

Bioequivalence Study of Clopidogrel 75 Mg Tablets in Healthy Male Volunteers

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Abstract

The marked inter-individual and ethnic variabilities in the metabolism of clopidogrel has been investigated. Although the pharmacokinetics (PK) of clopidogrel has been reported previously in Whites and Korean volunteers, the PK characteristics may not be fully extrapolated to the Chinese population. Little is known about the PK characteristics and relative bioavailability of clopidogrel in Chinese population. The present study was to assess the PK characteristics and relative bioavailability of clopidogrel in healthy Chinese volunteers. A single-dose, randomized-sequence, open-label, 2-period crossover study was performed in fasting healthy Chinese male volunteers. Eligible subjects were randomly assigned to receive a single 75-mg dose of the test or reference formulation of clopidogrel, followed by a 1-week washout period and administration of the alternate formulation. The plasma samples were collected and at 0 min (baseline), as well as at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 11, 14, 24 and 36 hours, respectively, after drug administration. The concentrations of both clopidogrel and SR26334 were detected by a validated liquid chromatography-tandem mass spectrometric method (LC-MS/MS). The formulations were considered to be bioequivalent if the 90% CIs for the log-transformed values were within the predetermined equivalence range (80%–125% for AUC and C_{max}). For clopidogrel, the 90% CIs for the log-transformed ratios of C_{max} and AUC_{0-t} were 90.26%–113.91% and 91.82%–103.27%, respectively. For SR26334, the 90% CIs were 85.23%–112.97% and 93.11%–103.67%, respectively. In conclusion, the present results show that the formulation of clopidogrel was of bioequivalence to the reference, which have been tested in fasting, healthy, male Chinese volunteers.

Keywords: Bioavailability; Bioequivalence; Pharmacokinetics; Clopidogrel

Introduction

Clopidogrel, a thienopyridine inhibitor of the platelet P2Y₁₂ adenosine diphosphate (ADP) receptor, has been widely used for the prevention of stent thrombosis in patients with acute coronary syndrome [1]. To exert an antiplatelet effect, clopidogrel requires conversion to an active thiol metabolite (15% of total metabolites) via the cytochrome P450 pathway (especially *CYP2C19*2*) [1,2]. But the active thiol metabolite of clopidogrel is labile and remains undetectable in plasma [3]. SR26334, the inactive metabolite of clopidogrel, is a stable and a major circulating metabolite (85% of total metabolites) [4]. The information on the absorption and disposition of clopidogrel after oral administration can be derived from the pharmacokinetics (PK) of both clopidogrel and SR26334 [5,6].

The marked inter-individual and ethnic variability's in the metabolism of some drugs has been investigated [7,8]. Several studies have shown that ethnicity is one of the important factors that could affect clopidogrel metabolism and its anti-platelet effect [9,10], in part because the allele frequency of *CYP2C19*2* variant in the Chinese population (30%) is twice than that in whites (14.7%) and blacks (17.3%) [7,9,11]. Although the PK characteristics of clopidogrel have been studied previously [12-15], all these studies were performed in whites, blacks or Korean volunteers. These PK characteristics may not be fully generalized or extrapolated to the Chinese people due to significantly greater frequencies of the *CYP2C19*2* variant alleles in Chinese subjects than white or black subjects [7,9,11]. However, the search of PubMed and Embase in November 30, 2011, using the terms such as *clopidogrel*, *bioavailability*, *pharmacokinetic* and *Chinese*, did not identify any published reports concerning the PK and bioavailability of clopidogrel in the Chinese population.

Recently, an oral tablet 25 mg formulation of clopidogrel was developed in China. Information regarding its PK and relative bioavailability was required to assess the efficacy before marketing it in China. Therefore, the aims of this study were to evaluate PK characteristics and the bioequivalence of the test formulation (Tian Long Pharmaceuticals Co., Ltd., Changsha, China) and the reference formulation (Plavix®, Hangzhou Sanofi-aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China) in healthy Chinese population, in order to determine whether any observed differences and exceeded regulatory guidelines for bioequivalence before the presence of the drug in Chinese market.

Subjects and Methods

Inclusion and exclusion criteria

Healthy male subjects were eligible based on the following criteria: aged 18 to 40 years; body mass index between 20 and 24. Additional inclusion criteria were nonsmoking status and normal findings on the clinical history. None of volunteers had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or any acute or

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chronic disease, or an allergy to any drugs. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications, chest radiography, electrocardiography, and laboratory tests (hematology, blood biochemistry, hepatic function, and urinalysis). All eligible subjects provided written informed consent prior to study initiation.

Study design and procedures

This study was a randomized sequence, open-label, 2×2 crossover design. The protocol was approved by the ethics and research committee of Wuhan Union Hospital, and the study was performed in accordance with the principles of the Declaration of Helsinki. Subjects arrived at the hospital the day before the study. A table of random numbers was used to assign subjects in a 1:1 ratio to receive a single 75 mg dose (administered with 250 mL of water) of the test or reference formulation of clopidogrel. Subjects did not eat anything and drank water only for 12 hours before the administration. After a one-week washout period, followed by administration of the initial formulation, the alternative formulation was administered.

Blood sampling

After 12 hours (overnight) fast and before administration of the drug, the blood was drawn for baseline (0 min) measurements. Additional samples were drawn and then put into the vacuum tubes with heparin sodium as an anticoagulant at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 11, 14, 24, and 36 hours, respectively, after the administration. The tubes were centrifuged at 4000rpm for 10 minutes. The separated plasma was stored at -70°C until analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [4].

Quantitative analyses of both Clopidogrel and SR26334 in plasma samples

The concentrations of clopidogrel and SR26334 were determined in our laboratory using a validated LC-MS/MS method [4]. The LC-MS/MS equipment was consisted of a Surveyor LC pump, a Surveyor auto-sampler, a Finnigan TSQ Discovery Max mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) equipped with electro spray ionization (ESI) source. In brief, 0.50 mL aliquot plasma samples were extracted, respectively, with 4 mL of diethyl ether-*N*-hexane (4:1, v/v). An isocratic HPLC method was performed on a Teknokroma C_{18} column (100 mm \times 2.1 mm i.d., 5 μm) with the mobile phase system consisting of methanol-water (0.1% formic acid) (80:20, v/v) at a flow rate of 0.20 mL/min. The column temperature was maintained at 35. The ion spray voltage and capillary temperature were set at 4000V and 320, respectively. The nitrogen sheath, ion sweep and auxiliary gasses were set at 10, 2 and 5 units, respectively. The transitions m/z 322.1 \rightarrow 212.1 for clopidogrel and m/z 308.1 \rightarrow 198.1 for SR26334 were monitored using multiple reactions monitoring (MRM) mode. The collision energies values of Clopidogrel and SR26334 were 10 and 15 eV, respectively.

Pharmacokinetic and statistical analyses

Pharmacokinetic parameters were calculated using DAS version 3.0 (ShangHai Traditional Chinese Medicine University, ShangHai, China). C_{max} and T_{max} were determined directly from the respectively observed plasma concentration-time data. AUC was calculated with use of the trapezoidal rule Using a power analysis (expected value, $\geq 1 - \beta = 0.8$), it was determined that the power of the analysis of variance (ANOVA) was >0.8 at a 90% CI according to the guidelines on bioequivalence testing indicating that 20 subjects would be sufficient for the purposes

of the study. To test the bioequivalence of the formulations, ANOVA for a 2×2 crossover design was performed on log-transformed C_{max} , AUC_{0-t} and $\text{AUC}_{0-\infty}$. The ratios of the log-transformed C_{max} , AUC_{0-t} and $\text{AUC}_{0-\infty}$ were obtained for both formulations, and ANOVA was performed using the F score. The probability of exceeding the limits of acceptance for bioequivalence (80%-125%) was obtained using two 1-sided *t* tests, as described by Schuirmann [16]. If the 90% CI of the test: reference ratio for AUC_{0-t} , $\text{AUC}_{0-\infty}$ and C_{max} were located within the 80% to 125% of the statistical interval. The two formulations would be considered to be bioequivalent.

Tolerability

Tolerability was assessed by monitoring vital signs (blood pressure and heart rate) at baseline (0 min) and at 1, 4 and 12 hours after the administration. Blood pressure and heart rate was measured using Omron intellectual electronic sphygmomanometer (Omron Company Ltd., Osaka, Japan). Laboratory tests (hematology, blood biochemistry, hepatic function, and urinalysis) were performed at baseline and after completion of the study. Subjects were interviewed during the study by doctor-in-charge concerning the occurrence of adverse events (AEs) such as abdominal pain, indigestion, constipation or diarrhea, rash, skin and mucous membrane bleeding, neutropenia and agranulocytosis after the start of the study was recorded in the case report form (CRF) regardless of the suspected relationship to the study drug.

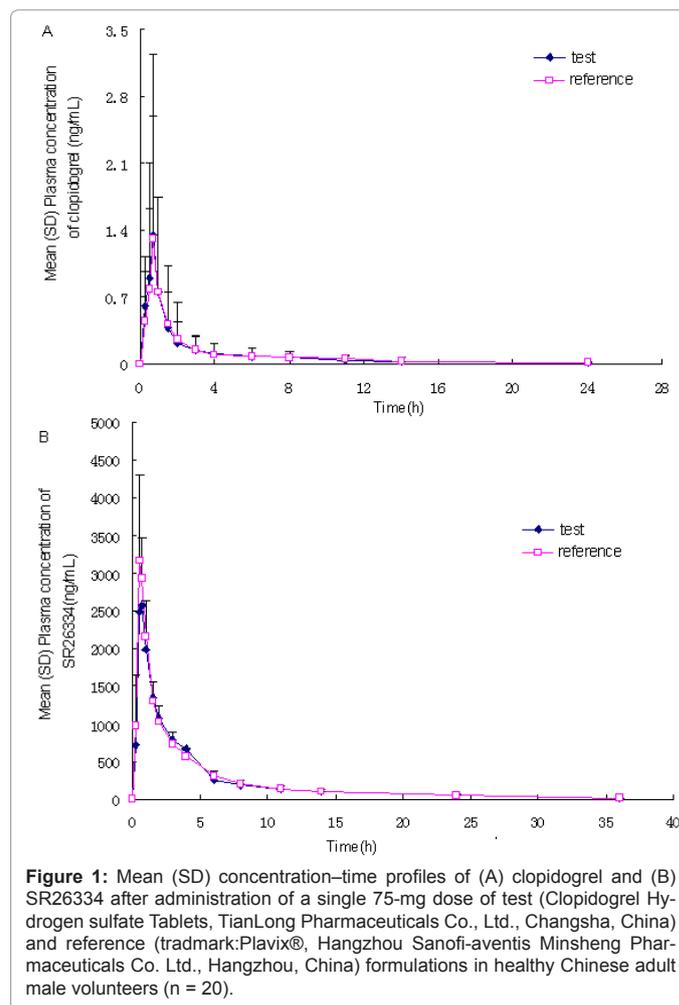


Figure 1: Mean (SD) concentration-time profiles of (A) clopidogrel and (B) SR26334 after administration of a single 75-mg dose of test (Clopidogrel Hydrogen sulfate Tablets, TianLong Pharmaceuticals Co., Ltd., Changsha, China) and reference (trademark: Plavix®, Hangzhou Sanofi-aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China) formulations in healthy Chinese adult male volunteers (n = 20).

clopidogrel			SR26334	
Parameter	Test	Reference	Test	Reference
C_{max} , ng/mL	1.86(1.52)	1.97 (1.92)	2993 (962)	3328(807)
T_{max} , h	0.6 (0.3)	0.7 (0.3)	0.68(0.20)	0.60(0.13)
$t_{1/2}$, h	6.9 (6.5)	6.5 (6.0)	8.3(1.4)	7.7(1.2)
AUC_{0-t} , ng·h/mL	2.37 (1.66)	2.36 (1.54)	8108(1861)	8475(1493)
$AUC_{0-\infty}$, ng·h/mL	2.48 (1.79)	2.43 (1.62)	8269(1690)	8624(1622)

Table 1: Pharmacokinetic properties of clopidogrel and SR26334 after a single 75-mg oral dose of 2 formulations of clopidogrel tablets in healthy Chinese male volunteers (n = 20). Data are mean (SD).

Results

LC/MS/MS method for quantitation

The linear range of clopidogrel and SR26334 were 0.005 to 5 ng/mL and 20 to 2500 ng/mL, respectively. For clopidogrel, the accuracy (the degree of closeness of the determined value to the nominal or known true value under prescribed conditions) of the range from 0.01 to 2 ng/mL was between 96.7% and 110.0%, and the inter-analysis and intra-analysis precision of the method was <9.2%. For SR26334, the accuracy of the range from 50 to 1200 ng/mL was between 104.1% and 108.7%, and the inter-analysis and intra-analysis precision of the method was <8.2%.

Pharmacokinetic properties

Twenty healthy male subjects (mean [range] age, 24.26 [22–29] years; mean [range] weight, 64.1 [55–71] kg; mean [range] height, 171 [163–185] cm) were enrolled in the study. The mean (SD) concentration-time profiles of clopidogrel and SR26334 after administration of the two formulations are shown in Figure 1. The PK properties of the two formulations are summarized in Table 1. On ANOVA, no period or sequence effects were observed for any PK property. The 90% CIs of the ratios (test: reference) for the log-transformed C_{max} (an index of the rate of absorption) and for AUC_{0-t} and $AUC_{0-\infty}$ (indexes of the extent of absorption), as well as the probability of exceeding the limits of acceptance for bioavailability and the power of the test are listed in Table 2 and meet the predetermined criterion for bioequivalence. Similar results were found for the data without log-transformation. For clopidogrel and SR26334, the relative bioavailability of test/reference formulation was 100.4% (mean AUC_{0-t}), 102.0% (mean $AUC_{0-\infty}$) and 95.6% (mean AUC_{0-t}), 95.8% (mean $AUC_{0-\infty}$), respectively.

Tolerability

No AEs were reported by subjects or found on analysis of vital signs or laboratory tests.

Discussion

To our knowledge, this is the first study to examine the PK properties and bioequivalence of two formulations of clopidogrel tablets in healthy Chinese male volunteers. We found that the 90% CIs of both clopidogrel and SR26334 were contained within the predefined bioequivalence criteria of 80% to 125% for the primary endpoint of AUC and C_{max} .

However, there are several reports in the literature regarding the PKs of clopidogrel and SR26334 in other ethnic groups, and the existing studies appear to differ [12–15]. For clopidogrel, Di et al. [12] reported that in a randomized-sequence, open-label, 2-period crossover study, clopidogrel 75mg administrated to 24 healthy, fasting, white volunteers of both sexes produced a geometric mean C_{max} of 877.76 pg/mL, AUC_{0-t} of 1911.53pg·h/mL. In a randomized, open-label, 2-period, single- and multiple-dose, comparative crossover study by Kim et al. [13], 24 healthy Korean male subjects received clopidogrel as a single 300-mg oral loading dose (day 1) followed by a 75-mg/d (once daily) maintenance dose on days 2 to 6. The mean values for C_{max} , T_{max} , and AUC_{0-t} with clopidogrel were 5.2 ng/mL, 0.9 hour, and 10.1 ng·h/mL, respectively. And the arithmetic values of SR26334 were as following: C_{max} , 10.9 µg/mL; AUC_{0-t} , 38.8 µg·h/mL; and $AUC_{0-\infty}$, 43.0 µg·h/mL. In another randomized, two-way, crossover, bioequivalence study of clopidogrel 75 mg tablets administrated to 32 fasting, healthy, male volunteers, El et al. [14] claimed that the arithmetic values of clopidogrel and SR26334, respectively were for C_{max} (4.39 ng/mL vs. 3.75µg/mL), AUC_{0-t} (11.98 ng·h/mL vs. 9.18µg·h/mL), $AUC_{0-\infty}$ (12.43 ng·h/mL vs. 9.72µg·h/mL), $t_{1/2}$ (6.06 h vs. 6.43h) and T_{max} (1 h vs. 0.75 h). But in a comparative, randomized, two-way cross-over study by Pawlowska et al. [15], 48 healthy male volunteers were administered a single dose of 150 mg (2 × 75 mg) of clopidogrel preparations under fasting condition with the following results: $AUC_{0-\infty}$: 1.96 ng·h/mL, AUC_{0-t} : 1.91 ng·h/mL, C_{max} : 1.44 ng/mL. The mean $AUC_{0-\infty}$, AUC_{0-t} , and C_{max} of clopidogrel were 0.98 ng·h/mL, 0.91 ng·h/mL and 0.72 ng/mL, respectively, as normalized to 75-mg dose, which were numerically lower than those in the above study.

In the present study, the C_{max} of clopidogrel and SR26334 were 1.86 ng/ml and 2993 ng/ml with the test formulation, respectively. For AUC_{0-t} , the corresponding mean values were 2.37 ng·h/mL and 8108 ng·h/mL, respectively, which appeared different from those previously

P for Exceeding the Limits of Acceptance for Bioavailability						
Compound	Parameter	Ratio Test/Reference, %	90% CI	<80%	>125%	Power
clopidogrel	C_{max}	94.4	90.26–113.91	0.001	<0.001	0.99
	AUC_{0-t}	100.4	91.82–103.27	<0.001	<0.001	0.99
	$AUC_{0-\infty}$	102.0	93.67–106.89	<0.001	<0.001	0.99
SR26334	C_{max}	90.1	85.23–112.97	0.002	<0.001	0.99
	AUC_{0-t}	95.6	93.11–103.67	<0.001	<0.001	0.99
	$AUC_{0-\infty}$	95.8	88.67–101.53	<0.001	<0.001	0.99

Table 2: Comparison of 90% CIs of natural log (ln)-transformed parameters for clopidogrel and SR26334 following administration of 2 formulations (Test/Reference) of clopidogrel tablets in healthy Chinese male volunteers.

reported. As is now widely accepted that there is marked inter individual variability in the metabolism of clopidogrel. The difference may be related to CYP2C19*2 genotype, which affects the metabolism of clopidogrel [17]. Significant variability in the frequencies of CYP2C19 allelic variants has been found among ethnic groups [7,9,11], which may explain ethnicity-specific responses to drugs.

As with any clinical trial, the current study had several limitations that should be considered. This was an open-label study, so it might not address objectively the efficacy profiles of the formulations tested. Because the data were only obtained from healthy male subjects who were administered a single dose, the PK characteristic of clopidogrel might differ in target populations. Therefore, the study results cannot be extrapolated to an older population or patients with a disease. The results of this study, might serve as a reference for future controlled studies of clopidogrel in the Chinese population. Also, the sample size was small; we are unable to predict the response of the drug at any time following alternative doses and/or administration interval because of the limited data. It did not examine steady-state PK of the two formulations and plasma concentration of the active metabolite of clopidogrel were not measured, because the active metabolite is chemically unstable and labile [3], we are limited in our ability to practically and quantitatively detect it in biological samples.

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