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Potential accumulation of protopanaxadiol-type ginsenosides in six-months toxicokinetic study of SHENMAI injection in dogs

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ABSTRACT

SHENMAI injection (SMI), derived from famous Shen Mai San, is a herbal injection widely used in China. Ginsenosides are the major components of SMI. To monitor the exposure level of SMI during long-term treatment, a 6-month toxicokinetic experiment was performed. Twenty-four beagle dogs were dived into four groups (n = 6 in each group): a control group (0.9% NaCl solution) and three SMI groups (2, 6 or 3 mg/kg). The dogs were i.v. infused with vehicle or SMI daily for 180 d. Blood samples for analysis were collected at specific time points as follows: pre-dose (0 h); at 10, 30, and 60 min during infusion; and at 10, 30, 60, 90, 120, 240, and 300 min post-administration. Concentrations of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in the plasma were determined simultaneously by liquid chromatography–tandem mass spectrometry. Non-compartmental parameters were further calculated and analyzed. Significant differences were found between the kinetic behavior of 20(*S*)-protopanaxadiol-type (PPD-type) and 20(*S*)-protopanaxatriol-type (PPT-type) ginsenosides. Increasing in the exposure level of PPD-type ginsenosides was observed in dogs during the experiment. Therefore, PPD-type ginsenosides are closely related to the immunity modulation effect of SMI. Increased PPD-type ginsenoside exposure level may present potential toxicity and induce drug-drug interaction risks during SMI administration. As such, PPD-type ginsenoside accumulation must be carefully monitored in future SMI research.

1. Introduction

Shenmai injection (SMI), which derived from Sheng Mai San, is widely used for the treatment of atherosclerotic coronary heart disease and viral myocarditis (Li et al., 2011). Based on previous research, ginsenosides in *P. ginseng* and ophiopogonin and ophiopogonone in *O. japonicus* are the active components of SMI (Yu et al., 2007; Li et al., 2016; Zeng et al., 2013). Ginsenosides have been determined as the most important pharmacological ingredient in SMI (Yu et al., 2007). As of this writing, over 30 kinds of ginsenosides have been discovered in the injection, of which dammarane-type ginsenosides are the major constituents (Li et al., 2016). Dammarane-type ginsenosides may be classified according to their structures as 20(*S*)-protopanaxadiol-type (PPD-type) or 20(*S*)-protopanaxatriol-type (PPT-type) ginsenosides (Liu et al., 2009). PPD-type ginsenosides possess sugar moieties at the C-3 and/or C-20 positions, whereas PPT-type ginsenosides have a hydroxyl group at C-3 and sugar moieties at C-6 and/or C-20 (Liu et al., 2009).

According to our earlier findings, the abundance of different kind of ginsenosides was quite different from one another. The abundance of PPD-type ginsenosides was found to be much higher than those of PPT-type ginsenosides (Yu et al., 2013, 2014). Furthermore, despite the availability of quantitative data on the absorption, distribution, metabolism, and excretion of several ginsenosides in SMI, some ginsenosides still require further quantification in terms of exposure level during repeated administration of SMI (Yu et al., 2007; Xia et al., 2008). Additionally, it's recently discovered that potential accumulation of PPD-type ginsenosides and negative regulating of immunity are closely related during one-month toxicokinetic study of SMI in rats (Yu et al., 2014). The duration of the administration of SMI seems vital in the relationship. Therefore, in present research, in order to determine the

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toxicokinetic profile of SMI after long-term administration in dogs, the exposure levels of different ginsenosides, including PPD-type ginsenosides (Rb1, Rb2, Rc, and Rd) and PPT-type ginsenosides (Rg1, Rf, and Re) were simultaneously determined. To further analyze the behavior of different ginsenosides in SMI, a six-month toxicokinetic research with simultaneously determination of ginsenosides in SMI was performed in present study (Yu et al., 2014).

2. Materials and methods

2.1. Chemicals and reagents

Ginsenosides Rb1 (purity > 95.9%), Rg1 (purity > 93.4%), Re (purity > 92.7%), and digoxin (purity > 99.9%) were purchased from National Institute for Food and Drug Control (Beijing, China). Ginsenosides Rb2 (purity > 98%), Rc (purity > 98%), and Rf (purity > 98%) were purchased from Shanghai YuanYe Co., Ltd. (Shanghai, China). The structure of Rg1, Rf, Re, Rc, Rd, Rb2, and Rb1 are shown in Fig. 1. SMI (50 mL per bottle, Lot No: 0907223) was provided by Chiatai Qingchunbao Pharmaceutical Co., Ltd. (Hangzhou, China). The contents of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in 50 mL SMI were 84.9, 40.0, 30.9, 18.1, 26.1, 28.6, and 25.8 mg, respectively, as determined by electrospray ionization (ESI)–liquid chromatography–tandem mass spectrometry (LC–MS/MS). One milliliter of SMI contained 2 g of crude drugs (1 g of *P. ginseng* and 1 g of *O. japonicus*). Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Tedia Company Inc. (Fairfield, USA). *N*-butanol (analytical grade) was purchased from Huadong Medicine Co., Ltd. (Hangzhou, China), and deionized water was obtained from a Milli-Q ultrapure water purification system.

2.2. Equipment and chromatographic conditions

A Quantum Access LC–MS/MS system (Thermo Electron Co., San Jose, CA, USA) including an auto-sampler, a quaternary pump, and a triple tandem quadrupole mass detector was used to analyze the plasma samples. Separation was performed on a Finnigan Surveyor™ HPLC system. Separation of analytes was achieved by a Thermo BDS HYPERSIL C18 column (150 × 2.1 mm, 5 μm) at 30 °C. Acetonitrile (A)–water (B) was used as the mobile phase at a flow rate of 200 μL/min. A gradient elution program was adopted as follows: 0–13 min, 70%–65% B; 13–13.5 min, 65%–10% B; 13.5–15.5 min, 10% B; 15.5–16 min, 10%–70% B; and 16–20 min, 70% B.

The mass spectrometer used was a Finnigan TSQ Quantum Discovery Max system equipped with an ESI source and operated in ESI positive ion mode. Quantification was performed using selected reaction monitoring (SRM) mode with transitions of m/z 1131 → 365.02 for Rb1, 1101 → 334.92 for Rb2, 1101 → 334.88 for Rc, 969 → 789.49 for Rd, 969 → 789.80 for Re, 823 → 365.12 for Rf, 823 → 643.48 for Rg1, and 803 → 386.72 for digoxin (internal standard, I.S.). The optimized MS parameters were as follows: spray voltage, 3000 V; sheath gas pressure, 35.0; ion sweep gas pressure, 0.0; auxiliary gas pressure, 5; capillary temperature, 394 °C; tube lens offset, −107; collision pressure, 1.5 mTorr; collision energies, 44 V (digoxin, Rf, Re, and Rd), 37 V (Rg1), 57 V (Rb1), 56 V (Rc), and 53 V (Rb2).

2.3. Animals

Beagle dogs with body weights ranging from 5.0 kg to 7.0 kg were obtained from Zhejiang Jiaxing Institute of Experimental Animals (Jiaxing, China). Dogs were housed under temperatures ranging from 16 °C to 26 °C and humidity ranging from 40% to 70%. Dogs were fed with a commercial diet and tap water *ad libitum* throughout the experiment period. All animal experiments were performed in accordance with the guidelines for animal experiments of Zhejiang Academy of Medical Sciences. The experiments were approved by ethical committee.

2.4. Experiment protocol

The dogs (12 males and 12 females) were randomly and equally divided into four groups (n = 6 in each groups), as follows: a control group (0.9% NaCl solution), a low-dosage group (2 g/kg), a medium-dosage group (6 g/kg), and a high-dosage group (20 g/kg). Each group was i.v. infused daily with vehicle and SMI for 180 d (6 mo). All three dosage levels were much higher than the normal clinical dosage (0.17–0.8 g/kg) of SMI.

General observation was performed daily during this research. Blood samples for toxicokinetic analysis were collected 1, 90, and 180 d (for batches 1, 2, and 3, respectively) after the beginning of the experiment. Blood samples of 1.5 mL were collected at the following time points: pre-dose (0 h); at 10, 30, and 60 min during the infusion; and at 10, 30, 60, 90, 120, 240, and 300 min post-administration. Plasma was transferred into heparinized plastic tubes after collection and centrifuged at 12,000 rpm for 2 min. The obtained plasma samples were stored at −80 °C.

2.5. Preparation of standard solution and quality control samples

Rb1, Rb2, Rc, Rd, Re, Rg1, and Rf and digoxin (I.S.) were dissolved in methanol at concentrations of 1.01 mg/mL, 1.01 mg/mL, 1.07 mg/mL, 1.01 mg/mL, 1.06 mg/mL, 1.09 mg/mL, 1.00 mg/mL, and 1.04 mg/mL. Working solutions of these analytes were obtained by further dilution. Plasma Rb1, Rb2, Rc, Rd, Re, Rg1, and Rf concentrations

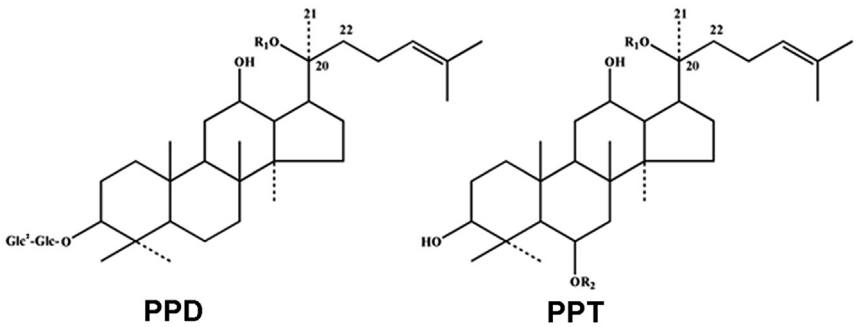

Fig. 1. Chemical structures of eight ginsenoside. (ginsenoside Rb1, R_1 = Glc^6-Glc; ginsenoside Rb2, R_1 = Glc^2-Arap; ginsenoside Rc, R_1 = Glc^6-Araf; ginsenoside Rd, R_1 = Glc; ginsenoside Re, R_1 = Glc, R_2 = O-Glc^2-Rha; ginsenoside Rf, R_1 = H, R_2 = O-Glc^2-Glc; ginsenoside Rg1, R_1 = Glc, R_2 = O-Glc; Glc = β-D-glucose; Arap = α-L-arabinose (pyranose); Araf = α-L-arabinose (furanose); Rha = α-L-rhannose).