China Pharmaceutical University[1], Nanjing First Hospital Affiliate To Nanjing Medical University[2], Nanjing, China

CYP2C19 681G > A polymorphism and pharmacokinetics of clopidogrel in Chinese healthy volunteers

Zou Jian-Jun[1,2], Ding Li[2], Tan Jie[1], He BangShun[1], Wang Guang-Ji[2], Wang Shu-Kui[1]

Received November 19, 2011, accepted December 16, 2011

Wang Shu-Kui, Central Laboratory, Nanjing First Hospital, Nanjing 210006, China
Wangshukui159@126.com

Pharmazie 67: 795–797 (2012) *doi: 10.1691/ph.2012.1810*

The aim of this study was to investigate the contribution of the most frequent single nucleotide polymorphism of CYP2C19 681G > A to the pharmacokinetics of clopidogrel in 20 healthy Chinese volunteers after administration of a single dose of clopidogrel 75 mg. The peak plasma concentration (C_{max}) was higher in the 681GA + 681AA group than that in the 681GG group (1.93 ± 1.77 vs. 1.65 ± 1.56 ng/mL, P = 0.613). The area under the curve to the last measurable concentration ($AUC_{0–36}$) and area under the curve extrapolated to infinity ($AUC_{0–\infty}$) of clopidogrel were lower in the 681GG group than that in the 681GA + 681AA group (2.25 ± 1.64 vs. 2.64 ± 1.69 ng h/mL, P = 0.465; 2.26 ± 1.65 vs. 2.67 ± 1.71 ng h/mL, P = 0.455) respectively. The oral clearance (Cl/F) was lower in the 681GA + 681AA group than that in the 681GG group (51.96 ± 36.13 vs. 54.47 ± 35.21 × 10^3 L/h, P = 0.829). The genetic polymorphism of CYP2C19 681G > A does not cause significant alterations in the pharmacokinetics of clopidogrel at a clinically relevant therapeutic dose in healthy Chinese volunteers.

1. Introduction

Clopidogrel, a thienopyridine inhibitor of the platelet P2Y12 adenosine diphosphate (ADP) receptor, is widely used for the prevention of stent thrombosis events in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary interventions with stenting (Yusuf et al. 2001). However, even with the use of such therapy, stent thrombosis (ST), a life-threatening event with severe clinical consequences, still occurs (Nguyen et al. 2005; Mega et al. 2009; Simon et al. 2009). Fast-growing evidence has well demonstrated that genetic variation may be a reason for this phenomenon (Nguyen et al. 2005; Mega et al. 2009; Simon et al. 2009). Of note, all clinical studies have almost consistently demonstrated that the loss-of-function CYP2C19 681G > A polymorphisms are associated with attenuated response to clopidogrel and increased risk for developing ST (Nguyen et al. 2005; Mega et al. 2009; Simon et al. 2009; Xie et al. 2011; Mega et al. 2010; Shuldiner et al. 2009; Hochholzer et al. 2010). However, all these studies were performed in white or black patients. The conclusion may not be fully generalized or extrapolated to the Chinese people due to the marked ethnic variabilities in the clopidogrel metabolism and its anti-platelet effect (Xie et al. 2011; Kim et al. 2008), in part because the polymorphism of CYP2C19 681G > A displays ethnic differences between Orientals and Caucasians (Zhou et al. 2000) and even among Orientals with different ethnic backgrounds (Shu et al. 2000). Moreover, the effect of CYP2C19 681G > A genetic polymorphisms on the pharmacokinetics of clopidogrel in Chinese subjects is still unknown so far. This study aims to study this aspect in healthy Chinese volunteers.

2. Investigations and results

2.1. CYP2C19 681G > A genotyping

The results of CYP2C19 681G > A genotyping are shown in Fig. 1. The 20 subjects were divided into two groups according to their CYP2C19 genotype: wild-type homozygotes, CYP2C19 681GG (n = 9); at least one 681G > A mutant allele (mutant heterozygotes, CYP2C19 681GA, n = 10; and mutant homozygotes, CYP2C19 681AA, n = 1).

2.2. Pharmacokinetics of clopidogrel

After an oral single dose of 75 mg clopidogrel, plasma concentration-time curves of clopidogrel according to the CYP2C19 681G > A genotypes are shown in Fig. 2. The pharmacokinetic parameters (C_{max}, $AUC_{0–36}$, $AUC_{0–\infty}$ and CL/F) of clopidogrel are shown in the Table and show no significant differences between the two genotyped groups.

3. Discussion

Our study shows that the pharmacokinetics of clopidogrel are not significantly altered by the CYP2C19 681G > A genetic variation. The mean $AUC_{0–36}$, $AUC_{0–\infty}$ and C_{max} values in the CYP2C19 681GA + 681AA mutant group were about 17%, 18% and 17% higher than corresponding values for the 681GG wild-type group. However, the oral elimination of clopidogrel (Cl/F) in the CYP2C19 681GA + 681AA mutant group was about 4.8% lower than in the 681GG wild-type group. These results were inconsistent with observations in a Korean population (Kim et al. 2008). Kim et al. showed that CYP2C19 polymorphisms have a significant effect on the pharmacokinetics of clopidogrel. They found that the AUC of poor metabolizers (PMs) was 1.8- and 2.9-fold higher than that of heterozygous extensive metabolizers (heteroEMs) and homozygous EMs (homoEMs), respectively (P = 0.013). The mean C_{max} of PMs was also 1.8- and 4.7-fold higher than that of heteroEMs and homoEMs, respectively (P = 0.008).

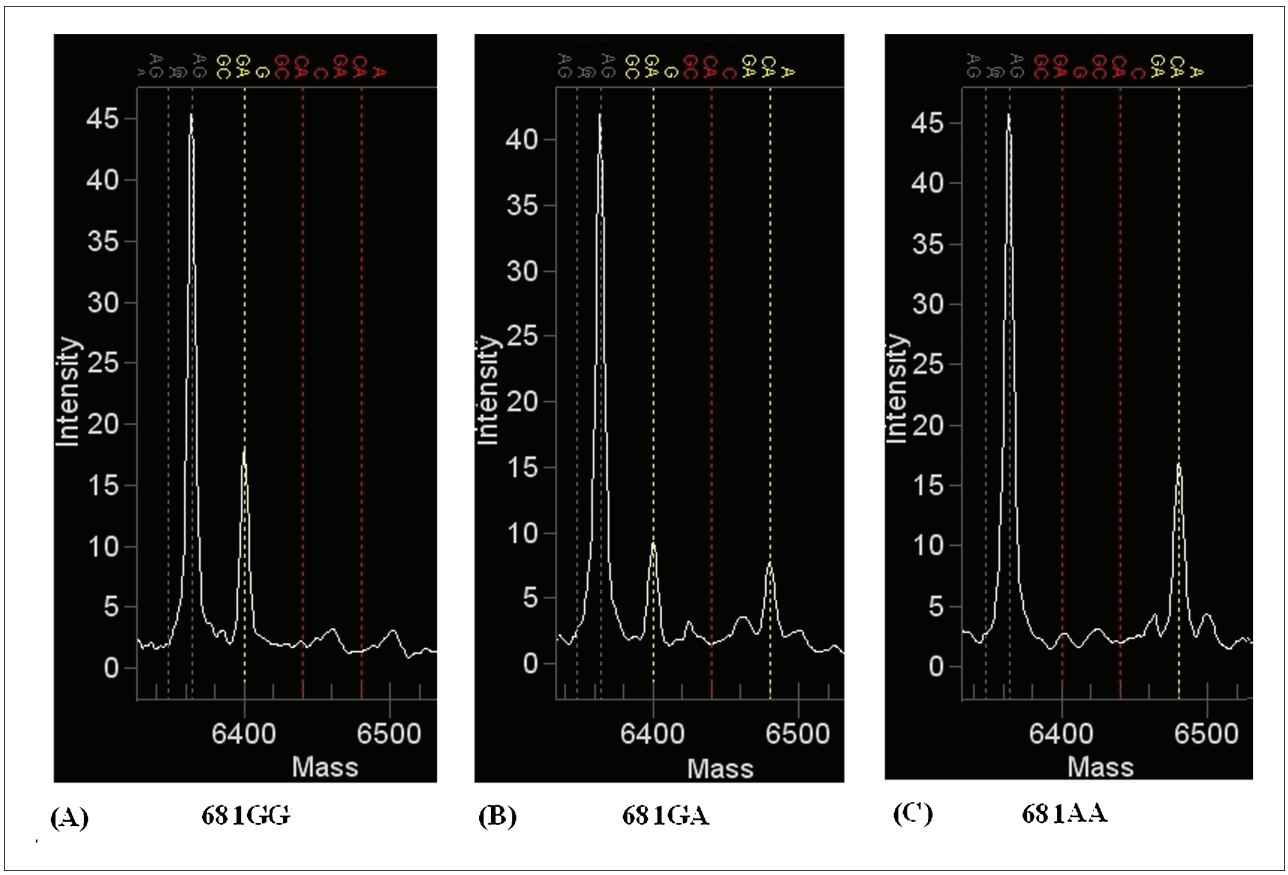

Fig. 1: Typical mass spectrograms for genotyping of CYP 681GG (A), 681GA (B) and 681AA (C)

Several studies have shown that ethnicity is one of the important factors that could affect clopidogrel metabolism (Xie et al. 2011; Li et al. 2009), in part because the allele frequency of CYP2C19*2 variant in the Chinese population (30%) is twice that in whites (14.7%) and blacks (17.3%) (Xie et al. 2011, 2001; Xie 1997). Moreover, the polymorphisms of CYP2C19 681G > A display ethnic differences even among Orientals with different ethnic backgrounds (Xie et al. 2001; Xie 1997).

The effect of CYP2C19 681G > A mutation on the pharmacokinetics of clopidogrel requires investigation. Our study was conducted in a small group of healthy subjects at a single dosage of 75 mg of clopidogrel. Further studies should be carried out on the effects of CYP2C19 genetic polymorphism to test whether multidose administration of clopidogrel in patients with ACS yields similar results.

Table: Pharmacokinetic parameters of clopidogrel in subjects with CYP2C19 681GA or 681AA genotypes and wild-type homozygotes (681GG) after a single oral dose of 75 mg clopidogrel

Parameters	681GG ($n = 9$)	681GA + 681AA ($n = 11$)	P
C_{max} (ng/mL)	1.65 ± 1.56	1.93 ± 1.77	0.613
AUC_{0-36} (ng h/mL)	2.25 ± 1.64	2.64 ± 1.69	0.465
$AUC_{0-\infty}$ (ng h/mL)	2.26 ± 1.65	2.67 ± 1.71	0.455
CL/F ($\times 10^3$ L/h)	54.47 ± 35.21	51.96 ± 36.13	0.829

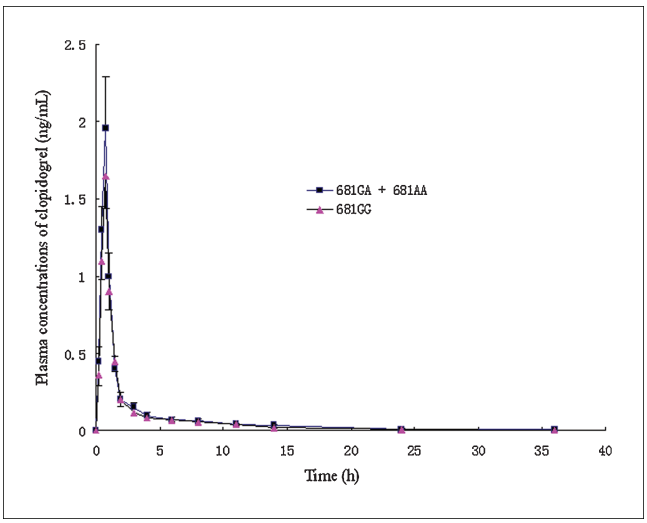

Fig. 2: The curve of mean time-concentration of clopidogrel in 20 healthy volunteers with different CYP2C19 681G > A genotypes after volunteers administrated a single oral dose of 75 mg clopidogrel. Data are expressed as mean ± SD

4. Experimental

4.1. Subjects

Twenty healthy volunteers, with a mean age of 23.6 years (± 2.7) (range 20–29 years) and a mean weight of 64.1 kg (± 5.1) (range 56–72 kg), were selected for this study. Nine CYP2C19 681GG homozygotes and 11 subjects with at least one 681G > A mutant allele (ten were 681GA heterozygotes and one was 681AA homozygotes) were recruited for the clopidogrel pharmacokinetic analysis. The study protocol was approved by the ethics committee of the Xijing Hospital of the Fourth Military Medical University, and written informed consents were obtained from all participants. Each subject was ascertained to be healthy by medical history, physical examination, routine blood and electrocardiographic tests. All of the subjects were non-smokers, abstained from drugs and did not take any coffee or alcohol for at least 1 week before entry into the study.

4.2. Drug administration and sample collection

Each subject received a single oral dose of 75 mg clopidogrel (Plavix®, Hangzhou Sanofi-Aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China) at 8:00 AM with 150 mL of warm water after overnight fasting. Meals were allowed 4 h later after clopidogrel administration. Serial venous blood samples (5 mL each) were drawn into collection tubes containing 3.8% sodium-citrate just before dosing (as baseline) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 11, 14, 24, and 36 h postdose. Blood samples was separated by centrifugation and immediately stored at −70 °C until assay.

4.3. CYP2C19 681 G > A genotyping

Genomic DNA was extracted using commercially available QIAamp DNA™ Blood Mini Kit (Qiagen, Venlo, the Netherlands). Primers were obtained from Sangon Biotech (Shanghai, China). Genotyping was performed in Shanghai Benegene Biotechnology Co., Ltd. (Shanghai, China) using the chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF) (Jackson et al. 2000) from MassARRAY Compact System (Sequenom, San Diego, CA, USA). To verify correct sample handling, genotyping was repeated in 20% of the randomly selected patients for all variants tested. Repeated genotyping revealed the identical results, and the call rate for CYP2C19 681 G > A was 100%, respectively.

4.4. Clopidogrel assay

Plasma concentrations of clopidogrel were determined using a validated liquid chromatography -tandem mass spectrometry (LC-MS/MS) method developed in our laboratory (Zou et al. 2009). In brief, clopidogrel and the internal standards (I.S), nateglinide were extracted from 0.50 mL of plasma sample using diethyl ether–*n*-hexane (4:1,v/v). LC separation was performed on a Teknokroma C_{18} column (100 mm × 2.1 mm i.d., 5 μm) with a mobile phase of methanol-water (0.1% formic acid) (80:20, v/v) at a flow rate of 0.20 mL/min. A Thermo Finnigan TSQ Discovery Max tandem mass spectrometer equipped with an electrospray ionization (ESI) source was used for mass detection. The collision energies (CE) of clopidogrel and I.S. were 10 and 20 V, respectively. The transitions *m/z* 322.1→212.1 for clopidogrel and *m/z* 318.3→166.2 for I.S were monitored in the multiple reaction monitoring (MRM) mode. Linearity was established over the concentration range of 0.005–5 ng/mL, the lower limit of quantification (LLOQ) of clopidogrel were 0.005 ng/mL.

4.5. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using DAS version 3.0 (ShangHai traditional Chinese medicine University, ShangHai, China). Maximum plasma concentrations (C_{max}) were determined directly from the respectively observed plasma concentration-time data. AUC was calculated with use of the trapezoidal rule. Total clearance (CL) was calculated by dose/$AUC_{0-\infty}$, where dose was 75 mg of clopidogrel.

4.6. Statistical analysis

The values of pharmacokinetic parameters were expressed as mean ± SD. Differences in pharmacokinetic data between the CYP2C19 681GG wild-type gene and the two mutated alleles 681GA and 681AA were evaluated statistically by independent-samples *t*-test, $P < 0.05$ was considered statistically significant. These statistical analyses were performed using the SPSS software for Windows (version 16.0, SPSS Chicago, IL).

Acknowledgments: This work was supported by the National Scientific Foundation of China grants (No.30901830), the Nanjing Science and Technology Agency (No.201001098), and the Nanjing Health Bureau (No.QYK10142).

References

Hochholzer W, Trenk D, Fromm MF, Valina CM, Stratz C, Bestehorn HP, Büttner HJ, Neumann FJ (2010) Impact of cytochrome P450 2C19 loss-of-function polymorphism and of major demographic characteristics on residual platelet function after loading and maintenance treatment with clopidogrel in patients undergoing elective coronary stent placement. J Am Coll Cardiol 55: 2427–2434.

Jackson PE, Scholl PF, Groopman JD (2000) Mass spectrometry for genotyping: an emerging tool for molecular medicine. Mol Med Today 6: 271–276.

Kim KA, Park PW, Hong SJ, Park JY (2008) The effect of CYP2C19 polymorphism on the pharmacokinetics and pharmacodynamics of clopidogrel: a possible mechanism for clopidogrel resistance. Clin Pharmacol Ther 84: 236–242.

Li YG, Ni L, Brandt JT, Small DS, Payne CD, Ernest CS 2nd, Rohatagi S, Farid NA, Jakubowski JA, Winters KJ (2009) Inhibition of platelet aggregation with prasugrel and clopidogrel: an integrated analysis in 846 subjects. Platelets 20: 316–327.

Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, Cannon CP, Danchin N, Giusti B, Gurbel P, Horne BD, Hulot JS, Kastrati A, Montalescot G, Neumann FJ, Shen L, Sibbing D, Steg PG, Trenk D, Wiviott SD, Sabatine MS (2010) Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. JAMA 304: 1821–1830.

Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS (2009) Cytochrome P-450 polymorphisms and response to clopidogrel. N Engl J Med 360: 354–362.

Nguyen TA, Diodati JG, Pharand C (2005) Resistance to clopidogrel: a review of the evidence. J Am Coll Cardiol 45: 157–1164.

Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, Steg PG, Ferrières J, Danchin N, Becquemont L (2009) Genetic determinants of response to clopidogrel and cardiovascular events. New Engl J Med 360: 363–375.

Shu Y, Zhou HH (2000) Individual and ethnic differences in CYP2C19 activity in Chinese populations. Acta Pharmacol Sin 21: 193–199.

Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA (2009) Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA 302: 849–857.

Xie HG (1997) Direct evidence for the higher frequency of CYP2C19 allelic heterozygotes in Chinese subjects than in white subjects. Clin Pharmacol Ther 62: 691–692.

Xie HG, Zou JJ, Hu ZY, Zhang JJ, Ye F, Chen SL (2011) Individual variability in the disposition of and response to clopidogrel: pharmacogenomics and beyond. Pharmacol Ther 129: 267–289.

Xie HG, Kim RB, Wood AJ, Stein CM (2001) Molecular basis of ethnic differences in drug disposition and response. Annu Rev Pharmacol Toxicol 41: 815–850.

Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK (2001) Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 345: 494–502.

Zhou HH, Liu ZQ (2000) Ethnic differences in drug metabolism. Clin Chem Lab Med 38: 899–903.

Zou JJ, Fan HW, Guo DQ, Li YB, Lin S, Zhu YB, Yu CY, Zhou J, Liu JH, Hu YF (2009) Simultaneous determination of clopidogrel and its carboxylic acid metabolite (SR26334) in human plasma by LC-ESI-MS-MS: application to the therapeutic drug monitoring of clopidogrel. Chromatographia 70: 1581–1586.