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Effects of Danshen capsules on the pharmacokinetics and pharmacodynamics of clopidogrel in healthy volunteers

Chun-hua Zhou[a,b], Meng Xu[c], Hai-bing Yu[d], Xiao-Ting Zheng[d], Zhang-Feng Zhong[d], Lan-tong Zhang[a,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Hebei Medical University, Shijiazhuang, Hebei province, China
[b] Department of Pharmacy, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei province, China
[c] Department of Pharmacy, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei province, China
[d] School of Public Health, Guangdong Medical University, Dongguan, Guangdong province, China

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ABSTRACT

Nowadays, the Herb-drug combination is becoming increasingly popular in China. However, the possible interaction induced by their combination was examined rarely. The aim of this study was to investigate the effect of multi-dose administration of Danshen capsules on clopidogrel pharmacokinetics and pharmacodynamics in healthy volunteers. A sequential, open-label, and two-period pharmacokinetic drug interaction study was designed to compare clopidogrel pharmacokinetic parameters before and after 7 days of administration of Danshen capsules in twenty healthy male volunteers. Co-administration of multiple doses of Danshen capsules caused increases in apparent oral clearance of clopidogrel and its metabolite by 96.5% and 73.7% and apparent volume of distribution by 94.2% and 75.1%, corresponding declines in C_{max} by 41.7% and 32.9%, AUC_{0-t} by 50.3% and 41.8%, and $AUC_{0-\infty}$ by 49.3% and 41.5% in human volunteers, respectively. Corresponding pharmacokinetic findings, co-administration of Danshen capsules with clopidogrel decreased the antiplatelet activity compared with individual agents. The results suggested that multiple dose administration of Danshen capsules could induce cytochrome P450 (CYP) isoenzymes, thereby increasing the clearance of clopidogrel. Therefore, caution should be taken when Danshen products containing lipophilic components are used in combination with therapeutic drugs metabolized by CYP3A4.

1. Introduction

Herbal medicines have received great attention as alternative medicines in recent years and are also referred to as a dietary supplement or health food. With the increasing uses of herbal medicines, the combination with therapeutic drugs is becoming more and more common, causing clinically important herb-drug interactions and adverse outcomes. However, up to now the potential herb-drug interactions are not always fully understood. Thus, it is very necessary to investigate the potential herb-drug interactions when combined administration so as to ensure the rational and safe use of drugs.

Clopidogrel bisulfate, an inactive thienopyridine prodrug, is 85% hydrolyzed *in vivo* by esterases to an inactive carboxylic acid derivative (Lins et al., 1999). The remaining drug undergoes oxidative biotransformation to its active thiol metabolite by a 2-step, CYP-dependent process in which CYP3A4 and CYP2C19 were involved (Kazui et al., 2010). It was reported that several drugs such as atorvastatin and omeprazole, due to their common requirements for CYP3A4 or CYP2C19 metabolism, had the competitive inhibition to the metabolism of clopidogrel, decreasing the conversion of the clopidogrel prodrug to the active metabolite and increasing the risk for cardiovascular events because of inadequate platelet P2Y12 receptor inhibition (Lau et al., 2003; Gilard et al., 2006). In the other hand, St. John's wort (SJW), a CYP2C19 and CYP3A4 inducer, enhances the CYP metabolic activity and antiplatelet effect of clopidogrel in hyporesponders (Lau et al., 2011).

Danshen (Salvia miltiorrhiza Bunge), a homology of medicine and food, is widely and traditionally used in the prevention and treatment of cardiovascular, cerebrovascular, and hepatic diseases in Asian countries especially in China (Zhou et al., 2005). Danshen is often co-administrated with other drugs in order to improve therapeutic efficacies, thus, the possible herb-drug interactions must be taken precaution to avoid severe damage in clinic. In fact, numbers of herb-drug interactions leading to adverse outcome were reported to involve Danshen when it was co-administered with therapeutic agents (Chen et al., 2017). For instance, Danshen gave rise to gross anticoagulation

* Corresponding author.
E-mail address: zhanglantong@263.net (L.-t. Zhang).

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and bleeding complications when it was combined with warfarin for anticoaglant treatment (Wu and Yeung, 2010; Liu et al., 2008). At present now, the most commercially available preparations from Danshen extract are primarily formulated with the ethanol extract, in which the diterpenoid tanshinones including cryptotanshinone, tanshinone IIA, and tanshinone I are the major components, accounting for approximately 95% of the total amount (Qiu et al., 2013). It was reported that tanshinones played significant roles in the inhibition and induction of several CYP450 isozymes (Zhou et al., 2012). Therefore, the possible of CYP-related herb-drug interactions should be paid attention to use Danshen preparations rich in tanshinones.

Currently, there is little knowledge about whether the Danshen capsules has a modulatory effect on the pharmacokinetic and pharmacodynamics of clopidogrel *in vivo*. In the present study, the effects of multi-dose administration of Danshen capsules made of ethanol extract on the pharmacokinetic behaviors as well as the anti-platelet aggregation activity were investigated in healthy volunteers, aiming to provide valuable information for the use of Danshen preparations in clinical practice.

2. Materials and methods

2.1. Materials and reagents

Clopidogrel Bisulfate Tablets were produced by Sanofi (Hangzhou) Phamaceutical Co., Ltd (China). Danshen capsules (DSC) were friendly provided by Hainan Selection Phamaceutical Co. Ltd (China). Cryptotanshinone and tanshinone IIA were purchased from Shanghai Shifeng Technology Co., Ltd. Clopidogrel hydrogen sulfate and internal standard (IS) ticlopidine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Racemic clopidogrel active metabolite (CAM) was purchased from the Toronto Research Chemicals Inc. (Toronto, Canada). The alkylating agent, 2-Bromo-3′-methoxyacetophenone (MPB), was obtained from J& K Chemicals (Beijing, China). Universal kit for sequencing reactions was provided by Beijing sino-era jiyin tech co.ltd, China. Platelet Aggregation Kit was purchased from Helena Laboratories, USA. Recombinant Human CYP3A4 (Creative BioMart, NY, USA) and human cryopreserved hepatocytes used in this study were provided by the Research Institute for Liver Disease Co. (Hebei, China). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA).

2.2. Subjects and ethical approval

Twenty male healthy Chinese volunteers (age range, 25–30 years; BMI range, 19–25 kg/m^2) were enrolled in the study after obtaining written informed consent. The clinical protocol and informed consent form were approved by the independent medical ethics committee of the Second Hospital of Hebei Medical University.Before entering the study, the health of all subjects was judged by a medical history, a physical examination, electrocardiogram, and laboratory tests (including complete blood count, blood biochemistry testing, and urinalysis). Besides, considering the effects of cytochrome P-450 polymorphisms on the metabolism of clopidogrel (Kim et al., 2008), the CYP2C19 genetic classification of the subjects was examined and only the subjects without carrying a genetic variant that diminished the pharmacokinetic and pharmacodynamic response to clopidogrel were enrolled. The subjects were required to be free from alcohol and medications for 2 weeks before and during the study period. Moreover, the caffeine-containing foods, orange juice, or other beverages were also need to be excluded during the study period.

2.3. Study design

A sequential, openlabel, and two-period trial was designed and conducted at the Second Hospital of Hebei Medical University. Each volunteer was orally given a single dose of 300 mg of clopidogrel followed by venous blood sampling (2 mL) at 0.00, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, and 24.0 h after drug administration for pharmacokinetic study. After a washout period of one week, all volunteers were orally taken Danshen capsules (4 capsules, tid) for 7 days. At the eighth day, each volunteer was co-administration of clopidogrel (300 mg) and Danshen capsules (4 capsules) and then 2 mL blood samples were collected into the tubes containing heparin sodium at different time points as above. Besides, blood samples were also collected into the tubes with trisodium citrate for pharmacodynamics study at 0, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h during each treatment period. The platelet aggregation of the samples was investigated within 4 h after obtaination.

For pharmacokinetic study, the above collected samples were immediately added 25 μL of MPB (500 mM) in acetonitrile to derivatize the active metabolite of clopidogrel. Next, the sample was gently mixed and centrifuged at 1500 g for 15 min at 4 °C. The separated plasma samples were frozen in polypropylene tubes at −80 °C until the time of analysis.

2.4. HPLC-DAD-MS/MS analysis

The powder of Danshen capsules (0.56 g) was extracted with 25 mL methanol in an ultrasonic bath for 15 min at room temperature. The extracted solution was prepared by the method of weight relief, by which the weight lost in the extraction procedure was compensated. After the centrifugation at 10,000 rpm for 10 min, the supernatant was diluted 4 times by methanol. After filtering through a 0.22 μm syringe filter, the sample was injected into HPLC-DAD-MS system.

Chromatographic separation was performed on a Symmetry C18 column (150 mm × 4.6 mm i.d., 5 μm) at 35 °C. The mobile phase consisted of eluent A (acetonitrile) and eluent B (water containing 0.1% formic acid). The optimized elution program consisted of the following steps: an isocratic elution of 30% A (0–3 min) and a linear gradient of 30–85% A (3–9 min). The peaks were recorded using PDA absorbance at 270 nm and the solvent flow rate was maintained at 1.0 mL/min. Mass spectrometry experiments were performed using an ESI source operated in both negative and positive ion modes. The main compounds were identified by comparing their ion chromatograms with those of references.

2.5. HPLC-MS/MS analysis

The samples were analyzed according to a previously validated method in our lab (Tian et al., 2016). Briefly, immediately after drawing, plasma samples (0.2 mL) were extracted with 0.6 mL of acetonitrile containing an internal standard (ticlopidine in a final concentration of 100.00 ng/mL) for protein precipitation. After centrifugation, the supernatant (10 μL) was injected onto the HPLC/MS/MS system. The separation was performed on a Diamonsil C18 column (4.6 mm × 150 mm, 5 μm) with a mobile phase of acetonitrile/1% formic acid (80/20) at a flow rate of 1.0 mL/min. All quantitative analyses were performed in ESI^+ MRM mode: the peak areas of m/z 322.2 → 212.0 for clopidogrel, m/z 504.3 → 354.1 for clopidogrel active metabolite and m/z 264.2 → 154.1 for ticlopidine (IS).

2.6. CYP3A4 activity

The incubation mixtures, which contained 0.5 mg of protein/mL of recombinant Human CYP3A4 (Creative BioMart, NY, USA), 5 ng/mL cryptotanshinone or tanshinone IIA, and 10 ng/mL clopidogrel, were reconstituted in 100 mM phosphate buffer (pH 7.4) and prewarmed for