



Inhibition of the Metabolism of Zidovudine (AZT) by Herbal Component Scutellarein

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SUMMARY. Herb-drug interaction strongly limits the clinical utilization of herbs and synthetic drugs. The inhibition of drug metabolism has been widely accepted to be an important reason for herb-drug interaction. The present study aims to investigate the inhibitory potential of herbal component scutellarein towards the metabolism of AZT which is the first U.S. government-approved antiretroviral drug for the successful treatment for the HIV/AIDS infectiousness, trying to predict potential scutellarein-AZT interaction. Noncompetitive inhibition of scutellarein towards AZT metabolism was demonstrated with the inhibition kinetic parameter (K_i) to be 19.6 μ M. *In vivo* scutellarein-AZT interaction seems not to occur *in vivo* maximum plasma concentration (C_{max}) after administration of 400 mg/kg scutellarin for rat. However, due to the complex factors affecting the *in vitro-in vivo* extrapolation (IV-IVE), the translation from *in vitro* data to *in vivo* situation should be given much attention.

INTRODUCTION

During the clinical therapy towards patients infected with HIV, the treatment guideline that co-administration of at least three antiretroviral (ARV) drugs was recommended, including the co-administration of synthetic drugs and herbal components ^{1,2}. Therefore, the drug-drug interaction (DDI) or herb-drug interaction (HDI) are difficult to avoid, which severely challenging the clinicians to treat HIV-infected patients. Among the factors resulting in DDI and HDI, pharmacokinetic factors (*e.g.* absorption, metabolism, excretion) are important factors inducing DDI and HDI.

Zidovudine (AZT) is the first U.S. government-approved antiretroviral drug for the successful treatment for the HIV/AIDS infectiousness, and has been sold under the names Retrovir and Retrovis. AZT is mainly metabolized by UDP-glucuronosyltransferase (UGT) 2B7 after absorption ³. AZT has narrow therapeutic window and short half-life time (0.5-3 h) ⁴. The *in vivo* exposure of AZT in serum can be signifi-

cantly affected by co-administrated compounds exhibiting inhibition potential towards UGT2B7-catalyzed metabolic reaction of AZT. In the previous studies, many drugs or herbal components have been demonstrated to exert strong inhibition towards metabolism of AZT, including demethylzeylasteral ⁵ and 20(S)-Protopanaxatriol (Ppt) ⁶.

Scutellarein is an important flavonoid isolated from *Erigeron breviscapus* (Vant.) Hand-Mazz extracts, and has been observed to be an important serum component after administration of scutellarin ⁷. The structure of scutellarein contains multiple phenol hydroxy groups which have been demonstrated to be vulnerable to glucuronidation and exhibit strong inhibition towards UDP-glucuronosyltransferases (UGTs) isoforms ^{8,9}. Previous literatures have shown that scutellarein underwent UGT isoforms-catalyzed glucuronidation and exhibited strong inhibition towards several UGT isoforms, including UGT1A1, 1A6, 1A9, and 2B7 ^{10,11}.

KEY WORDS: Herb-drug interaction, Scutellarein, Zidovudine.

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The present study aims to investigate the inhibition of scutellarein towards the metabolism of AZT. *In vitro* human liver microsomes (HLMs)-catalyzed AZT glucuronidation reaction was used to evaluate the inhibition potential of scutellarein. The inhibition type was determined, and the inhibition kinetic parameter (K_i) was calculated.

MATERIALS AND METHODS

Chemicals and reagents

Scutellarein (purity $\geq 98\%$), 3'-azido-3'-deoxythymidine (AZT), Tris-HCl, alamethicin and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were obtained from Sigma-Aldrich (St Louis, MO). HPLC grade acetonitrile was obtained from Merck, and all aqueous solutions were prepared using ultrapure Milli-Q water ($>18\text{ M}\Omega$). Human liver microsomes (HLMs) were prepared from human liver tissues as previously described ⁵.

Evaluation of scutellarein's inhibition towards HLMs-catalyzed metabolism of AZT

Scutellarein was dissolved in DMSO at 20 mmol/L as stock solution. The incubation system (total volume = 200 μL) is consisted of 50 mM Tris-HCl (pH = 7.4), HLMs (final concentration = 0.5 mg/mL), 5 mM UDPGA, 5 mM MgCl_2 , 50 $\mu\text{g}/\text{mg}$ protein alamethicin, and AZT (the concentration is corresponding to the apparent K_m value). 30 min incubation time was used. After centrifugation at $20000\times g$ for 10 min at 4 $^\circ\text{C}$, aliquots of supernatants were transferred for HPLC analysis. The HPLC column was eluted at 1mL/min with a mobile phase of acetonitrile:aqueous (v/v = 12/88). The aqueous phase contained 0.4 mL concentrated H_3PO_4 di-

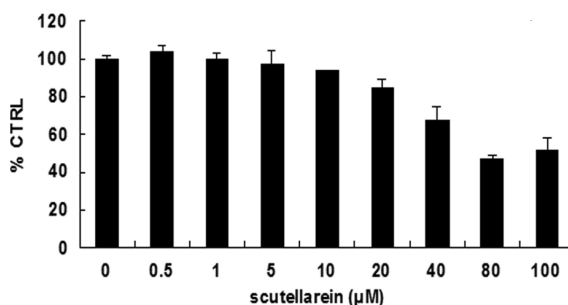


Figure 1. The dose-dependent inhibition behaviour of scutellarein towards human liver microsomes (HLMs)-catalyzed. The experiments were carried out for triplicate, and the data were given as mean plus standard deviation (S.D.).

luted to 1 L with water (pH = 2.4). Ultraviolet detection was at 267 nm. The reaction velocity (v) was determined at various concentrations of AZT and scutellarein. Dixon plot and Lineweaver-Burk plot were employed to determine the inhibition type, and the inhibition kinetic parameter (K_i) was calculated using second plot drawn with the slopes from Lineweaver-Burk plot versus the concentrations of scutellarein.

RESULTS

Dose-dependent inhibition of scutellarein towards the metabolism of AZT catalyzed by human liver microsomes (HLMs) was observed, with the activity of AZT glucuronidation inhibited by -3.6, -0.2, 3.2, 6.4, 15.1, 33.2, 53, and 48.7% at 0.5, 1, 5, 10, 20, 40, 80, and 100 μM of scutellarein (Fig. 1). The intersection point in Dixon plot (Fig. 2A) and Lineweaver-Burk plot (Fig. 2B) was located in the horizontal axis, indicating the noncompetitive inhibition of scutellarein towards the metabolism of AZT. The K_i value was calculated to be 19.6 μM (Fig. 3).

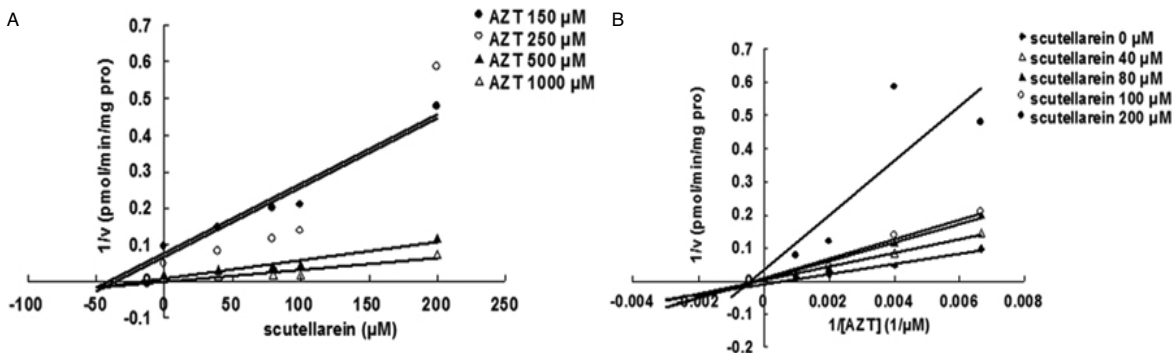


Figure 2. Determination of inhibition kinetic type using Dixon plot (A) and Lineweaver-Burk plot (B). Each point represents the mean value of two replicates.

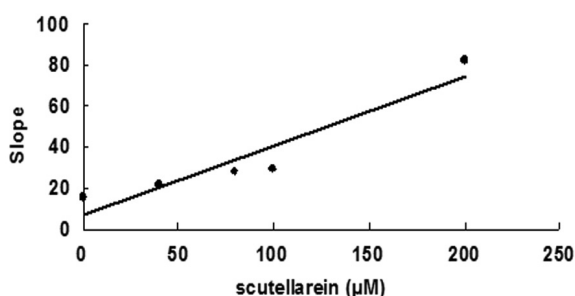


Figure 3. Determination of the inhibition parameter (K_i) using second plot with the slopes from Lineweaver-Burk plots towards the concentrations of scutellarein.

DISCUSSION

Herbs are often co-administered with therapeutic drugs, raising the potential herbs-drug interaction. For example, enhanced anticoagulation and bleeding occurred when patients undergoing long-term warfarin therapy also take *Salvia miltiorrhiza* (danshen)¹². The administration of *Allium sativum* (garlic) can significantly decrease the area under the plasma concentration-time curve (AUC) and maximum plasma concentration of saquinavir (http://www.lets-drug.com/meds_interaction/248-5540-0/). *Panax ginseng* (ginseng) induced the reduction of the concentrations of alcohol (ethanol) and warfarin in blood^{13,14}.

Given that interactions between herbal medicines and prescribed drugs can occur and may lead to serious clinical consequences. The present study aims to evaluate the interaction between scutellarein and AZT. Noncompetitive inhibition was demonstrated for the inhibition of scutellarein towards HLMs-catalyzed AZT metabolism with the K_i value to be 19.6 μM . Whether this inhibition effect can result in the *in vivo* scutellarein-AZT interaction can be decided by not only the *in vitro* inhibition kinetic parameter, but also the *in vivo* concentration of scutellarein. The *in vivo* maximum plasma concentration (C_{max}) was reported to be 0.2 mg/L (0.7 μM) after administration of 400 mg/kg scutellarein for rat¹⁵. With this concentration, the *in vivo* scutellarein-AZT interaction seems not to

occur. However, many complex factors might influence the translation from *in vitro* data to *in vivo* situation. For example, the herbs in different batches might contain different quantities of scutellarein. Therefore, the *in vitro-in vivo* extrapolation process should be given much attention.

REFERENCES

1. Tseng, A. & M. Foisy (2012) *Curr. Infect. Dis. Rep.* **14**: 67-82.
2. Fulco, P.P., M.M. Zingone & R.T. Hiqqinson (2008) *Pharmacotherapy* **28**: 945-9.
3. Barbier, O., D. Turgeon, C. Girard, M.D. Green, T.R. Tephly, D.W. Hum, *et al.* (2000) *Drug Metab. Dispos.* **28**: 497-502.
4. Hoetelmans, R.M. (1999) *Antivir. Ther.* **4** (Suppl. 3): 29-41.
5. Zhuang, L., G.H. Wang, J.W. Zhao, X.Q. Ji & L.H. Chen (2012) *Lat. Am. J. Pharm.* **31**: 1370-2.
6. Xing, J. & W. Che (2012) *Lat. Am. J. Pharm.* **31**: 628-31.
7. Gao, C., H. Zhang, Z. Guo, T. You, X. Chen & D. Zhong (2012) *Drug Metab. Dispos.* **40**: 2009-20.
8. Song, J.H., Z.Z. Fang, L.L. Zhu, Y.F. Cao, C.M. Hu, G.B. Ge, *et al.* (2013) *J. Pharm. Pharmacol.* **65**: 521-7.
9. Dong, R.H., Z.Z. Fang, L.L. Zhu, G.B. Ge, X.B. Li, C.M. Hu, *et al.* (2013) *Xenobiotica* **43**: 133-9.
10. Wang, Y., H. Ao, Z. Qian & Y. Zheng (2011) *Xenobiotica* **41**: 538-48.
11. Ma, G.Y., Y.F. Cao, C.M. Hu, Z.Z. Fang, X.Y. Sun, M. Hong, *et al.* (2013) *Phytother. Res.* doi: 10.1002/ptr.4990.
12. Chan, T.Y. (2001) *Ann. Pharmacother.* **35**: 501-4.
13. Haron, M.H., B. Avula, I.A. Khan, S.K. Mathur & A.K. Dasmahapatra (2013) *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **157**: 287-97.
14. Lee, S.H., Y.M. Ahn, S.Y. Ahn, H.K. Doo & B.C. Lee (2008) *J. Altern. Complement Med.* **14**: 715-21.
15. Xing, J.F., H.S. You, Y.L. Dong, J. Lu, S.Y. Chen, H.F. Zhu *et al.* (2011) *Acta Pharmacol. Sin.* **32**: 655-63.