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Pharmacokinetics of clopidogrel in healthy Chinese volunteers

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There are marked ethnic variabilities in the metabolism of clopidogrel. The pharmacokinetic (PK) characteristics of clopidogrel have been studied previously in whites or Korean volunteers, but these PK characteristics may not be fully extrapolated to the Chinese people. Little is known about the PK characteristics of clopidogrel in Chinese population. The aim of this study was to evaluate the pharmacokinetic profiles of clopidogrel in 20 healthy Chinese volunteers after administration of a single dose of clopidogrel 75 mg. The peak plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration versus time curve from time 0 h to 36 h ($AUC_{0–36}$), elimination half-life ($t_{1/2}$), clearance rate (CL/F) and apparent volume of distribution (Vd) were (1.804 ± 1.706) ng/ml, (0.7 ± 0.3) h, (2.465 ± 1.693) ng·h/ml, (7.3 ± 7.0) h, (53.09 ± 34.65) × 10^3 L/h and (447.1 ± 440.8) × 10^3 L, respectively.

1. Introduction

Clopidogrel, an thienopyridine inhibitor of the platelet P2Y12 adenosine diphosphate (ADP) receptor, is widely used for the prevention of stent thrombosis in patients with acute coronary syndrome undergoing percutaneous coronary intervention (PCI) (Simon et al. 2009). To exert an antiplatelet effect, clopidogrel requires conversion to an active thiol metabolite via the cytochrome P450 pathway (especially by CYP2C19*2) (Simon et al. 2009; Tobias et al. 2011). Several studies have shown that ethnicity is one of the important factors that could affect clopidogrel metabolism and its anti-platelet effect (Xie et al. 2011; Li et al. 2009). The pharmacokinetic (PK) characteristics of clopidogrel have been studied previously in whites, blacks or Korean volunteers (Di et al. 2010; Kim et al. 2009; El et al. 2009; Pawlowska et al. 2009), but little is known about the PK characteristics of clopidogrel in Chinese population. Those PK characteristics may not be fully generalized or extrapolated to the Chinese people, 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. 2001, 2011; Xie 1997). Recently, the search of PubMed and Embase in September 28, 2011, using the terms such as *clopidogrel, pharmacokinetic* and *Chinese,* did not identify any published reports concerning the PK of clopidogrel in the Chinese population. Therefore, the aim of this study was to evaluate PK characteristics of clopidogrel in healthy Chinese population.

2. Investigations and results

2.1. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis for clopidogrel

The method was validated and found to be linear over the concentration range of 0.005 to 5 ng/ml ($r^2 = 0.9947$) for clopidogrel. The accuracy was between 96.7% and 110.0%, and the inter-analysis and intra-analysis precision of the method were < 9.2%. A stability study showed that clopidogrel and the internal standard were stable in plasma at room temperature for at least 4 hours, as well as for 3 weeks at −70 °C.

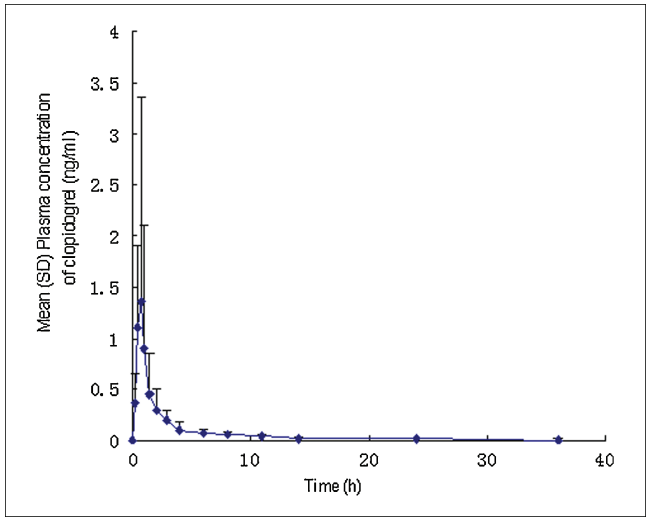

Fig.: Mean (SD) concentration–time profiles of clopidogrel after administration of a single 75-mg dose of clopidogrel in healthy Chinese male volunteers (n = 20)

2.2. Pharmacokinetics

The mean concentration-time profile of clopidogrel after administration of a single dose of clopidogrel 75 mg is shown in the Fig. The peak plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration versus time curve from time 0 h to 36 h ($AUC_{0–36}$), elimination half-life ($t_{1/2}$), clearance rate (CL/F) and apparent volume of distribution (Vd) were 1.804 ± 1.706 ng/ml, 0.7 ± 0.3 h, 2.465 ± 1.693 ng·h/ml, 7.3 ± 7.0 h, 53.09 ± 34.65 × 10^3 L/h and 447.1 ± 440.8 × 10^3 L, respectively.

3. Discussion

To our knowledge, this is the first study to examine the PK properties of clopidogrel in healthy Chinese volunteers. There are several reports in the literature regarding the PKs of clopidogrel in other ethnic groups, but the existing studies appear to differ. Di et al. (2010) reported that clopidogrel 75 mg administrated to 24 healthy, fasting, white volunteers of both sexes produced a geometric mean C_{max} of 877.76 pg/ml, $AUC_{0\text{-}t}$ of 1911.53 pg·h/ml. In a randomized, open-label study, 24 healthy Korean male subjects received clopidogrel as a single 300-mg oral loading dose, The mean values for C_{max}, T_{max}, and $AUC_{0\text{-}t}$ with clopidogrel were 5.2 ng/ml, 0.9 h, and 10.1 ng·h/ml, respectively (Kim et al. 2009). In another randomized study of clopidogrel 75 mg tablets administrated to 32 fasting, healthy, male volunteers, the arithmetic values of C_{max}, $AUC_{0\text{-}t}$, $t_{1/2}$ and T_{max} were 4.39 ng/ml, 11.98 ng·h/ml, 6.06 h and 1 h, respectively (El et al. 2009). But in a randomized, cross-over study (Pawlowska et al. 2009), 48 healthy male volunteers were administered a single dose of 150 mg (2×75 mg) of clopidogrel under fasting condition with the following results: C_{max}: 1.44 ng/ml; $AUC_{0\text{-}t}$: 1.91 ng·h/ml. As normalized to 75-mg dose, the mean C_{max} and $AUC_{0\text{-}t}$ of clopidogrel were 0.72 ng/ml and 0.95 ng·h/ml, respectively, which were numerically lower than those in the above study.

In the present study, C_{max} and $AUC_{0\text{–}36}$ of clopidogrel were 1.804 ng/ml and 2.465 ng·h/ml, respectively, which appeared different from those previously reported. The difference may be related to the CYP2C19*2 genotype. Significant variability in the frequencies of CYP2C19 allelic variants has been found among ethnic groups (Xie et al. 2001, 2011; Xie 1997), which may affect the metabolism of clopidogrel (Kim et al. 2008) and explain ethnicity-specific responses.

The current study had several limitations that should be considered. First, because the data were only obtained from healthy young subjects who received a single dose of clopidogrel, 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. Second, the steady-state PK of clopidogrel were not examined. Third, plasma concentrations of the active metabolite of clopidogrel were not measured, because the active metabolite is chemically unstable and labile (Pereillo et al. 2002), thus being hard to practically and quantitatively detect in biological samples.

4. Experimental

4.1. Materials

Clopidogrel standard reference (lot no.: 20061103; purity: 99.7%) was purchased from Beijing Nordhuns Chemical Technology Co. (Beijing, China); The internal standard (I.S., nateglinide, lot no. 100286–200701; purity: 99.8%) was purchased from Jiangsu Deyuan Pharmaceuticals (Lianyungang, China); Clopidogrel tablets (Plavix®, containing 75 mg clopidogrel, lot no.: 0903052) were kindly provided by Hangzhou Sanofi-aventis Minsheng Pharmaceuticals Co. Ltd. (Hangzhou, China). Reagent: methanol for HPLC, Tedia Company; Formic acid, diethyl ether and *n*-hexane for AR, Nanjing Chemical Reagent Co., Ltd.; Water was deionized and purified using a Milli-Q system (Millipore,Bedford, MA, USA). Instrument: 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 an electrospray ionization (ESI) source.

4.2. Drug administration and sample collection

The study protocol was approved by the ethics committee of the Xijing Hospital of the Fourth Military Medical University and performed in accordance with the principles of the Declaration of Helsinki. Male subjects were eligible based on the following criteria: aged 18 to 40 years; body mass index between 20 and 24. They were selected after passing a clinical screening procedure including a physical examination and laboratory tests, which included hematology, blood biochemistry, and urine analysis. No volunteers had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or an allergy to any drugs. All eligible subjects provided written informed consent prior to study initiation. Volunteers were hospitalized at 9:00 p.m. 1 day before this study and fasted 10 h before drug administration. At 8:00 a.m., volunteers were administered a single dose of clopidogrel 75 mg with 250 ml water. During the 24 h period after drug administration, no strenuous physical or mental activity was permitted. No other food was permitted during the 'in-house' period but liquid consumption was allowed *ad libitum* after lunch (with the exception of alcohol, soda, and coffee drinks, as well as juices). 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 after dosing. The blood samples were centrifuged at 4000 r for 10 min, and plasma samples were separated and stored at −70 °C until required for analysis.

4.3. Determination of plasma clopidogrel concentrations

The concentrations of clopidogrel were determined in our laboratory using a validated LC-MS/MS method (Zou et al. 2009). In brief, A 0.50 ml aliquot plasma sample were extracted with 4 ml of diethyl ether-*n*-hexane (4:1, v/v). LC separation was performed on a Teknokroma Nucleosil® C_{18} column (ID 2.1 mm × 100 mm, dp 5 μm) with a mobile phase of methanol-water (containing 0.1% formic acid) (80: 20, v/v) at a flow rate of 0.20 ml/min. The column temperature was maintained at 35 °C. The ion spray voltage and capillary temperature were set at 4000 V and 320 °C, respectively. The nitrogen sheath, ion sweep and auxiliary gasses were set at 10, 2 and 5 psi, respectively. The transitions *m/z* 322.1→212.1 for clopidogrel and *m/z* 318.3→166.2 for I.S. were monitored using multiple reaction monitoring (MRM) mode. The collision energies values of clopidogrel and I.S. were 10 and 20 V, respectively.

4.4. Data processing of plasma pharmacokinetics

Pharmacokinetic parameters for clopidogrel in plasma were calculated using DAS software version 3.0 (ShangHai traditional Chinese medicine University, ShangHai, China). C_{max} and T_{max} were obtained directly from visual inspection of the curves. $AUC_{0\text{-}t}$ was calculated using the trapezoidal rule. Other parameters such as $t_{1/2}$, CL/F and Vd were obtained directly from the results of DAS 3.0 software.

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