogrel response due to decreased formation of the active metabolite. Some patients with *CYP2C19* variance have affected conversion of clopidogrel to its active metabolite, resulting in higher SR26334, which has no effect on the treatment of clopidogrel, but less active metabolite.[26] Clinically, it can result in a higher relative risk of adverse cardiovascular events by a factor of 1·53–3·69 among the carriers of the loss-of function alleles as compared with the non-carriers.[8] For Chinese people, related studies[27] have shown that *CYP2C19* genetic polymorphism can affect the pharmacokinetic response of clopidogrel. In China, almost one-third of the population carries *CYP2C19* allelic variant. Moreover, a recent meta-analysis, which obtained data from nine pharmacogenetic investigations of clopidogrel involving 9685 cases who had an ACS or underwent PCI, has validated these fundamental findings.[28] For the pharmacogenetic studies, *CYP2C19* wild-type, *CYP2C19**2 and

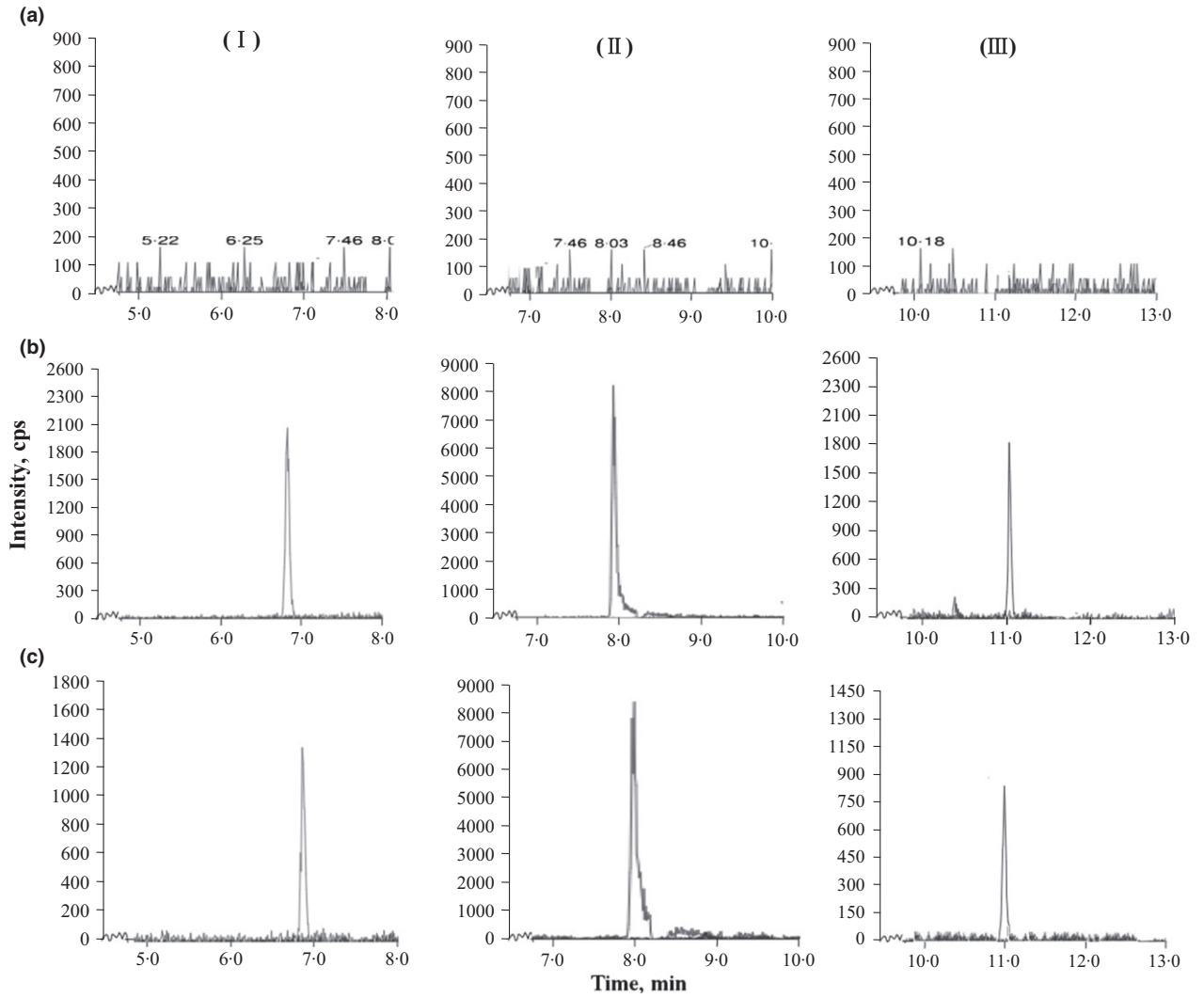

Fig. 2. Typical chromatograms recorded on the MRM transitions: (a) blank plasma; (b) blank plasma spiked with clopidogrel at 1 ng/mL, SR26334 at 20 ng/mL (LLOQ) and the IS at 10 ng/mL; (c) plasma sample collected from a patient at 8 h after the oral administration of a tablet with 75 mg clopidogrel; I: SR26334, II: quercetin, III: clopidogrel.

*CYP2C19**3 alleles were chosen as the most potent determinants of clopidogrel pharmacokinetics. Although other important *CYP2C19* gene variants encode an inactive enzyme (i.e. *CYP2C19**4 and *5), they are rare in most other groups.[29]

According to mean plasma concentrations ratio values of the clopidogrel to SR26334 (EM 0·559, IM 0·499 and PM 0·348), the plasma concentrations ratio of EM was higher by 22% than IM group, and IM was higher by 43% than PM. This provides an experimental basis for individualized dosing. Specifically, assuming that administered daily dose of clopidogrel for IM was the recommended 75 mg, the dose of EM group should theoretically be 60 mg, and PM group should be 105 mg. Of course, this is estimated only by the data obtained in this experiment and specific dosages need to be verified by taking a large number of researches.

Using plasma concentrations of the clopidogrel and its metabolite (SR26334) seemed to be rational for the following reasons: (i)

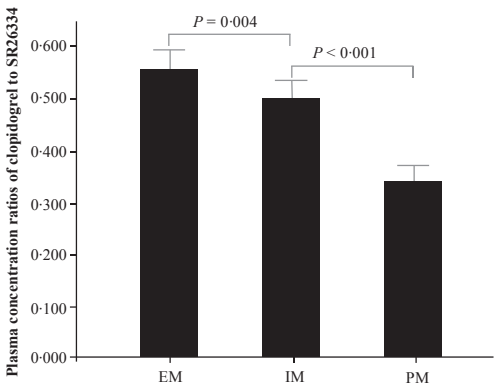

Fig. 3. Plasma concentration ratios of clopidogrel to SR26334 in relation to *CYP2C19* genotypes.

Table 2. Plasma concentration ratio values of clopidogrel to its inactive metabolite SR26334 across *CYP2C19* genotypes.

		IM		PM		
CYP2C19 genotypes	EM *1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
Median value	0·559	0·511	0·459	0·405	0·293	0·224
IQR	0·201–0·723	0·301–0·724	0·289–0·636	0·274–0·521	0·162–0·471	0·209–0·235

Table 3. Univariate and multivariable linear regression analyses of clinical and genetic factors for the plasma concentrations ratio of clopidogrel to SR26334.

	Univariate		Multivariable	
Independent variable	OR (95% CI)	*P*	OR (95% CI)	*P*
*CYP2C19**2 or *3 allele carriage	0·343 (0·121–2·157)	0·043*	0·335 (0·112–0·863)	0·038*
Age	1·031 (0·984–1·064)	0·093	1·045 (0·993–1·113)	0·072
Sex	1·163 (0·542–2·520)	0·697	1·583 (0·535–4·574)	0·349
Body weight	1·028 (0·885–1·149)	0·773	0·985 (0·823–1·171)	0·891
Diabetic	1·198 (0·468–3·070)	0·706	0·949 (0·317–2·842)	0·925
Hypertensive	1·034 (0·485–2·174)	0·923	1·411 (0·513–3·862)	0·515
hs-CRP	1·422 (0·125–16·252)	0·773	2·587 (0·125–53·837)	0·538
HDL-cholesterol	1·248 (0·466–3·137)	0·627	2·116 (0·391–11·434)	0·385
Glycated haemoglobin	1·668 (0·522–5·337)	0·387	0·984 (0·102–9·621)	0·993

Whole model: R square=0·131 (adjusted R square=0·115).
*Significant at $P < 0·05$.

the degree of platelet aggregation has a relationship with clopidogrel metabolic processes in outpatients, so the plasma concentrations ratio values of clopidogrel to SR26334 can reflect the clinical efficacy of clopidogrel;[30] (ii) the plasma concentrations of clopidogrel and SR26334 have been widely accepted as an appropriate surrogate for drug effect in many clinical studies;[31] (iii) as the active metabolite is unstable in blood, levels of exposure and the pharmacokinetics of clopidogrel have been indirectly determined by the inactive carboxylic acid derivative of clopidogrel[32] or by simultaneous measurements of clopidogrel and its carboxylic acid metabolite.

Study limitations

Our study, which was performed in a single centre, still had some limitations. Firstly, the sample size was relatively limited. Clearly, we need more studies on independent samples of sufficient size to further elucidate the potential predictive role of the genetic functional variants *CYP2C19**2 (681G>A) and *CYP2C19**3 (636G>A) of cytochrome CYP2C19 on the development of adverse vascular events in the more general population. In a larger population, the results would have been more reliable. In addition, it would be helpful to clarify whether the genotyping of other polymorphisms (e.g. *CYP2C19**17) may further improve prognostication based on genetic data. Secondly, we do not have an accurate approach to determine the active metabolite of clopidogrel in plasma directly, although we used the plasma concentration ratio values of clopidogrel to SR26334 measured by HPLC–MS/MS as an indirect marker of the active metabolite. Finally, apart from CYP2C19, we cannot exclude the effect of other drug metabolism enzymes, such as CYP1A2, 3A, 2B6 and 2C9, on clopidogrel response.

CONCLUSION

In summary, this study was conducted in 366 subjects with ischaemic heart disease receiving maintenance therapy of 75 mg clopidogrel daily. Polymorphism of *CYP2C19* was significantly associated with plasma concentration ratios of clopidogrel to SR26334. Clopidogrel metabolism was regulated by *CYP2C19*, which can predict the drug concentration of patients. These findings demonstrate the desirability for incorporating genetic factors when designing individual antiplatelet treatment.

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DECLARATION OF CONFLICTING INTERESTS

Authors declare that they have no conflict of interest.

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