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

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A B S T R A C T

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 increases 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 Thunb., 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 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 (IC50 = 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.

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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,
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 azapeptid 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-administered 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 a 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/NewSh5H.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, p-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-Glu-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 M. 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% H3PO4 (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 OD600 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 MgCl2 (10 mM), Escherichia coli was ruptured with pulsed mode sonication. The homogenate was resuspended
buffer A containing Triton X-100 (1%, v/v). After centrifugation, the pellet was re-dissolved in buffer B containing 8 M urea, 10 mM Tris (pH 7.5), and 10 mM dithiothreitol (DTT), and then centrifuged at 20,000 g for 1 h. HIV-1 protease in supernatant was enriched through a DEAE Sephacel column (GE Healthcare, USA), and dialyzed against a solution (pH 3.5) containing 1 mM DTT, 1% (v/v) glycerol, and 10 mM sodium acetate at 4 °C overnight. The solution was adjusted to pH 4.4, and further dialyzed against 10 mM sodium acetate (pH 3.5) at 4 °C overnight. Purified HIV PR solution was concentrated through ultrafiltration in an Amicon Centricon (M, 10,000 cutoff) and stored at 4 °C before use (Lin et al., 1994).

2.3.2. HIV-1 protease inhibition assay

The proteolytic activity of HIV-1 protease was measured by using the synthetic peptide substrate (acetyl-Ser-Gln-Asn-Tyr-Pro-Val-Val-amide) as described previously (Wan et al., 1996). In brief, the assay was performed in a total volume of 60 μl dodecyl acetate buffer (50 mM; pH 5.5) containing 10 μM substrate (10 μM), 15 μl HIV-1 protease (0.45 μg/ml) and 10 μl herbal extract or ATP or blank solution. The reaction was carried out for 2 h at 37 °C and terminated by adding TFA solution (5.5%, 10 μl). After centrifugation at 10,000g for 5 min, the supernatant was subjected to analysis by Agilent 6530 Accurate-Mass Q-TOF LC/MS equipped with Agilent ZORBAX RRHD Extend Plus C18 column (100 mm × 2.1 mm, 1.8 μm). The column temperature was maintained at 40 °C. The mobile phase consisted of (A) 0.1% formic acid and (B) acetonitrile at a flow rate of 0.35 ml/min with gradient elution: 5% A from 0 min to 0.25 min, 5–40% A from 0.25 to 5.5 min, and 40–95% A from 5.5 to 7 min. Mass spectrometry was performed in positive electrospray ionization (ESI) mode. The substrate of HIV-1 protease was detected at 847.43 m/z [M + H]+. The inhibition rate was calculated as follows:

\[
\text{Inhibition (\%)} = \left(1 - \frac{E}{P}\right) \times 100
\]

where \( E \) represents peak area of HIV-1 protease substrate in the presence of a tested sample, and \( P \) represents peak area of HIV-1 protease substrate only.

2.4. Enzymatic kinetic study for rat CYP3A2 inhibition

Rat liver microsomes (RLM) were prepared according to the previous report (Or et al., 2012). Inhibition of SH formula on CYP3A2 activity in RLM was determined by measuring the formation of 1'-hydroxymidazolam (1'-OH MDZ) from midazolam (MDZ), a probe substrate of rat CY3A2. In brief, reaction was performed in 250 μl phosphate buffer (50 mM, pH 7.4) with NADPH-regenerating system containing 5 mM MgCl2, 1 mM NADP, 10 mM G-6-P, and 2 units/ml G-6-PD. For IC50 assay, a fixed concentration (50 μM) was used to assess the inhibition potency of SH formula and ketoconazole (positive control) on rat CYP3A2; for enzymatic kinetic study, various concentrations of SH formula (0–500 μg/ml) and MDZ (25–200 μM) were used. Twenty-minute incubation was initiated by the addition of 1 mg/ml RLM, and stopped by addition of ice-cold acetonitrile (250 μl). Ten microliters of Diazepam (50 μg/ml) was added as the internal standard. The mixture was centrifuged, and supernatant was subjected to HPLC analysis. The formation of 1'-OH MDZ was analyzed by Waters Acquity UPLC system (Waters, MA, USA) equipped with an Acquity UPLC BEH C8 column (100 mm × 2.1 mm, 1.7 μm) protected by an Acquity UPLC BEH C8 VanGuard Pre-column (5 mm × 2.1 mm, 1.7 μm). The column was maintained at 50 °C. The mobile phase consisted of 65% (A) 10 mM KH2PO4 (pH 7.4) and 35%
(B) acetonitrile (ACN). The flow rate was set at 0.3 ml/min, and the injection volume was 2 μl. The analytes were monitored by a photodiode array detector (PDA) at the wavelength of 245 nm (Eeckhoudt et al., 1998). Each concentration of SH formula or MDZ was measured in quadruplicate. Ketoconazole served as positive control.

2.5. In vivo pharmacokinetic study in rats

2.5.1. Animals

Male Sprague–Dawley rats (280–310 g) were provided by the Laboratory Animal Services Centre, The Chinese University of Hong Kong (CUHK). Animals were kept with free access to food and water under a 12:12-h light-dark cycle. Before the pharmacokinetic study, animals were fasted overnight. Animals were under general anesthesia by urethane (20% in normal saline, w/v; 1.2 g/kg) during the procedures for blood sample collection. All experiments have been approved by the CUHK Animal Experimentation Ethics Committee (AEEC no. 13/001/MIS-5) according to the guidance of Department of Health, Hong Kong SAR.

2.5.2. Pharmacokinetic studies of ATV

Rats were randomly separated into three groups including vehicle control (1% CMC-Na solution) group, positive control (ketoconazole, 20 mg/kg, suspended in 1% CMC-Na) group and SH formula group. According to the body surface areas of human and rat, the oral dose of SH formula in rats was 500 mg/kg (suspended in 1% CMC-Na), which was equal to its clinical dose (83 mg/kg). Meanwhile, ATV was dissolved into the working concentration (3.5 mg/ml) with 50% propylene glycol in normal saline. Before intravenous administration of ATV, rats were orally administrated with vehicle solution, ketoconazole or SH formula. One-hour later, rats were anaesthetized by urethane, and ATV (7 mg/kg) was intravenously administrated. After intravenous administration, blood samples (0.3 ml) were collected from posterior orbital venous plexus into heparinized tubes at 0, 5, 10, 15, 30, and 45 min, and 1, 2, 4 and 6 h. Plasma samples were obtained from whole blood samples by centrifugation at 970 g for 10 min, and immediately frozen at −80 °C before analysis (Fukushima et al., 2009; Kobuchi et al., 2013).

2.5.3. Sample preparation

Sample preparation was performed according to previous method with some modifications (Fukushima et al., 2009; Kobuchi et al., 2013). Briefly, 20 μl of Indinavir (IDV, 200 μM in methanol) as the internal standard and 100 μl of ZnSO₄ solution (2% in 50% methanol–water, w/v) were added to 100 μl plasma sample, and vigorously mixed for 15 s. Distilled water (300 μl) was added, and vortexed for another 30 s, and then centrifuged at 10,778g for 5 min. The upper phase was loaded on a solid phase extraction (SPE) column which was preconditioned with methanol (1 ml) followed by distilled water (1 ml). Washing of samples was done by adding distilled water (1 ml). After being eluted by methanol (1 ml), all samples were dried under nitrogen stream at 40 °C. The residues were reconstituted with 100 μl mobile phase and then 10 μl aliquot was subjected to LC–MS analysis.

2.5.4. LC–MS analysis

Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS system was used for LC–MS analysis with an Agilent ZORBAX RRHD Extend Plus C₁₈ column (100 mm × 3 mm, 1.8 μm) maintained at 40 °C. The mobile phase consisted of (A) 0.1% formic acid and (B) acetonitrile at a flow rate of 0.35 ml/min with the following gradient: 5% B from 0 min to 0.25 min; 5-95% B from 0.25 to 5.5 min; 95% B from 5.5 min to 7 min; and 5% B from 7 min to 9 min. Mass spectrometry was performed utilizing dual electrospray ionization (ESI) in positive mode.

The electrospray voltage was +3.5 kV, and the flow rate of the nebulizing gas (N₂) was set at 9 L/min at 300 °C. ATV and IDV were detected at 705.39 and 614.37 m/z [M+H]⁺, respectively. The concentration of ATV in each plasma sample was quantified by calculating from its standard curve using the peak area ratio of ATV to IDV (Crommentuy et al., 2004). Each calibration curve was individually analyzed by using least square weighted (1/k²) linear regression. A typical equation of the calibration curve of ATV was Y = 10.792X + 0.0605 with good correlation coefficient (r > 0.9945). Its intra- and inter-batch precisions also met acceptable relative standard deviation (R.S.D. < 15%) and accuracy ranged from 85% to 115%.

2.6. Data analysis

IC₅₀ value was calculated by fitting non-linear regression analysis, and statistical analysis was performed by student t-test, one-way ANOVA or two-way ANOVA followed by Bonferroni’s Post-Hoc test where appropriate with GraphPad Prism 4.0 (GraphPad Software, USA). Graphical inspections from Primary Lineweaver–Burk plot, Dixon plot and Secondary Lineweaver–Burk plot were used to illustrate the inhibition types, Ki and αKi values (Feng et al., 2012; Shou et al., 2001). Pharmacokinetic parameters of ATV, including half-life (t½α and t½β), maximum drug concentration (Cmax), area under curve (AUC₀–ₗ and AUC₀–∞), clearance (CL), and mean retention time (MRT₀–ₗ and MRT₀–∞), were calculated using a two-compartmental model with DAS 2.0 pharmacokinetic software (Anhui Provincial Center for Drug Clinical Evaluation, Hefei, China). Combination effect of these SH formula and ATV was evaluated by the Combination Index (CI) method, and CI was calculated using Loewe additivity model as follows:

$$CI = \frac{d_1}{ED_{50}^{[1]} \cdot \frac{d_2}{ED_{50}^{[2]}}}$$

where d₁ and d₂ are the concentrations of the two test drugs added together at which 50% inhibition is achieved. ED₅₀⁽¹⁾ and ED₅₀⁽²⁾ represent the half maximal inhibitory concentration of test drugs in HIV-1 protease inhibition assay. CI < 1 = 1, and > 1 indicate synergism, additivity, and antagonism, respectively (Koizumi and Iwami, 2014).

3. Results

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

IC₅₀ values of SH formula and ATV on HIV-1 protease activity were 217.0 μg/ml and 38.6 ng/ml (Fig. 2a and b), respectively. Three concentrations of SH formula (41.7, 83.3, and 166.7 μg/ml) and ATV (16.7, 25, and 33.3 ng/ml) were chosen to evaluate the inhibition efficacy of various combinations. The results demonstrated that all combinations increased inhibition activity of ATV by 52–50.6% on HIV-1 protease activity when compared to the same concentration of ATV alone (Fig. 2c), and the combinations of ATV (16.7 and 33.3 ng/ml) with SH formula (41.7–166.7 μg/ml) showed stronger inhibition effects on HIV-1 protease activity when compared to the corresponding concentrations of SH formula and ATV (Fig. 2c), indicating possible synergistic or additive effects.

To further evaluate the combination effects of SH formula and ATV, their Loewe additivity was exploited to assess their combination index (CI) value. CI values (Table 1) of SH formula at concentration of 83.3 μg/ml and 166.7 μg/ml with ATV were 1.08 and 1.19, respectively, showing that combination of SH formula and ATV almost possess additive effects on HIV-1 protease inhibition (Chou, 2006).
3.2. Enzymatic kinetic study for rat CYP3A2 inhibition

SH formula concentration-dependently decreased the formation of 1-OH MDZ in RLM. As determined with 50 μM MDZ, IC₅₀ value of SH formula was 119.8 μg/ml (Fig. 3e). Since the straight line was intersected at the negative x-axis in the primary Lineweaver–Burk plot and Dixon plot, the inhibitory effect of SH formula on CYP3A2-mediated MDZ hydroxylation fits to a competitive inhibition mode (Fig. 3a and b). Calculated from the Secondary Lineweaver–Burk plots, Kᵢ and αKᵢ values of SH formula inhibition on rat CYP3A2 were 98.2 μg/ml and 104.7 μg/ml, respectively (Fig. 3c and d). As calculated from Kᵢ and αKᵢ values, the α value (α = 0.94) was approximately equal to 1 which further confirmed that the inhibition was competitive (Feng et al., 2012; Shou et al., 2000). The IC₅₀ value of the positive control ketoconazole on rat CYP3A2 was 11 μM (0.59 μg/ml) with MDZ (50 μM) as the substrate (Fig. 3f).

3.3. In vivo pharmacokinetic study

After intravenous administration with or without oral co-administration of SH formula (500 mg/kg), the concentration-time curves of ATV in plasma are shown in Fig. 3. The Cₘₐₓ (about 5 μg/ml) values of ATV in control group and SH formula group appeared at 5 min, and then the concentration of ATV in plasma dramatically decreased to 1.7 μg/ml from 5 min to 60 min. Subsequently, the concentration of ATV decreased slowly to 100 ng/ml from 120 min to 360 min, fitting into a two-compartmental pharmacokinetic model. The pharmacokinetic parameters of ATV were calculated by the DAS (Table 2), while the parameters in the control group were similar with previous studies (Fukushima et al., 2009; Kobuchi et al., 2013). The blood concentrations and pharmacokinetic parameters between control group and SH formula group were not significantly different, as analyzed by student t-test or two-way ANOVA. This means that oral administration of SH formula may not affect the metabolism of ATV. However, between the positive control group and control group, the blood concentrations (from 5 min to 120 min) and pharmacokinetic parameters showed significant differences (Fig. 3 and Table 2), indicating that ketoconazole, as a known CYP3A inhibitor, inhibited CYP3A2-mediated ATV metabolism and decreased its clearance in vivo.
4. Discussion

In TCM practice, herbs are always used in different combinations as compound formulae to treat different ailments. Therefore TCM formulas are thought to exhibit a wider range of actions in boosting immunity as compared to single-entity drugs, including increasing the number of white blood cells, boosting natural killer cell activity, and selectively stimulating cytokines, etc. (Zou et al., 2012). Chinese herbal formulae have been prevalently used by people suffering from HIV/AIDS with the expectation of reducing symptoms, improving quality of life, and alleviating side effects of current anti-HIV drugs (Zou et al., 2012). However, haphazard uses of CAM and prescribed drugs can potentially attenuate drug efficacy and/or enhance toxicity. This is particularly important for people living with HIV/AIDS who use HAART to maintain a prolonged suppression on HIV replication. Thus,

![Graph with data points and lines](image)

**Table 2**
Pharmacokinetic parameters of atazanavir (i.v., 7.0 mg/kg) in rats in different pre-treatment groups (n=6–8; Mean ± S.E.M.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>SH formula</th>
<th>Ketoconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2\alpha}$ (h)</td>
<td>0.18 ± 0.11</td>
<td>0.11 ± 0.03</td>
<td>0.37 ± 0.16</td>
</tr>
<tr>
<td>$t_{1/2\beta}$ (h)</td>
<td>0.7 ± 0.06</td>
<td>0.72 ± 0.06</td>
<td>0.92 ± 0.05*</td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>0.67 ± 0.26</td>
<td>0.86 ± 0.39</td>
<td>0.92 ± 0.12</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>1.42 ± 0.13</td>
<td>1.42 ± 0.08</td>
<td>0.79 ± 0.07***</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (mg/L h)</td>
<td>4.72 ± 0.41</td>
<td>4.57 ± 0.27</td>
<td>8.48 ± 0.63***</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (mg/L h)</td>
<td>5.19 ± 0.43</td>
<td>5.04 ± 0.29</td>
<td>9.17 ± 0.69***</td>
</tr>
<tr>
<td>MRT$_{0-\infty}$ (h)</td>
<td>0.88 ± 0.03</td>
<td>0.88 ± 0.02</td>
<td>1.04 ± 0.04**</td>
</tr>
<tr>
<td>MRT$_{0-\infty}$ (h)</td>
<td>0.89 ± 0.03</td>
<td>0.9 ± 0.03</td>
<td>1.07 ± 0.05**</td>
</tr>
<tr>
<td>IC$_{50}$ (µg/ml)</td>
<td>119.8 µg/ml</td>
<td>119.8 µg/ml</td>
<td>IC$_{50}$ = 119.8 µg/ml</td>
</tr>
<tr>
<td>IC$_{50}$ (µg/ml)</td>
<td>119.8 µg/ml</td>
<td>119.8 µg/ml</td>
<td>IC$_{50}$ = 119.8 µg/ml</td>
</tr>
</tbody>
</table>

* $p < 0.05$ indicate significant difference to control group.
** $p < 0.01$ indicate significant difference to control group.
*** $p < 0.001$ indicate significant difference to control group.
for drug safety reasons, it is necessary to evaluate the potential herb–
drug interaction between CAM and antiretrovirals.

The herbal components of SH formula include Astragalus membra-
naeus, traditionally used as tonic for the weakness and chronic illness,
may enhance host defense against HIV and Morus alba mainly effective
on the suppression of HIV-1 replication through inhibition of HIV-1
protease (Du et al., 2003; Fu et al., 2014). However, considering the
efficient effects achieved by antiretrovirals, SH formula in AIDS treat-
ment may be a useful adjuvant for current treatment with anti-HIV
drugs. In this study, the inhibition activity of SH formula (417.7–
1667.7 μg/ml) combined with ATV (167.3–333 ng/ml) on HIV-1 protease
activity was measured according to their respective EC50 values. All
combination treatments with SH formula and ATV showed stronger
inhibition on HIV-1 protease activity than either SH formula or ATV
used alone (Fig. 2c). The nearly additive results were achieved when
SH formula was combined with ATV using Loewe additivity model.
Comitant use of SH formula and ATV indicates possible additive
effects on HIV protease inhibition. Considering the advantages of SH
formula in other respects in AIDS treatment, beneficial effects may be
obtained by concomitantly using SH formula and ATV. Thus, the
pharmacokinetics-based herb–drug interaction potential between SH
formula and ATV was further evaluated for drug safety consideration.

Previous studies have revealed the pivotal roles of CYP3A in the
metabolism of ATV (Ruster and CLOTET, 2005), and the plasma
concentration of ATV is increased when it is co-administrated with
ritonavir clinically. In this study, the interaction between SH formula
and ATV was investigated in vitro and in vivo in the laboratory. An in
vivo inhibition assay in RLM demonstrated that SH formula possessed
very weak inhibition on CYP3A2 (IC50 = 2312 μg/ml) in competitive
inhibition mode, which suggested a weak herb–drug interaction
between SH formula and ATV. The in vitro potential effect of SH formula
on the metabolism of ATV was further explored in rats. After oral
administration of SH formula followed by intravenous administration
of ATV, the pharmacokinetic parameters of ATV were comparable with
those of control group (Table 2 and Fig. 3), suggesting that the effect of
SH formula (500 mg/kg) on the metabolism of ATV was almost
negligible in vivo. This was consistent with the weak inhibition of SH
formula in vitro. This finding indicated that concomitant administra-
tion of SH formula along with ATV did not lead to metabolism-based
herb–drug interactions in rats. Previous clinical studies have demonstrated that CD4+ T cells and
natural killer cells proliferation were enhanced when SH formula was
used in people with HIV/AIDS. Our findings demonstrated for the
first time that concomitant administration of SH formula and ATV
exhibited nearly additive effects on HIV-1 protease activity without
causing adverse pharmacokinetics-based herb–drug interactions. It is
therefore concluded that the co-administration of SH formula and
ATV may therefore benefit people with HIV/AIDS. A proper clinical
trial using the combination therapy should be considered in the
future to confirm these findings.

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