In vitro evidence of baicalein's inhibition of the metabolism of zidovudine (AZT)

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Abstract

Background: Herb-drug interaction (HDI) has been regarded as a key factor limiting the clinical application of herbs and drugs.

Aims: Potential baicalein-zidovudine (AZT) interaction was predicted in the present study.

Methods: *In vitro* evaluation of baicalein's inhibition towards human liver microsomes (HLMs)-catalyzed metabolism of zidovudine (AZT) was performed. Dixon and Lineweaver-Burk plots were used to determine the inhibition kinetic type, and second plot with the slopes from Lineweaver-Burk plot versus the concentrations of baicalein was employed to calculate the inhibition parameter (K_i). In combination with the *in vivo* concentration of baicalein, *in vitro-in vivo* extrapolation (IVIVE) was carried out to predict *in vivo* baicalein-AZT interaction.

Results: Competitive inhibition of baicalein towards AZT metabolism was demonstrated, and the K_i value was calculated to be 101.2 μM. The value of AUC_i/AUC was calculated to be 2.

Conclusion: Potential baicalein-AZT interaction was indicated in the present study, indicating the need for monitoring when AZT is co-administrated with baicalein or baicalein-containing herbs.

Keywords: Baicalein, zidovudine (AZT), metabolism, herb-drug interaction

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Introduction

Herbs are defined as the plants used for flavoring, food, medicine, or perfume. Historically, herbs have been widely used as the origin of modern pharmacotherapy [1]. Herbs have been utilized for thousands of years in Asia and South America, and are enjoying more and more popularity in Europe and North America [2,3]. The co-administration of herbs has been reported to occur among 16% prescription drug users in the United States [4]. In the clinic, the major reasons for the increased utilization of herbs in combination with prescribed drugs are that herbs have been always believed to be safe.

Pharmacokinetics-based herbs-drug interactions appear inevitable clinically and strongly limit the utilization of herbs. For example, patients received oral warfarin (Coumadin, DuPont Pharmaceuticals), 5mg daily, for the first 3 consecutive days duringweek 1. Beginning in week 2, patients were randomly assigned to receive either oral AG, 1.0 g, or placebo b. i. d. (= 2 g/day), for 3 consecutive weeks. During week 4, all patients again received oral warfarin, 5 mg daily, for the first 3 consecutive days. The INR index significantly after 2 weeks of ginseng administration, which was closely correlated with the reduced peak plasma level of warfarin [5].

Zidovudine (AZT) is a nucleoside analog reverse-transcriptase inhibitor (NRTI) to treat HIV/AIDS , and has been approved and prescribed under the name Retrovir [6]. AZT has been demonstrated to undergo UDP-glucuronosyltransferase (UGT) 2B7-catalyzed glucuronidation elimination after its absorption into the serum [7]. To date, many herbal components have been demonstrated to exhibit the inhibition towards the metabolism of AZT, such as demethylzeylasteral and 20(S)-protopanaxatriol (ppt) [7,8].

Baicalein is the hydrolyzed product and main effective substance basis of baicalin which is a flavonoid isolated from Scutellariae radix, and has been demonstrated to exhibit various biochemical and pharmacological activities, including anti-inflammatory, anti-tumor, anti-allergic, and anti-oxidation effects [9,10]. As a herbal component exhibiting many pharmacological activities, the co-exposure possibility between baicalein and AZT is very high. Therefore, the aim of the present study was to evaluate the inhibition potential of baicalein towards the metabolism of AZT. The inhibition kinetic type and parameter (K_i) were determined, and *in vitro-in vivo* extrapolation (IVIVE) was carried out.

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Materials and Methods

Chemicals and reagents

3'-azido-3'-deoxythimidine (AZT), Tris-HCl, alam-

ethicin and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St Louis, MO). Baicalein was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pooled human liver microsomes (HLMs) were purchased from BD Gentest (Woburn, MA). All other reagents were of HPLC grade or of the highest grade commercially available.

Inhibition evaluation of HLMs-mediated AZT glucuronidation by baicalein

Baicalein was dissolved in methanol at 20 mmol/L as stock solution. The incubation mixture (200 μL total volume) contained HLMs (final concentration=0.5 mg/mL), 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH=7.4), 50 μg/mg protein alamethicin, and AZT (the concentration is corresponding to the apparent Km value). The incubation time was 30 min. The mixture was kept on ice until it was centrifuged at 20000 ×g for 10 min at 4 °C. Aliquots of supernatants were transferred for HPLC analysis. For the analysis AZT glucuronidation, the HPLC column was eluted at 1 mL/min with a mobile phase of acetonitrile aqueous (v/v = 12:88). The aqueous phase contained 0.4 mL concentrated H_3PO_4 diluted to 1 L with water (pH 2.4). Ultraviolet detection was at 267 nm.

Furthermore, the reaction was determined at multiple concentrations of AZT and baicalein. Dixon plot and Lineweaver-Burk plot were used to determine the inhibition kinetic type, and the second plot was further employed to calculate the inhibition kinetic parameter (K_i).

Prediction of in vivo baicalein-drug interaction magnitude

The baicalein-AZT interaction magnitude is affected by both *in vitro* inhibition kinetic parameters (K_i) and *in vivo* concentration of baicalein. The most common equation for drug-drug interaction prediction was employed to predict the AUC alteration of AZT caused by co-administration of baicalein.

$AUC_i/AUC=1+[I]_{in\ vivo}/K_i$

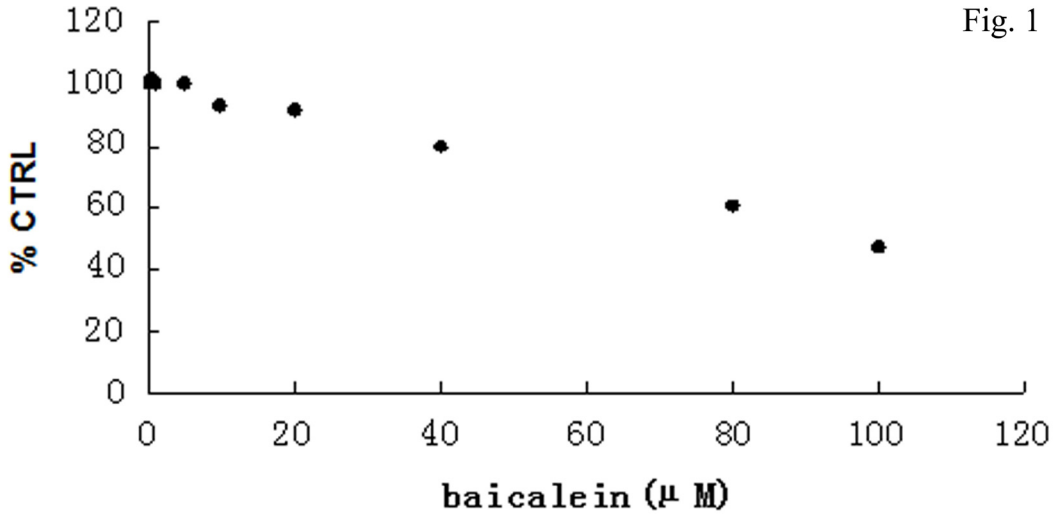
The terms are defined as follows: AUC_i/AUC is the predicted ratio of *in vivo* exposure of AZT with co-administration of baicalein vs. that in control situation. K_i is the *in vitro* reversible inhibition constant, $[I]_{in\ vivo}$ is the *in vivo* concentration of baicalein. In this study, the maximum plasma concentration (C_{max}) of baicalein was used.

Results

The dose-dependent inhibition behaviour of baicalein towards AZT glucuronidation was observed. The HLMs-catalyzed AZT glucuronidation activity was inhibited by -1.1%, 0.1%, 0.2%, 7.7%, 9.5%, 20.9%, 40%, and 53.4% for 0.5, 1, 5, 10, 20, 40, 80, and 100 μM of baicalein (Fig. 1).

Fig. 1 The concentration-dependent inhibition of baicalein towards the metabolism of zidovudine (AZT). The data point represents the mean of duplicate experiment.

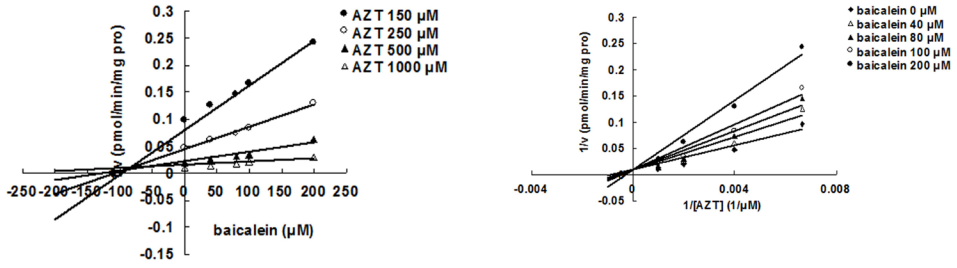
Fig. 1



Dixon plot was drawn using the 1/reaction velocity (v) versus the concentration of baicalein, and Lineweaver-Burk plot was drawn with the 1/reaction velocity (v) versus 1/the concentration of AZT. For the inhibition of baicalein towards the metabolism of AZT, the intersection point was located in the second quadrant in Dixon plot (Fig. 2A), and the verticle axis in Lineweaver-Burk plot (Fig. 2B).

Fig. 2 Determination of inhibition kinetic type for baicalein's inhibition towards the metabolism of zidovudine (AZT). (A) Dixon plot for baicalein's inhibition towards the metabolism of zidovudine (AZT); (B) Lineweaver-Burk plot for baicalein's inhibition towards the metabolism of zidovudine (AZT). The data point represents the mean of duplicate experiment.

Fig. 2

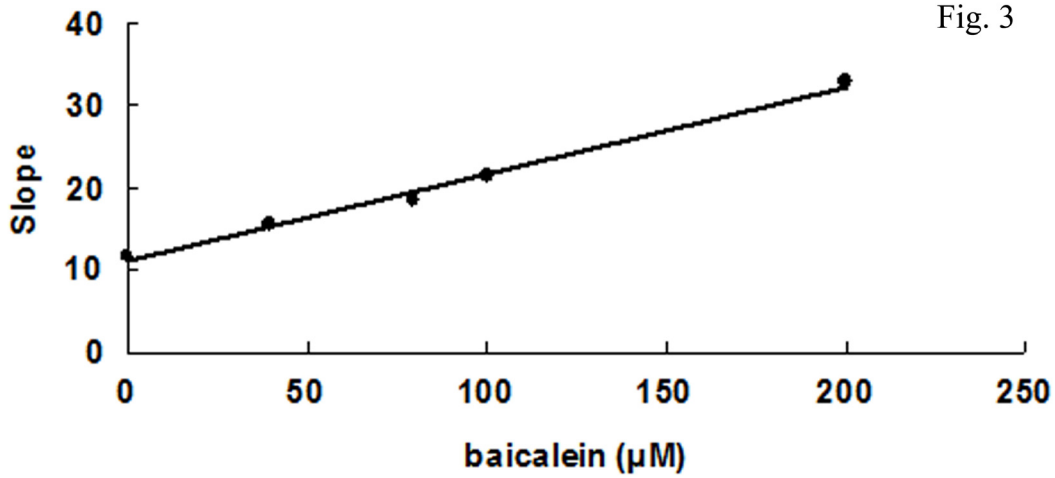


(A) (B)

The second plot with the slopes from Lineweaver-Burk plot versus baicalein's concentrations was performed to determine the inhibition parameter (Ki) to be 101.2 μM. The maximum concentration of baicalein in plasma was reported to be approximately 100 μM after i.v. administration of 2.2 mg/kg or 5.5 mg/kg of baicalein for rats [11]. Using this value in combination the K_i value, the value of AUC_i/AUC was calculated to be 2.

Fig. 3 Calculation of inhibition kinetic parameter (K_i) using the second plot drawn with the slopes from Lineweaver-Burk versus the concentrations of baicalein.

Fig. 3



Discussion

Drug-metabolizing enzymes (DMEs)-based clinical drug-drug interaction (DDI) and herb-drug interaction (HDI) limited the utilization of many clinical drugs and R&D process of potential drugs. For example, the strong inhibition of noscapine towards cytochrome P450 2C9 and 3A4 strongly limited the utilization of noscapine as an efficient antitussive drug and the development of this compound as a potential anti-tumor drug [12]. The inhibition of herbal components liquiritigenin and isoliquiritigenin towards the metabolism of hypnotic agent propofol limited the co-utilization of these two herbal components and propofol [13].

The dose beyond the efficient concentration of AZT can induce the genotoxicity and minimum cell death [14]. AZT has a narrow therapeutic index, and slight alteration of AZT concentration will exceed the minimal toxicity concentration of AZT. The present study reported the AUC_i/AUC value was 2 through *in vitro-in vivo* extrapolation (IVIVE) using the *in vitro* K_i value and *in vivo* concentration of baicalein. This result can be affected by the following factors: 1) *In vitro* incubation factors: previous literature has reported that the addition of bovine serum albumin (BSA) can significantly cause the reduction of K_i value for fluconazole inhibition of AZT glucuronidation by HLM [15]. Therefore, the K_i value calculated in the present study might underestimate the baicalein-AZT interaction due to the lack of BSA in our present incubation system. 2) *In vivo* factors: The present study using the *in vivo* concentration after i.v. adminstration of single purified compound. When the administration route is different (e.g., oral gavage (p.o.), intraperitoneal injection (i.p.), etc.) or the herbs containing baicalein is given, the in vivo concentration of baicalein might be different which can further complicate the IVIVE results.

In conclusion, the baicalein-AZT interaction was indicated in the present study using *in vitro* inhibition experiment, which challenges the co-utilization of AZT and baicalein or baicalein-containing herbs.

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