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Herb–drug interaction between an anti-HIV Chinese herbal SH formula and atazanavir *in vitro* and *in vivo*

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ABSTRACT

Ethnopharmacological relevance: With the prevalent use of highly active antiretroviral therapy (HAART) for AIDS patients since 1996, the mortality of HIV/AIDS patients has been remarkably decreased. With long-term use of HAART, drug resistance and side effects of antiretrovirals have been frequently reported, which not only reduce the efficacy, but also decreases the tolerance of patients. Traditional herbal medicine has become more popular among HIV/AIDS patients as adjuvant therapy to reduce these adverse effects of HAART. SH formula is a Chinese herbal formula consisting of five traditional Chinese herbs including *Morus alba* L., *Glycyrrhiza glabra* L., *Artemisia capillaris* Thumb., *Astragalus membranaceus* Bge., and *Carthamus tinctorius* L. SH formula is clinically used for HIV treatment in Thailand. However, the possible pharmacokinetic interactions between these Chinese herbs and antiretroviral drugs have not been well documented. The aim of this study was to investigate the potential herb-drug interaction between SH herbal Chinese formula and the antiretroviral drug atazanavir (ATV).

Materials and methods: The combination effect of SH formula and ATV on HIV protease was studied in HIV-1 protease inhibition assay *in vitro*. The inhibition of SH formula on rat CYP3A2 was assessed by detecting the formation of 1′–OH midazolam from midazolam in rat liver microsomes *in vitro*. The *in vivo* pharmacokinetic interaction between SH formula and ATV was investigated by measuring time-dependent plasma concentrations of ATV in male Sprague–Dawley rats with liquid chromatography–mass spectrometry.

Results: Through the *in vitro* HIV-1 protease inhibition assay, combination of SH formula (41.7–166.7 μg/ml) and ATV (16.7–33.3 ng/ml) showed additive inhibition on HIV-1 protease activity than SH formula or ATV used alone. *In vitro* incubation assay indicated that SH formula showed a weak inhibition (IC_{50}=231.2 μg/ml; Ki=98.2 μg/ml) on CYP3A2 activity in rat liver microsomes. *In vivo* pharmacokinetic study demonstrated that SH formula did not affect the metabolism of ATV in rats.

Conclusions: Our study demonstrated for the first time that there is no metabolism-based herb-drug interaction between SH formula and ATV in rats, but this combination enhances the inhibition potentials against HIV protease activity. This observation may support the combinational use of anti-HIV treatment in human.

1. Introduction

With the expectation of reducing drug toxicity, alleviating side effects and minimizing anti-human immunodeficiency virus (HIV) drug resistance, people living with HIV/AIDS often choose complementary and alternative medicine (CAM) to complement highly active antiretroviral therapy (HAART) (Littlewood and Vanable,

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2011). However, improper use of CAM may lead to treatment failure or drug resistance due to pharmacodynamic and/or pharmacokinetic interactions (Liu et al., 2005). Atazanavir (ATV) is a potent azapeptide HIV protease inhibitor approved for the treatment of HIV infection by the U.S. Food and Drug Administration in 2003. ATV prevents viral replication by binding to HIV protease and blocking proteolytic cleavage of protein precursors that are necessary for the productions of infectious HIV viral particles (Fuster and Clotet, 2005). Because of its excellent oral bioavailability and pharmacokinetic parameters, ATV can be used for once-daily dosing which is convenient to patients (Fuster and Clotet, 2005). ATV is mainly metabolized by CYP3A4 in humans (Fuster and Clotet, 2005), and so it is often co-administrated with ritonavir, another HIV protease inhibitor with CYP3A4 inhibition activity, to extend its bioavailability (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2014).

After screening of more than 1000 Chinese medicinal herbs for anti-HIV activity, SH formula (also called "Si-Ai-Te-San" in Chinese) has been developed under the principles of traditional Chinese medicine (TCM) (Luo et al., 1995; Luo, 1998; Luo and Wang, 1999). SH underwent a clinical trial in 2013, and is now approved by the Ministry of Public Health of Thailand for clinical use. SH formula consists of five traditional Chinese herbs including *Glycyrrhiza glabra* L., *Artemisia capillaris* Thumb., *Morus alba* L., *Astragalus membranaceus* Bge., and *Carthamus tinctorius* L. (Kusum et al., 2004; Sangkitporn et al., 2005). *Morus alba*, a traditional anti-viral herbal medicine, serves as major component for the inhibition of HIV replication; while, *Astragalus membranaceus* modulates the host immunity to intensify the inhibition of HIV replication (Du et al., 2003; Liu, 2009; Chinese Pharmacopoeia Commission, 2010; Fu et al., 2014). *Artemisia capillaris* and *Carthamus tinctorius* play the roles as assistant drugs to enhance the effects of two major ingredients, and *Glycyrrhiza glabra* as a unique guide drug moderates the characteristics of other herbs (Liu, 2009; Wang et al. 2013). According to the manufacturer's information, SH formula inhibits HIV protease activity, and increases cell proliferation in natural killer cells (http://www.shidea.net.cn/NewsSH5.asp?id=102). Previous clinical research data demonstrated decreased HIV viral load in 14–35% of HIV-positive patients when SH formula was used alone; while combination treatment of SH formula and nucleoside reverse transcriptase inhibitors (e.g. zidovudine and zalcitabine) also showed a greater antiviral activity than antiretrovirals used alone (Kusum et al., 2004; Sangkitporn et al., 2005).

Due to the therapeutic efficacy of SH formula by itself in anti-HIV treatment, we decided to investigate the combinational uses of SH formula in combination with antiretrovirals. Understanding the herb–drug interactions between SH formula and antiretrovirals is indispensable for the safe use of SH formula in clinical practice. However, there is no report on it. Two components of SH formula, namely *Glycyrrhiza glabra* and *Astragalus membranaceus*, have inhibitory effects on CYP3A4 activity (Pandit et al., 2011; Pao et al., 2012). It is speculated that SH formula may affect the metabolism and pharmacokinetic profiles of CYP3A4-metabolizing drugs by decreasing CYP3A4 activity. Rat CYP3A2 is highly homologous to human CYP3A4, and possess similar enzymatic activity of CYP3A4. The aim of this study was to evaluate the herb–drug interaction potentials between SH formula and ATV through CYP3A2 inhibition assay *in vitro* and pharmacokinetic studies of ATV in rats *in vivo*. The combinational inhibition of SH formula and ATV on HIV protease was also investigated.

2. Materials and methods

2.1. Materials and chemicals

Sodium diethyl-dithiocarbamate, D-glucose 6-phosphate (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PD), β-nicotinamide adenine dinucleotide phosphate (NADP), HIV-1 protease substrate acetyl-Ser-Gln-Asn-Tyr-Pro-Val-Val amide, urethane, Tris–HCl and sodium carboxyl methyl cellulose (CMC-Na) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Midazolam (Dormicum® Injection 5 mg/ml) and diazepam were obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). 1′-hydroxymidazolam was purchased from Cayman Chemical Company (Michigan, USA). Indinavir (IDV) was purchased from Santa Cruz Biotechnology, Inc. (Texas, USA). Atazanavir (ATV) was supplied by Shanghai Biochempartner Co., Ltd. (Shanghai, China). Ketoconazole was purchased from Cascade Biochem Ltd. (Dublin, Ireland). Acetonitrile, methanol and ethyl acetate were purchased from Labscan Analytical Sciences (Bangkok, Thailand). SH formula powder was provided by Yunnan SH-IDEA Pharmaceutical Co. (Kunming, China).

2.2. Preparation and quality control of SH formula

The raw herbs of *Glycyrrhiza glabra*, *Artemisia capillaris*, *Morus alba*, *Astragalus membranaceus*, and *Carthamus tinctorius* were authenticated according to the Chinese Pharmacopoeia 2010. Their voucher specimens were kept in the Kunming Institute of Botany, Chinese Academy of Sciences, China. The production procedures of SH formula by Yunnan SH-IDEA Pharmaceutical Co. (Kunming, China) were performed according to the standard operating procedures. Briefly, *Morus alba, Carthamus tinctorius* and *Artemisia capillaris*. (15:1:1, w/w/w) were powdered, mixed and then extracted twice by refluxing with 80% ethanol for three hours. After concentrated, the crude extract was re-suspended in water, and extracted by petroleum and ethyl acetate twice, respectively. After concentrated again, the ethyl acetate fraction was obtained (Fr.1). The raw herbs of *Glycyrrhiza glabra* and *Carthamus tinctorius* were extracted by refluxing with 70% ethanol in the ratio of 9:2 (w/w), and then extracted by ethyl acetate. Subsequently, the water-soluble part was extracted by n-butanol as the n-butanol fraction (Fr.2). Finally, Fr.1 and Fr.2 were fully mixed and dried to produce the powder of SH formula.

Product quality was monitored by high performance liquid chromatography (HPLC) (Fig. 1). In brief, morusin, as the main anti-HIV component in *Morus alba*, was selected as the chemical marker for quality control. Morusin was prepared in methanol in series of concentrations, and 50 mg of SH formula (batch number: 200910302) was dissolved in methanol (10 ml). Samples were analyzed using the Agilent 1100 HPLC system with photodiode array detector (Agilent Corporation, Santa Clara, CA, USA). The separation was achieved by Agilent Zorbax SB-C18 column (250 mm × 4.6 mm i.d., 5 μm). The mobile phases consisted of 0.2% H_3PO_4 (A) and acetonitrile (B) in a gradient elution: 30–50% B, 0–10 min; 50–65% B, 10–20 min; 65–80% B, 20–30 min; and 80–100% B, 30–35 min. The flow rate was 1.0 ml/min and the column temperature was set at 27 °C. The content of morusin in the powder of SH formula was no less than 1.12 mg/g, which was calculated against the standard curve using its chemical standard.

2.3. In vitro combinational inhibition of SH formula and ATV on HIV-1 protease activity

2.3.1. Preparation of recombinant HIV-1 protease

The expression and purification of HIV-1 PR were performed according to the previous study (Miller et al., 1994). In brief, *Escherichia coli* transformed with plasmid pET-HIV-1 protease were incubated in LB (luria broth) medium, and isopropyl-β-D- thiogalactopyranoside (IPTG) was added when OD_{600} reached 0.6. Three hours later, *Escherichia coli* was collected and suspended in ice-cold buffer A [10 mM Tris (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, and 2 mM EDTA] for 20-min incubation in ice bath. After adding Nonidet P-40 (0.1%, v/v) and $MgCl_2$ (10 mM), *Escherichia coli* was ruptured with pulsed mode sonication. The homogenate was resuspended in ice-cold