Regular Article

No Effect of \textit{SLCO1B1} and \textit{CYP3A4/5} Polymorphisms on the Pharmacokinetics and Pharmacodynamics of Ticagrelor in Healthy Chinese Male Subjects

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Received August 27, 2016; accepted November 3, 2016

Ticagrelor is a direct-acting P2Y12 receptor antagonist. It is rapidly absorbed and partly metabolized to the active metabolite AR-C124910XX by CYP3A4 and CYP3A5. Three genetic loci (\textit{SLCO1B1}, \textit{CYP3A4}, and \textit{UGT2B7}) were reported to affect ticagrelor pharmacokinetics. This study aimed to investigate the possible effects of \textit{SLCO1B1} and \textit{CYP3A4/5} genetic polymorphisms on the pharmacokinetics and pharmacodynamics of ticagrelor in healthy Chinese male volunteers. Eighteen healthy male volunteers who participated in pharmacogenetics study of ticagrelor were genotyped for \textit{SLCO1B1} rs113681054, \textit{SLCO1B1}\textsuperscript{*5} (rs4149056), \textit{CYP3A4*1G} (rs2242480), and \textit{CYP3A5*3} (rs776746). All subjects received a single 180 mg loading dose of ticagrelor and then series blood samples were collected from 0 to 48h. Plasma concentrations of ticagrelor and AR-C124910XX were determined by the high performance liquid chromatography-tandem mass spectrometry method. Inhibition in platelet aggregation (IPA) was assessed and the area under the time–effect curve (\textit{AUEC}) for the IPA was calculated as pharmacodynamic parameters. No significant difference in ticagrelor pharmacokinetics among genotypes of the two genes was observed. The \textit{AUEC} did not differ significantly among genotypes of candidate single nucleotide polymorphisms (SNPs). Our data suggest that common genetic variants in \textit{SLCO1B1} and \textit{CYP3A4/5} may have no effect on the pharmacokinetics and pharmacodynamics of ticagrelor in healthy Chinese volunteers.

Key words ticagrelor; pharmacokinetics; pharmacodynamics; \textit{SLCO1B1}; \textit{CYP450}

Ticagrelor is the first reversible P2Y12 receptor inhibitor that provides faster onset/offset of pharmacological action and more consistent platelet inhibition compared to clopidogrel. It is recommended for combination therapy with aspirin in patients presenting with acute coronary syndrome (ACS) and undergoing percutaneous coronary intervention (PCI). In the PLATelet inhibition and patient Outcomes (PLATO) trial, ticagrelor was observed to reduce the primary composite end point of cardiovascular death, myocardial infarction, or stroke, compared with clopidogrel.\textsuperscript{1)}

As compared with clopidogrel, ticagrelor does not require metabolic activation to exert its antiplatelet effects. The major active metabolite of ticagrelor, AR-C124910XX (ARC), is formed \textit{via} the hepatic CYP enzyme, CYP3A4 and CYP3A5.\textsuperscript{2)} ARC is present in the blood at about 30–40% of the concentration of ticagrelor with a similar antiplatelet activity. Like other antiplatelet drugs, ticagrelor also shows interindividual variation in platelet inhibitory response in patients with ACS.\textsuperscript{3)}

At present, no genetic determinants for the ticagrelor pharmacodynamics (PD) are known,\textsuperscript{4–6} although several genetic loci (\textit{SLCO1B1}, \textit{UGT2B7}, and \textit{CYP3A4}) were reported to affect the pharmacokinetics (PK) of ticagrelor in Caucasian patients with ACS.\textsuperscript{7)} However, single nucleotide polymorphisms (SNPs) in the PK related genes showed no effects on efficacy or safety of ticagrelor.\textsuperscript{3)} Our previous studies revealed that a missense variant rs5911 polymorphism in integrin alpha 2b (\textit{ITGA2B}) is associated with \textit{ex vivo} antiplatelet activity of ticagrelor in Chinese healthy subjects while \textit{P2Y12} polymorphisms had no effect.\textsuperscript{8,9)}

As PK of ticagrelor is influenced by \textit{SLCO1B1} rs113681054, \textit{SLCO1B1}\textsuperscript{*5} (rs4149056), and \textit{CYP3A4} (rs62471956 and rs56324128) polymorphisms in Caucasian population,\textsuperscript{7)} we hypothesized that these polymorphisms may affect the PK/PD of ticagrelor in Chinese subjects. Whereas the minor allele frequencies of rs62471956 and rs56324128 polymorphisms were less than 5% in Caucasian population\textsuperscript{7)} and 0% in Han Chinese subjects from the 1000 Genomes project (www.1000genomes.org). Therefore, another functional SNP \textit{CYP3A4*1G} (rs2242480) with a minor allele frequency of 25\% was included in the present study. The aim of our present study was to investigate the effects of \textit{SLCO1B1} rs113681054, \textit{SLCO1B1}\textsuperscript{*5} (rs4149056), \textit{CYP3A4*1G} (rs2242480), and \textit{CYP3A5*3} (rs776746) polymorphisms on the PK/PD of ticagrelor in healthy Chinese subjects.

MATERIALS AND METHODS

Subjects and Study Design A total of 18 healthy young male subjects who participated in pharmacogenetics study of ticagrelor were recruited. All subjects were non-smokers and healthy as determined by medical history, physical examination, blood pressure, electrocardiogram and clinical laboratory tests. Subjects were excluded if they had a history or evidence

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of hepatic, renal, gastrointestinal, or hematologic abnormalities, any other acute or chronic diseases, or an allergy to any drugs. No medications, herbal medicine, alcohol, citrus juice, or beverages containing caffeine were permitted for 15 d prior to the study and for the duration of the study. The study protocol was approved by the Ethical Committee of Institute of Clinical Pharmacology, Central South University, China. Written informed consent was obtained from the study participants before their enrollment. The clinical research admission was approved by Chinese Clinical Trial Registry (registration number ChiCTR-OPN-15006160).

Following a 12 h overnight fast, subjects received a single oral dose of 180 mg ticagrelor (two 90 mg tablets, AstraZeneca, Sweden) with 250 mL of water and remained fasted until 4 h after dosing, when lunch was provided. Blood samples (5 mL) were collected in a total volume of 50 mL containing tubes prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h post dose. Tubes were gently inverted and placed in an ice bath until centrifugation. After centrifugal separation (1500×g, 10 min, 4°C), plasma was immediately stored at −40°C until analysis. All samples were processed within an hour. All subjects were under careful observation by a group of experienced physicians and nurses for emergency medical treatment during the entire trial.

SNPs Genotyping
Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol/chloroform protocols. Genotyping of the SLC01B1 rs113681054, SLC01B1*5 (rs4149056), CYP3A4*1G (rs2242480), and CYP3A5*3 (rs776746) polymorphisms were performed by direct PCR sequencing using an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The amplifications were performed in a total volume of 50 µL containing 10 ng genomic DNA, 2.5 mM of each deoxynucleoside triphosphate (dNTP), 5 µL 10×PCR buffer, 20 µM each of primers, and 2.5 U Taq DNA Polymerase. Thermocycling conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 30 s. The reactions were terminated by an additional extension step at 72°C for 5 min. Further details on the selected SNPs and the nucleotide sequences of the primers were listed in Table 1.

Determination of Plasma Ticagrelor and AR-C124910XX Concentrations
The concentrations of ticagrelor and its major active metabolite AR-C124910XX (ARC) in plasma samples were determined by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) by using of the Waters QuattroMicro API (Waters, Milford, MA, U.S.A.). Ticagrelor, ARC, and the deuterium-labeled internal standard (IS) ticagrelor-d7 were obtained from ALSACHIM (Illkirch-Graffenstaden, Alsace, France). A Waters Alliance 2695 liquid chromatographic system (Waters, Milford, MA, U.S.A.) equipped with a Hypurity C18 column (Thermo Hypersil-Keystone, 150 mm×2.1 mm, i.d. 5 µm) was applied for separation of plasma samples. The column temperature was set at 30°C. The mobile phase consisted of acetonitrile and 10 mmol/L ammonium acetate at a ratio of 60:40 (v/v), and was delivered at a flow rate of 0.3 mL/min. Ticagrelor-d7 was used at a concentration of 1.012 µg/mL. Briefly, 50 µL mobile phase and 50 µL IS were added into 500 µL of plasma. The mixture was vortexed for 30 s and extracted with 2 mL methyl tert-butyl ether by vortex mixing for 1 min. After centrifugation at 4000 rpm for 10 min, the deproteinized supernatant was transferred and evaporated to dryness under nitrogen stream. The residue was redissolved in 150 µL mobile phase. After centrifugation at 13000 rpm for 5 min, an aliquot of 20 µL of the supernatant was injected directly into the HPLC-MS/MS system. MS/MS conditions: electrospray ionization+multiple reaction monitoring; capillary, 3.5 kV; source temperature, 100°C; desolvation temperature, 400°C; cone gas flow, 50 L/h; and desolvation gas flow, 450 L/h. The ion transitions monitored were as follows: m/z 522.9 to m/z 152.9 for ticagrelor, m/z 478.9 to m/z 152.9 for ARC, and m/z 530.2 to m/z 152.9 for IS. These transitions represent the product ions of the [M+H]+ ions. The limits of quantification for ticagrelor and ARC were 0.019 and 2.899 ng/mL, and the correlation coefficient for ticagrelor and ARC calibration curves were 0.9997 and 0.9992, respectively. The analytical intra-day coefficients of variation for ticagrelor and ARC were ranged from 1.2 to 5.1% and from 1.4 to 5.1%, inter-day coefficients of variation ranged from 2.2 to 6.8% and from 2.2 to 5.5%, respectively (n=6). The analytical intra-day relative error for ticagrelor and ARC were ranged from −0.3 to 8.3% and from −7.4 to 3.4%, inter-day relative error ranged from 0.5 to 2.6% and from −7.9 to 5.6%, respectively (n=6).

Pharmacokinetic Analysis
Individual PK parameters of ticagrelor and ARC were estimated by noncompartmental methods with WinNonlin Version 4.1 (Pharsight, Mountain View, CA, U.S.A.). The peak observed drug concentration (Cmax) and the first time of its occurrence (tmax) were taken directly from the concentration–time profile. The plasma elimination half-life (t1/2) was calculated as 0.693/λz, where λz is the apparent terminal phase elimination rate constant estimated by linear regression of the logarithmically transformed concentration data. The area under the plasma concentration–time curve within the dosing interval (AUC0–t) was calculated by the linear trapezoidal rule and extrapolation to infinite time (AUC0–∞) by the addition of C/t1/2, where C is the plasma concentration.
centration at 48h. Apparent oral clearance (CL/F) of ticagrelor was calculated as dose divided by $AUC_{0-\infty}$.

**Measurement of Platelet Aggregation** Platelet aggregation was measured using a platelet aggregometer (LBY-NJ4, Pulisheng Instrument Co., Ltd., China) as described previously. The citrated (3.2%) blood samples were collected before and at 2, 4, 8, and 24 h, respectively, after ticagrelor dosing. The initial 5 mL blood was discarded to avoid measuring platelet activation induced by needle puncture. The citrated blood were centrifuged for 10 min at $150 \times g$ to obtain platelet rich plasma (PRP) and at 2, 4, 8, and 24 h, respectively, after ticagrelor dosing. The initial 5 mL blood was discarded to avoid measuring platelet activation induced by needle puncture.

The citrated blood were centrifuged for 10 min at $150 \times g$ to obtain platelet rich plasma (PRP). Platelet aggregation was measured for 5 min in response to 20 µM ADP and expressed as the maximal percentage change of light transmission from baseline using PPP as a reference. The inhibition of platelet aggregation (IPA) was calculated from the observed maximal aggregation (MPA) at each scheduled time-point by the following formula:

$$IPA = \left( \frac{MPA_{\text{predose}} - MPA_{\text{postdose}}}{MPA_{\text{predose}}} \right) \times 100\%$$

The area under the time–effect curve ($AUEC$) for the IPA of ticagrelor was calculated from the time vs. IPA value curve, using the linear trapezoidal rule.

**Statistical Analysis** Statistical analyses were performed by the SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL, U.S.A.). Data were expressed as the mean±standard deviation (S.D.). Normality of distribution was assessed using the Shapiro–Wilk $W$-test. PK/PD parameters that did not follow a normal distribution were log-transformed for statistical analysis and then back-transformed for data presentation. PK/PD parameters among genotypic cohort were compared using a one-way ANOVA or Kruskal–Wallis test, followed by the post hoc Bonferroni test for multiple comparisons. Student $t$-test or Wilcoxon rank-sum test was used to compare PK/PD parameters between the genotype groups. Effect of SLC10A1 haplotypes with a minimal frequency of 5% on platelet aggregation was analyzed using the THESIAS software. Difference in PK/PD parameters between the most frequent haplotype (reference) and others was compared. A $p$-value of less than 0.05 was considered to be statistically significant.

**RESULTS**

**Pharmacokinetics** Eighteen healthy Chinese male volunteers participated in our study. Mean age and weight were 22 years (range: 20–26 years) and 63 kg (range: 55–76 kg), respectively. All subjects remained compliant during the study and completed the study. The single dose of ticagrelor was well tolerated and no adverse events were observed.

Following oral administration of ticagrelor, the box plots of $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, $AUC_{0-48}$, $AUC_{0-\infty}$, and CL/F of ticagrelor and/or AR-C were shown in Fig. 1. The mean±S.D. and min–max of $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, $AUC_{0-48}$, $AUC_{0-\infty}$ and CL/F of ticagrelor were 1286.8±532.1 ng/mL (537.4–2482.8 ng/mL), 2.0±0.9 h (1.0–4.0 h), 8.6±1.0 h (7.0–10.3 h), 8765.4±3203.6 ng·h/mL (3874.9–15275.7 ng·h/mL), 8933.3±3296.6 ng·h/mL (3974.9–15643.0 ng·h/mL), 22.6±7.9 L/h (11.5–45.3 L/h), respectively, in the overall subjects. The mean±S.D. and min–max of $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, $AUC_{0-48}$, and $AUC_{0-\infty}$ of AR-C were 3977.7±1251.5 ng/mL (2274.4–662.2 ng/mL), 2.6±0.7 h (2.0–4.0 h), 10.2±2.6 h (6.5–18.4 h), 4248.9±1019.1 ng·h/mL (2523.9–6690.8 ng·h/mL), 4454.8±1207.6 ng·h/mL (2618.9–7959.4 ng·h/mL), respectively, in the overall subjects. The coefficients of variation of $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, $AUC_{0-48}$, $AUC_{0-\infty}$, and CL/F for ticagrelor were 41.4, 45.1, 11.3, 36.5, 36.9, and 35.0%, respectively. The coefficients of variation of $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, $AUC_{0-48}$, and $AUC_{0-\infty}$ for AR-C were approximately 31.5, 27.6, 25.6, 24.0, and 27.1%, respectively.

The genotype distributions of the candidate SNPs were as follows: SLC10A1 rs113681054 TT genotype ($n=4$), TC genotype ($n=1$), CC genotype ($n=3$); SLC10A1 rs4149056 TT genotype ($n=15$), TC genotype ($n=2$), CC genotype ($n=1$); CYP3A4 rs2242480 GG genotype ($n=7$), GA genotype ($n=8$),
AA genotype (n=3); and CYP3A5 rs776746 GG genotype (n=6), GA genotype (n=10), AA genotype (n=2). The plasma concentration versus time profiles of ticagrelor and ARC were compared among the SLCO1B1 and CYP3A4/5 genotype groups after administration of a 180 mg loading dose of ticagrelor (Figs. 2–4); the pharmacokinetic parameters were summarized in Table 2. An overall differences in AUC\text{0–48} of ARC was observed among the SLCO1B1 rs113681054 genotypes (p=0.093). The AUC\text{0–48} of ARC trended to be higher in rs113681054 heterozygotes as compared with TT/CC homozygotes. No significant difference in C\text{max}, t\text{max}, t\text{1/2}, AUC\text{0–48}, AUC\text{0–\infty}, and CL/F of ticagrelor and/or ARC was observed among SLCO1B1 haplotypes (Table 3).

Pharmacodynamics The average IPAs were 84.1±4.7, 82.3±4.8, 78.9±4.6, and 57.5±11.3% at 2, 4, 8, and 24 h, respectively, after ticagrelor administration (Fig. 5). Maximal inhibition (E\text{max}) was observed at 2 h after dosing. No significant differences were observed in IPA among genotypes of the SNPs within 2 h of a loading dose (SLCO1B1 rs113681054 TT vs. TC vs. CC genotype: 82.3±4.2 vs. 85.3±5.0 vs. 82.1±3.8%, p=0.418; SLCO1B1 rs4149056 TT vs. TC/CC genotype:
83.9±4.9 vs. 85.1±3.6%, p=0.714; CYP3A4 rs2242480 GG vs. GA vs. AA genotype: 83.5±4.4 vs. 84.6±4.9 vs. 84.3±6.2%, p=0.921; and CYP3A5 rs776746 GG vs. GA vs. AA genotype: 85.5±2.4 vs. 83.0±5.7 vs. 85.9±4.2%, p=0.522). The impact of candidate SNPs on AUEC after ticagrelor loading dose were depicted in Table 4. No significant differences were observed in AUEC among genotypes of the SNPs. Also, no differences were observed in AUEC among SLCO1B1 haplotypes (Table 3).

**Correlation between Ticagrelor PK and PD** When we assessed whether there was a correlation between the ticagrelor PK (Cmax and AUC0–48) and PD (Emax and AUEC), we observed a weak negative correlation between Cmax and Emax (r=-0.144) and a very weak positive correlation between AUC0–48 and AUEC (r=0.011, Fig. 6). The correlations between them were not significant (p>0.05).

**DISCUSSION**

In this study, we evaluated the possible effect of SLCO1B1 rs113681054, SLCO1B1*5 (rs4149056), CYP3A4*1G (rs2242480), and CYP3A5*5 (rs776746) polymorphisms on the oral PK/PD parameters of ticagrelor in 18 healthy Chinese male subjects. We observed remarkable interindividual variations in pharmacokinetics parameters, including Cmax, tmax, AUC0–48, AUC0–∞, and CL/F of ticagrelor after a single oral dose of 180mg. There were no differences in PK/PD parameters of ticagrelor observed among genotypes of these candidate polymorphisms. Also, SLCO1B1 haplotypes exhibited no effect on ticagrelor PK/PD.

The PK/PD profiles of ticagrelor have been evaluated in healthy volunteers and in patients with ACS, coronary artery disease, and atherosclerosis. Ticagrelor and ARC were identified as the major circulating components in the plasma. The results from the present study in healthy Chinese subjects demonstrated that ticagrelor was rapidly absorbed and exhibits a rapid onset of action. These findings are consistent with those single and multiple-ascending dose studies in healthy subjects.

SLCO1B1 encodes the organic anion transporter polypeptide (OATP1B1) expressed on the sinusoidal membrane of human hepatocytes. It mediates the hepatic uptake of many endogenous substances and xenobiotics, including many drugs. SLCO1B1*5 was associated with impaired hepatic uptake and increased plasma concentrations of most OATP1B1 substrates, thus increasing the risk of simvastatin-induced myopathy. Although our results showed that rs4149056 TT (SLCO1B1*1/*1) homozygotes seemed to show increased CL values and decreased Cmax, AUC0–48, and AUC0–∞ values of ticagrelor than carriers of the C allele (SLCO1B1*5), there was no difference in PK parameters of ticagrelor observed among rs4149056 genotypes. Another SLCO1B1 variant rs113681054, reported only in recent genome-wide association study (GWAS), also showed no significant effect on PK/PD variables of ticagrelor in the present study. Our study was not consistent with the previous finding, which suggested an association of SLCO1B1 variants (rs113681054 and rs4149056) and ticagrelor as well as AR-C124910XX steady-state area under the curve in Caucasian patients with ACS.

In this GWAS based on Caucasian populations, rs113681054 is in linkage disequilibrium (LD) with the functional variant rs4149056 (r²=0.76) that results in decreased OATP1B1 transporter activity. However, rs113681054 exhibited low degree of LD with rs4149056 (r²=0.145) and high degree of LD with rs11045879 as well as rs4149081 (r²=0.923 for both) in 103 Han Chinese subjects from the 1000 Genomes project. The rs11045879 and rs4149081 polymorphisms have been associated with methotrexate plasma concentration and toxicity. Neither rs113681054 nor rs4149056 affected efficacy and safety endpoints of patients treated with ticagrelor in the PLATO trial.

In agreement with the findings from PLATO trial, we also observed that these two variants exhibited no influence on ticagrelor PK/PD parameters in our study.

Ticagrelor is mainly metabolized by CYP3A enzymes, of which CYP3A4 and CYP3A5 are responsible for the formation of ARC. The CYP3A4 variants (rs62471956 and
Table 2. Pharmacokinetic Parameters of Ticagrelor and AR-C124910XX for Different Genotypes

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotype/n</th>
<th>Ticagrelor</th>
<th>AR-C124910XX</th>
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<tr>
<td></td>
<td></td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>$t_{\text{max}}$ (h)</td>
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<td>SLC01B1</td>
<td>TT/4</td>
<td>1300.4±454.9</td>
<td>1.5±0.6</td>
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<tr>
<td></td>
<td>CC/3</td>
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<td>2.5±1.3</td>
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<tr>
<td></td>
<td>p</td>
<td>0.837</td>
<td>0.357</td>
</tr>
<tr>
<td>SLC01B1</td>
<td>TT/15</td>
<td>1246.1±538.7</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.485</td>
<td>0.343</td>
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<tr>
<td>CYP3A4</td>
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<td>AA/3</td>
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<tr>
<td></td>
<td>p</td>
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<td>0.964</td>
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<td>CYP3A4</td>
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<tr>
<td></td>
<td>p</td>
<td>0.353</td>
<td>0.943</td>
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Table 3. The Effect of SLC01B1 Haplotypes on Ticagrelor Pharmacokinetics and Pharmacodynamics

<table>
<thead>
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<th>Haplotype</th>
<th>Ticagrelor</th>
<th>AR-C124910XX</th>
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<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>$t_{\text{max}}$ (h)</td>
</tr>
<tr>
<td>A</td>
<td>709.0±760.4</td>
<td>0.8±3.2</td>
</tr>
<tr>
<td>B</td>
<td>512.9±781.3</td>
<td>1.3±2.7</td>
</tr>
<tr>
<td>C</td>
<td>755.8±716.6</td>
<td>1.2±2.5</td>
</tr>
<tr>
<td>p*</td>
<td>0.579/0.915</td>
<td>0.690/0.705</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±S.D. *p values of haplotypes B and C as compared with the corresponding reference haplotype.

rs56324128) were reported to be associated with ticagrelor steady-state area under the curve in Caucasian patients with ACS.\(^7\) The minor allele frequencies were 3.4% (rs62471956) and 0.52% (rs56324128), respectively, in the previous study.\(^7\) However, the frequencies of wild type alleles were 100% for both SNP in 208 Han Chinese from the 1000 Genomes project. Given CYP3A4*1G (rs2242480) and CYP3A5*3 (rs776746) are the most frequent functional variants in Chinese population, they were included in the present study. Moreover, CYP3A4 haplotypes containing CYP3A4*1 allele were closely linked to CYP3A5*3.\(^23\) CYP3A4*1G is located in CYP3A4 intron 10 and could regulate enhancer and promoter activity.\(^24\) CYP3A4*1G was reported to increase CYP3A metabolic activity, thus affecting the PK and lipid-lowering efficacy of atorvastatin,\(^25,26\) tacrolimus PK,\(^27,28\) and analgesic effect of fentanyl.\(^29,30\) CYP3A5*3 resulted in a truncated protein with loss of CYP3A5 expression\(^31\) and was reported to be associated with plasma levels of cyclosporine and tacrolimus in transplant patients\(^32–34\) and alprazolam PK/PD in healthy subjects.\(^35\) However, we failed to observe the influence of CYP3A4*1G and CYP3A5*3 variant on PK/PD of ticagrelor in our study. It may be explained that CYP3A4/5 is responsible for the metabolism of ticagrelor to the active metabolite ARC, which is approximately equipotent to ticagrelor in antiplatelet effect. In accordance with our study, no association between CYP3A4*1G and CYP3A5*3 and clopidogrel PK and/or PD was observed elsewhere.\(^36–41\) On the contrary, CYP3A4*1G allele was reported to be associated with better clopidogrel responsiveness in Spanish patients with stable coronary artery disease\(^42\) and in Chinese patients with ischemic stroke.\(^43\) Evidence also revealed that CYP3A5*3 was associated with clopidogrel response and clinical outcomes.\(^44–46\)

Currently, few studies investigated the association of genetic polymorphisms with ticagrelor response. The polymorphisms in P2Y12, P2Y1, and ITGB3 have no effect on platelet aggregation in ticagrelor-treated patients with atherosclerotic
The CYP2C19 and ABCB1 polymorphisms were not associated with clinical outcomes of treatment with ticagrelor for ACS, lack of association for CYP2C19 polymorphisms was further confirmed by Tantry et al. Our previous study showed that ITGA2B rs5911 polymorphism is associated with in vitro antiplatelet activity of ticagrelor. Although SNPs in SLCO1B1, CYP3A4, and UGT2B7 were reported to affect ticagrelor PK, none of these SNPs was associated with clinical outcomes in patients treated with ticagrelor. Consequently, all previous association studies were based solely on Caucasian population and failed to find the genetic determinants of ticagrelor response.

There are several limitations that deserve attention. The sample size might not allow the identification of genetic variants that exhibit small effects. Presumably, a substantially larger sample size would be required to fully evaluate the effect of these polymorphisms. Only light transmission aggregometry was applied to determine platelet reactivity in our present study. In addition, only healthy subjects were recruited in the present study. Therefore, P2Y12 receptor specific test such as vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) assay should be adopted to determine the association of these candidate polymorphisms with ticagrelor response in patients with ACS or those undergoing PCI. Inclusion of clinical outcomes from these patients would increase the impact of our findings.

In conclusion, our results indicated that SLCO1B1 rs113681054, SLCO1B1*5 (rs4149056), CYP3A4*1G (rs2242480), and CYP3A4*5 (rs776746) polymorphisms had no effect on the PK/PD of ticagrelor in healthy Chinese volunteers. Given our study was limited by the sample size, further investigations should be conducted to verify our findings.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


