doi.org/10.36721/PJPS.2020.33.1.REG.049-052.1

Influence of a herbal preparation on the pharmacokinetics of highly active antiretroviral therapy drugs in rats

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Abstract: Shenlin Fuzheng Capsule (SLFZC) is a herbal preparation used for HIV/AIDS in Guangxi, China. This study was designed to evaluate the influence of SLFZC on the pharmacokinetics of highly active antiretroviral therapy (HAART) drugs, zidovudine (3'-azido-3'-deoythymidine, AZT), 2',3'-dideoxy-3'-thiacytidine (3TC) and efavirenz (EFV). Thirty-six male SD rats were divided into three groups. Group A was given a combination of AZT, 3TC and EFV (AZT/3TC/EFV). Group B rats were given AZT/3TC/EFV simultaneously with SLFZC. Group C rats were given AZT/3TC/EFV 2h prior to SLFZC. Blood samples were collected at fixed time intervals. Plasma concentration of each antiretroviral drug was tested for calculation of pharmacokinetic parameters. There was significant difference among groups with respect to $t_{1/2}$ for AZT (F=3.371, P<0.05), but the Student-Newman-Keuls (SNK) pairwise multiple comparison procedure showed no statistical differences in all pairwise comparisons (P>0.05). There were no significant differences among groups in terms of C_{max}, T_{max}, $AUC_{0\text{-}12h}$ and CL for AZT, and $t_{1/2}$, C_{max}, T_{max}, $AUC_{0\text{-}12h}$ and CL for 3TC and EFV, respectively. The results indicate that SLFZC has little impact on pharmacokinetic properties of AZT, 3TC and EFV.

Keywords: Pharmacokinetics, highly active antiretroviral therapy (HAART), herb, HIV, AIDS

INTRODUCTION

Although highly active antiretroviral therapy (HAART) has been successful in suppressing HIV replication and providing significant clinical benefits in HIV-infected patients, it cannot completely eradicate HIV from the body and may cause severe side effects. With the expectation of boosting immunity, treating symptoms, improving quality of life and alleviating side effects related to HAART, some patients seek complementary and alternative medicine (CAM) combined with HAART or taken alone (Liu, 2007; Chinsembu, 2016; Shedlin *et al.*, 2013). CAM is popular in these patients, even more than half of them reported use of CAM therapies currently or in the past (Duggan *et al*., 2001; Jernewall *et al*., 2005).

Traditional Chinese medicine (TCM) is a major stream of CAM. Since 2004, the State Administrative Bureau of Traditional Chinese Medicine has launched a National Free TCM HIV/AIDS Treatment Program for people with HIV infection and AIDS, which has financed nineteen provinces in China by 2015, including Guangxi Province. Shenlin Fuzheng Capsule (SLFZC) is the most commonly used herbal preparation in HIV/AIDS patients in Guangxi.

SLFZC is made by Ruikang Hospital affiliated to Guangxi University of Chinese Medicine, with permission of the local authorities to be used as complementary treatments for HIV/AIDS patients. As a hospital-made preparation, which refers to a drug made by a hospital rather than by a pharmaceutical company and prescribed only in this hospital, it doesn't need as highly rigorous assessments as those registered drugs always do before clinical use.

SLFZC contains rude extracts from Pilose Asia bell Root, Membranous Milkvetch Root, Large head Atractylodes Rhizome, *Gynostemma pentaphyllum*, Black Ants and *Lucid ganoderma*. Previous clinical researches showed that use of SLFZC combined with HAART can relieve some side effects related to HAART, such as fatigue and headache (Su *et al*., 2013). However, it's demonstrated that some herbs in conjunction with HAART may cause herb-drug interactions, leading to treatment failure or HIV drug resistance (Liu *et al*., 2005; Fasinu *et al*., 2015). Some clinicians have been expressing concerns that SLFZC may mitigate these side effects by lowering blood concentrations of HAART drugs. This study was designed to evaluate the influence of SLFZC on the pharmacokinetics of zidovudine (3'-azido-3'-deoythymidine, AZT), 2',3'-dideoxy-3'-thiacytidine (3TC) and efavirenz (EFV) that constitute a frequently used 3-drug HAART combination.

MATERIALS AND METHODS

Materials and reagents

AZT tablet was purchased from Northeast Pharmaceutical Group Co., Ltd (Shenyang, China). EFV tablet was purchased from Merck Sharp & Dohme (Australia) Pty.

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Ltd. (North Ryde, Australia). 3TC tablet was purchased from Baker Biological Pharmaceutical Co., Ltd. (Hefei, China). AZT standard (purity 99.7%), 3TC Standard (purity 99.7%) and internal standard Telmisartan (purity 99.3%) were purchased from National Institute for Food and Drug Control (Beijing, China). EFV standard (purity 98%) was purchased from Toronto Research Chemicals (Toronto, Canada). Methanol (HPLC grade), acetonitrile (analytical grade) and formic acid (analytical grade) were purchased from Merck Serono Co., Ltd., China (Beijing, China).

Animals

36 male Sprague Dawley rats, 4-7 weeks old and weighting 250-300g, were kept under temperature-controlled conditions (21°C) with a standard 12-h light/12-h dark cycle. Rats were forced to fast one hour before drug treatment. Food and water were continued after the administration of the drug. The study protocol was reviewed and approved by the Ethics Committee of Guangxi University of Chinese Medicine.

Analysis of pharmacokinetic parameters

The rats were randomly divided into three groups (A, B and C) of twelve animals each, which were given intragastrically HAART with or without SLFZC. Group A was given AZT/3TC/EFV. Group B was given AZT/3TC/EFV and SLFZC simultaneously. Group C was given AZT/3TC/EFV 2h prior to the administration of SLFZC. AZT, 3TC and EFV were administered once daily at a dose of 31.5, 31.5 and 63.0 mg/kg, respectively, and SLFZC at 155 mg/kg once daily. The doses for rats were converted from the human doses based on body surface area.

Blood sample of 0.4mL was collected via orbital sinus puncture in heparinized tubes before treatment and 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h after treatment. A mix containing 96μL of plasma, 4μL of internal standard solution and 800μL of methanol was centrifuged at 12000 rpm (12235 g) for 10 min. The supernatants were evaporated for drying by nitrogen at 37°C and dissolved in 80μL mobile phase solvents, and submitted to the Varian 320-MS high performance liquid chromatography coupled to electrospray ionization-triple quadruple tandem mass spectrometry (HPLC-ESI-MS/MS, Varian Technologies, USA) for measurement of drug concentrations.

The pharmacokinetic parameters, including the area under the plasma concentration-time curve from zero to 12 h ($AUC_{0\text{-}12h}$), plasma clearance (CL), elimination half-time (t1/2), maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were calculated using Drug and Statistics (DAS) version 2.0 pharmacokinetic program (Chinese Pharmacology Society, Beijing, China) based on non-compartmental analysis.

STATISTICAL ANALYSIS

Statistical analysis was carried out by SPSS 16.0. Pharmacokinetic parameters were compared among groups using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls (SNK) multiple comparison test where appropriate. *P* value less than 0.05 was considered significant.

RESULTS

AZT blood concentration promptly increased after administration, reaching a maximum between 5 and 10 min, and then decreased rapidly. 3TC and EFV reached maximum concentrations at approximately 20 min and decreased thereafter (Fig. 1).

There was significant difference among groups regarding $t_{1/2}$ for AZT ($P<0.05$), but no statistical significance was found in SNK multiple comparison ($P=0.061$). The one-way ANOVA showed that there were no significant differences among groups in terms of C_{max}, T_{max}, $AUC_{0\text{-}12h}$ and CL for AZT, and $t_{1/2}$, C_{max}, T_{max}, $AUC_{0\text{-}12h}$ and CL for 3TC and EFV, respectively (Table 1).

DISCUSSION

The combination of two nucleoside reverse transcriptase inhibitors (NRTI) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) is recommended as initial HAART regimen in China. The regimen 3TC/AZT/EFV has been considered as one of the most tolerated formulations with longest follow-up time (Sun *et al*., 2015). It has been reported that some herbs can have an impact on cytochrome P450 (CPY) enzymes, and thus increase the toxicity of HAART drugs or decrease their efficacy (Larson *et al*., 2014; Thomford *et al*., 2016). With the widely use of HAART combined with SLFZC in Guangxi, China, there is a growing concern that SLFZC may interact with HAART, leading to incomplete virological response and HIV drug resistance. In the present study, we used HPLC-ESI-MS/MS method by which we can synchronously evaluate the impact of SLFZC on the pharmacokinetics of AZT, 3TC and EFV. Considering that some HIV/AIDS patients take SLFZC 2h after HAART as advised by their doctors, and some take both simultaneously, we also observed the difference between these two modes of combined administration.

We found that the pharmacokinetic parameters of the three antiretroviral drugs did not differ among groups, except for the $t_{1/2}$ of AZT having a positive response in one-way ANOVA analysis ($P=0.047$). However, significance was not found in SNK multiple comparison procedure. These findings indicated that SLFZC has little impact on the overall pharmacokinetic parameters of AZT, 3TC and EFV whether SLFZC is administered simultaneously with HAART or 2h later, therefore, it does

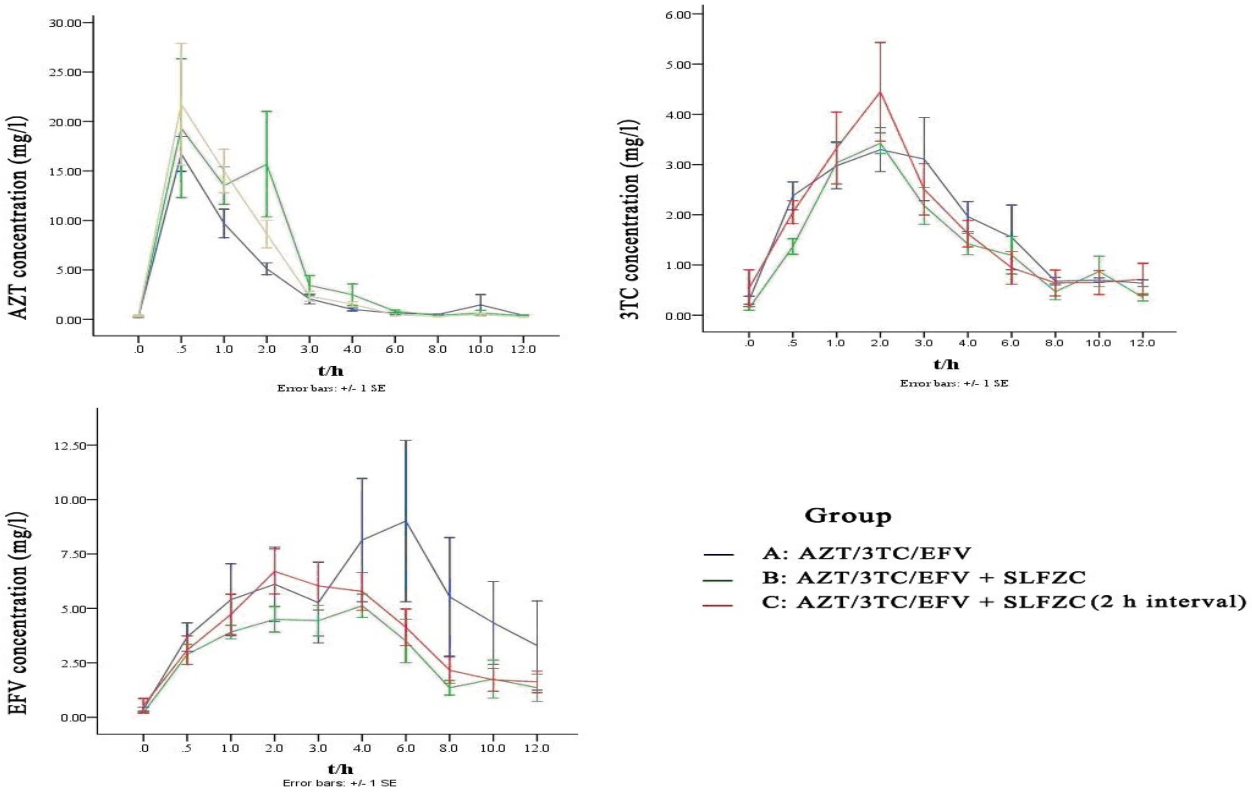

Fig. 1: AZT, 3TC or EFV concentration-time curves following a single dose of HAART, HAART and SLFZC simultaneously, or HAART 2h prior to SLFZC administration.

Table 1: Comparison of pharmacokinetic parameters of HAART drugs among groups, $\bar{x} \pm s$

Drugs and Groups	Pharmacokinetic parameters				
	$t_{1/2}$ (h) [a]	C_{max} (mg/l) [a]	T_{max} (h) [a]	$AUC_{0\text{-}12h}$ (mg/l*h) [b]	CL (l/h/kg) [a]
AZT					
Group A	5.90±0.49	16.74±0.42	0.61±0.02	27.79±1.27	1.02±0.02
Group B	3.55±0.42	18.02±2.15	0.91±0.05	34.42±1.66	0.91±0.07
Group C	3.13±0.39	18.55±3.40	0.77±0.02	30.89±1.80	1.07±0.11
F-statistic	3.371	0.074	3.033	0.573	0.299
P-value	0.047	0.929	0.063	0.570	0.744
3TC					
Group A	6.25±0.79	4.95±0.27	1.94±0.25	18.11±1.35	1.34±0.03
Group B	5.15±0.85	3.60±0.04	1.79±0.10	15.10±1.40	1.85±0.06
Group C	3.68±0.74	4.71±0.32	1.59±0.11	15.69±1.77	1.93±0.24
F-statistic	1.021	1.253	0.249	0.574	1.617
P-value	0.375	0.303	0.782	0.570	0.219
EFV					
Group A	3.48±0.37	6.49±1.40	2.00±0.37	25.08±2.41	2.47±0.31
Group B	4.29±2.43	6.40±0.20	3.24±0.29	32.92±1.54	1.65±0.16
Group C	5.50±1.88	7.49±0.53	2.77±0.15	38.91±1.76	1.37±0.24
F-statistic	0.353	0.215	1.410	1.189	1.784
P-value	0.706	0.808	0.261	0.319	0.187

not mitigate side effects related to HAART by lowering their blood concentrations. The results also suggested that use of SLFZC may not decrease anti-HIV effect of HAART and may not increase the risk of developing resistance to the drugs contained in the HAART combination.

It is well demonstrated that TCM drugs are good at improving immune function and fitness of patients, which we suppose is the reason why some side effects can be reduced when SLFZC and HAART are co-administered. Its deeper mechanisms may be difficult to be elucidated because of the complexity of drug interactions, TCM compositions and their physic-chemical properties.

CONCLUSIONS

In summary, the present study showed that SLFZC has little impact on the pharmacokinetic parameters of AZT, 3TC and EFV that constitute a common 3-drug HAART regimen. It may mitigate side effects related to HAART by other mechanisms rather than lowering blood concentrations of HAART drugs.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant 81360258) and the Natural Science Foundation of Guangxi Province (grant 2014GXNSFAA118175).

REFERENCES

Chinsembu KC (2016). Ethnobotanical study of plants used in the management of HIV/AIDS-related diseases in Livingstone, Southern Province, Zambia. *Evid. Based Complement. Alternat. Med.*, 4238625.

Duggan J, Peterson WS, Schutz M, Khuder S and Charkraborty J (2001). Use of complementary and alternative therapies in HIV-infected patients. *AIDS Patient Care STDS*, **15**(3): 159-167.

Fasinu PS, Gurley BJ and Walker LA (2015). Clinically relevant pharmacokinetic herb-drug interactions in antiretroviral therapy. *Curr. Drug Metab*., **17**(1): 52-64.

Jernewall N, Zea MC, Reisen CA and Poppen PJ (2005). Complementary and alternative medicine and adherence to care among HIV-positive Latino gay and bisexual men. *AIDS Care*, **17**(5): 601-609.

Larson EC, Hathaway LB, Lamb JG, Pond CD, Rai PP, Matainaho TK, Piskaut P, Barrows LR and Franklin MR (2014). Interactions of Papua New Guinea medicinal plant extracts with antiretroviral therapy. *J. Ethnopharmacol.*, **155**(3): 1433-1440.

Liu J (2007). The use of herbal medicines in early drug development for the treatment of HIV infections and AIDS. *Expert Opin. Investig. Drugs*, **16**(9): 1355-1364.

Liu JP, Manheimer E and Yang M (2005). Herbal medicines for treating HIV infection and AIDS. *Cochrane Database Syst. Rev.*, **20**(3): CD003937.

Shedlin MG, Anastasi JK, Decena CU, Rivera JO, Beltran O and Smith K (2013). Use of complementary and alternative medicines and supplements by Mexican-origin patients in a U.S.-Mexico border HIV clinic. *J. Assoc. Nurses AIDS Care*, **24**(5): 396-410.

Su QJ, Li YZ, Wu WQ, Lu ZZ, Zhang YP, Liu ZW, Li X and Deng X (2013). Treating HIV/AIDS patients by Shenlin Fuzheng Capsule and Qingdu Capsule: an analysis of clinical effectiveness. *Zhongguo Zhong Xi Yi Jie He Za Zhi.*, **33**(11): 1476-1480.

Sun J, Liu L, Shen J, Qi T, Wang Z, Song W, Zhang R and Lu H (2015). Reasons and risk factors for the initial regimen modification in Chinese treatment-naïve patients with HIV infection: A retrospective cohort analysis. *PLoS One*, **10**(7): e0133242.

Thomford NE, Awortwe C, Dzobo K, Dzobo K, Adu F, Chopera D, Wonkam A, Skelton M, Blackhurst D and Dandara C (2016). Inhibition of CYP2B6 by Medicinal Plant Extracts: Implication for Use of Efavirenz and Nevirapine-Based Highly Active Anti-Retroviral Therapy (HAART) in Resource-Limited Settings. *Molecules*, **21**(2): 211.