In vitro evidence for bakuchiol's influence towards drug metabolism through inhibition of UDP-glucuronosyltransferase (UGT) 2B7

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Abstract

Background: Inhibition of drug-metabolizing enzymes (DMEs) has been regarded as one of the most important reason for clinical drug-drug interaction.

Aim: The aim of the present study is to evaluate the inhibition of bakuchiol towards UDP-glucuronosyltransferase (UGT) 2B isoforms.

Methods: *In vitro* recombinant UGT2B-catalyzed 4-methylumbelliferone glucuronidation was used as the probe reaction. Dixon plot and Lineweaver-Burk plot were employed to determine the inhibition kinetic type, and nonlinear regression of data was utilized to calculate the inhibition kinetic parameter (K_i). *In vitro-in vivo* extrapolation (IVIVE) was carried out to predict *in vivo* inhibition magnitude.

Results: Among the tested UGT2B isoforms, UGT2B7 was inhibited by the strongest intensity. The noncompetitive inhibition was demonstrated by the results obtained from Dixon plot and Lineweaver-Burk plot. The K_i value was calculated to be 10.7 μM. In combination with the reported concentration after an intravenous administration of bakuchiol (15 mg/kg) in rats, the high risk of *in vivo* inhibition of bakuchiol towards UGT2B7-catalyzed metabolism of drugs was indicated.

Conclusion: All these results provide an important information for the risk evaluation of the clinical utilization of bakuchiol.

Keywords: bakuchiol, drug-drug interaction, drug-metabolizing enzymes

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Introduction

Drug metabolism plays a key role in the breakdown and safe elimination of the parent compounds. Drug metabolism can be categorized into phase I and phase II metabolism[1]. Biotransformation of parent drug during phase I metabolism involves the addition of hydroxyl, carboxyl, amino, or thiol groups [2]. The drug-metabolizing enzymes involved in phase I metabolism contain cytochrome P450 (CYP) and flavin-containing monooxygenases (FMO) [3]. Furthermore, phase II reactions will increase the hydrophilicity, facilitating the excretion of metabolites into the sinusoidal circulation or bile. The metabolic enzymes contain UDP-glucuronosyltransferases (UGTs), N-acetyltransferase 2 (NAT2), and glutathione Stransferase (GST) [4].Besides the detoxification properties of drug metabolism, some metabolic behaviours of drugs might result in the toxicity. For example, acetaminophen (APAP) can underwent CYP3A4, 1A2 and 2E1-mediated bioactivation to form reactive metabolite Nacetyl pbenzoquinoneimine (NAPQI) which plays a key role in the hepatotoxicity of APAP [5].

Human UGT superfamily can be categorized into two families (UGT1 and UGT2) and three subfamilies (UGT1A, UGT2A and UGT2B). Some UGT isoforms are highly expressed in the liver, including UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17 and 2B28. Additionally, some UGT isoforms have been detected only in the gastrointestine (GI) tract, including UGT1A7, 1A8 and 1A10 [6]. Many factors can influence the activity of UGTs, including the nuclear receptors (e.g., pregnane X receptor, liver X receptor, farnesoid X receptor, etc.) and microRNAs [7,8].

The activity of UGTs can directly be inhibited by clinical drugs, and the severe clinical results might occur. For example, sorafenib, the approved drug for the treatment of renal cell and unresectable hepatocellular carcinomas, has been reported to induce hyperbilirubinemia through the inhibition of UGT1A1 activity [9]. Additionally,

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unconjugated hyperbilirubinemia induced by indinavir might be resulted from the inhibition of indinavir towards UGT1A1 activity [10].

Bakuchiol, a prenylated phenolic monoterpene isolated from the seeds of Psoralea corylifolia L. (Leguminosae), has been reported to exert a variety of pharmacological activities, such as anti-microbacterial activity and inhibition of iNOS expression [11,12]. Bakuchiol has been demonstrated to have anti-cancer activity [13]. Bakuchiol has been reported to exhibit regulation effects towards the enzyme activity. For example, bakuchiol has been demonstrated to protect mitochondrial respiratory enzyme activities against both NADPH-dependent and dihydroxyfumarate-induced peroxidation injury[14], indicating the good interaction between bakuchiol and enzymes. Therefore, the present study aims to evaluate bakuchiol's inhibition towards UGT2B isoforms, trying to indicate the potential influence of bakuchiol towards the metabolsim of xenobiotics and endogenous substances mediated by UGT2B isoforms.

Methods

Chemicals and reagents

Bakuchiol was purchased from SichuanWeikeqi Bio-technology Co. Ltd (Sichuan,
China), and the purity of bakuchiol was demonstrated to be more than 98% by HPLC analysis. 4-methylumbelliferone (4MU), 4-methylumbelliferone β-D-glucuronide, and UDP-glucuronic acid (trisodium salt) were purchased from Sigma-Aldrich. Recombinant UGT2B7, 2B15 and 2B17 were obtained from BD Gentest Corp. (Woburn, MA, USA). Acetonitrile and methanol (HPLC-grade) were purchased from Merck. High purity water was obtained from Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). All other reagents were of analytical grade.

Inhibition evaluation of bakuchiol towards recombinant UGT2B activity

The inhibition capability of bakuchiol towards UGT2B activity was evaluated as previously described [15]. 4-MU was used as probe substrate to determine bakuchiol's inhibition towards UGT2B isoforms, including UGT2B7, 2B15 and 2B17. The incubation mixture contained recombinant UGT2B isoforms, 5 mM $MgCl_2$, 5 mM UDPGA, 50 mM Tris-HCl (pH=7.4), 4-MU, and bakuchiol. After 5-min pre-incubation, the reaction was initiated by the addition of UDPGA, and the reaction time was 120 min. The reaction was terminated by adding 100 μL acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. The mixture was centrifuged at 20000 × g for 10min and an aliquot of supernatant was transferred to an autoinjector vial for chromatographic analysis. The concentrations of 4-MU and recombinant UGT2B isoforms, and the analytical conditions were used as previously described [16,17]. In brief, the concentration of recombinant enzymes was 0.05, 0.75 and 0.5 mg/mL for UGT2B7, 2B15, and 2B17, and the used concentration of 4-MU was 350 μM for UGT2B7, 250 μM for UGT2B15 and 2000 μM for UGT2B17.

Determination of IC_{50} values

The IC_{50} values were defined as the concentrations of bakuchiol exhibiting 50% inhibition. The IC_{50} values were determined through plotting dose response curves of enzyme activity versus the concentrations of bakuchiol.

Determination of the inhibition kinetic type and parameters

The reaction velocity was determined at the different concentrations of 4-MU and bakuchiol, Dixon plot and Lineweaver-Burk plot were used for the determination of inhibition kinetic type. The inhibition kinetic parameters (K_i) were calculated through nonlinear regression using the equations for competitive inhibition (1), noncompetitive inhibition (2).

$V=Vmax*[S]/(Km/(1+([I]/Ki))+[S])$ **(1)**

$V=Vmax*[S]/(Km+[S])*(1+([I]/Ki))$ **(2)**

Where the items are defined as followed: V is the reaction velocity, [S] and [I] are the concentrations of substrate and inhibitor, respectively. Km value is the substrate concentration in which the velocity reached to half of the maximum velocity (Vmax) of the reaction. Ki value is the inhibition constant.

Prediction of in vivo **risk from** in vitro **data**

The $[I]/K_i$ value has been widely regarded as the common method to estimate the *in vivo* risk due to the inhibition of drug-metabolizing enzymes [18,19]. When this value is more than 1, the possibility of drug-drug interaction is high.

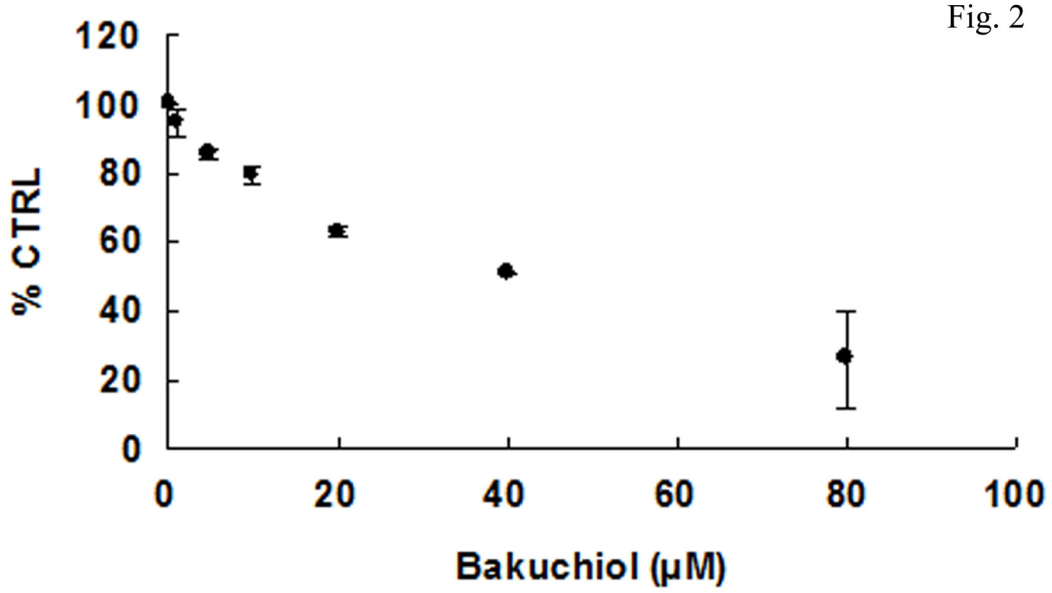
Results

As shown in Fig. 1, bakuchiol exhibited dose-dependent inhibition towards all the tested UGT2B isoforms, including UGT2B7, UGT2B15, and UGT2B17. The inhibitory potential of three concentrations of bakuchiol towards these three UGT2B isoforms was evaluated. At 1 μM of bakuchiol, the activity of UGT2B7, 2B15, and 2B17 was inhibited by 3.5%, -3.9%, and 1.9%,

respectively. At 10 μM of bakuchiol, the activity of UGT2B7, 2B15, and 2B17 was inhibited by 26.3%, -7.8%, and 10.0%. The activity of UGT2B7, 2B15 and 2B17 was inhibited by 89.4%, 58.3%, and 6.7% at 100 μM of bakuchiol.

Fig. 1 Initial screening of bakuchiol's inhibition towards UGT2B isoforms. Three concentrations (1, 10, 100 μM) were selected. The experiments were performed in duplicate, and the data were given as mean.

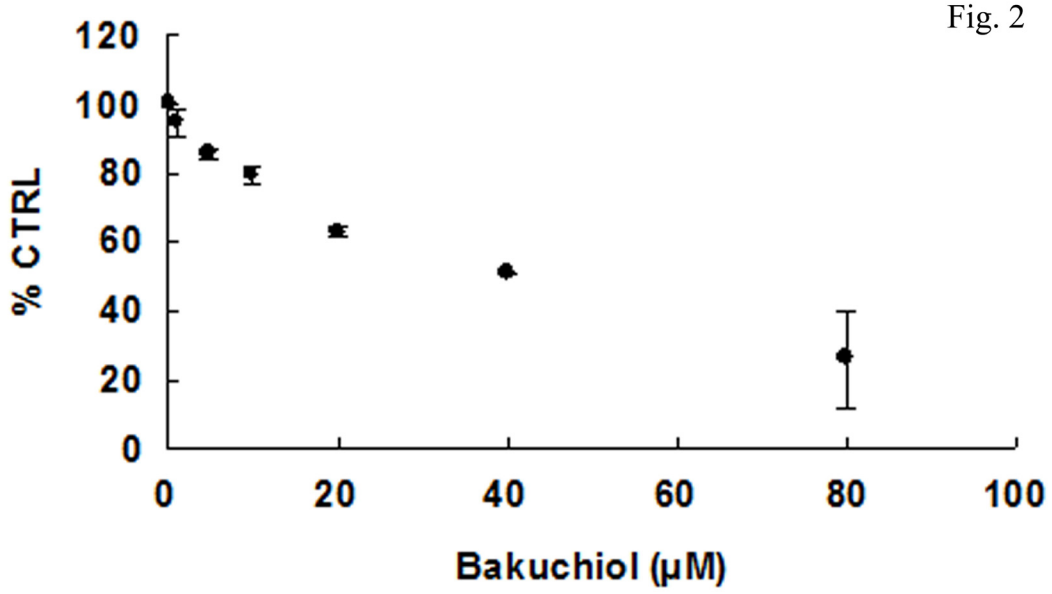
Fig. 2



Due to the strongest inhibition of bakuchiol towards UGT2B7, more concentrations of bakuchiol were selected for the determination of IC_{50} value, and the results were given in Fig. 2. Bakuchiol exhibited concentration-dependent inhibition towards UGT2B7, with the activity of UT2B7 inhibited by 5.3%, 14.5%, 20.5%, 36.9%, 49.1%, and 74.1%, for 1, 5, 10, 20, 40, and 80 μM, respectively. The IC_{50} value was calculated to be 40.9±0.5 μM.

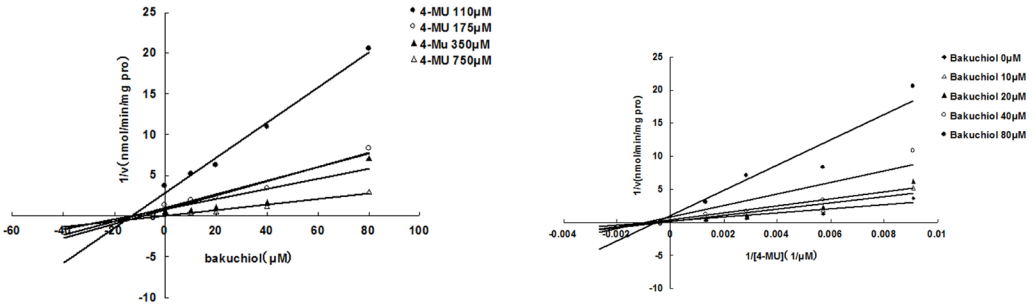
Fig. 2 IC_{50} curve of bakuchiol's inhibition towards UGT2B7. The experiments were performed in duplicate, and the data were given as mean±S.D..

Fig. 2



The inhibition type can be categorized into competitive and noncompetitive inhibition, and a combination of Dixon plot and Lineweaver-Burk plot can be the most common method to determine the inhibition type. The results (Fig. 3A & 3B) indicated noncompetitive inhibition of bakuchiol towards UGT2B7. Nonlinear regression of data using noncompetitive fitting equation was furtherly performed to calculate the inhibition kinetic parameter (K_i) to be 10.7 μM. The *in vivo* maximum concentration of bakuchiol was reported to be 4090.6 ng/ml (16μM) after an intravenous administration of bakuchiol (15 mg/kg) in rats [20]. Arbitrarily supposed that the concentration of bakuchiol in human was equal to that in rats, the [I]/Ki was calculated to be 1.5.

Fig. 3 Determination of inhibition kinetic type. (A) Dixon plot of bakuchiol's inhibition towards UGT2B7. (B) Lineweaver-Burk plot of bakuchiol's inhibition towards UGT2B7. The data point represents the mean value of two experiments.



(A) (B)

Fig. 3

Discussion

UGT2B7, arguably regarded as the most important UGT isoforms, can catalyze the glucuronidation many drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids [21]. Some previous reports have indicated that the inhibition of UGT2B7 activity can elevate the exposure of some drugs mainly undergoing UGT2B7-metabolized elimination. For example, efavirenz (EFV), the non-nucleoside reverse transcriptase inhibitor (NNRTI) agent to treat HIV infection, can induce 225% elevation of zidovudine (AZT) exposure in plasma through inhibition of UGT2B7-catalyzed AZT glucuronidation [22].

UGT2B7 was highly expressed in all tissues, including liver, small intestine, colon, kidney, and bladder [23]. Therefore, bakuchiol can affect the activity of UGT2B7 in all these tissues. It should be noted that the maximum plasma concentration of bakuchiol was used for *in vitro-in vivo* extrapolation (IVIVE), which was suitable for IVIVE for liver UGT2B7. However, the concentration of bakuchiol in other tissues was significantly different with that in the liver. For example, the concentration of bakuchiol in the intestine was higher than that in the liver given that bakuchiol needed to undergo the absorption and first-pass metabolism. Therefore, the *in vivo* inhibition magnitude might be higher for the inhibition of bakuchiol towards intestinal UGT2B7. Another important reason to influence the *in vitro-in vivo* extrapolation (IVIVE) is the elimination ratio due to UGT2B7-catalyzed metabolic elimination. In the present study, the ratio was arbitrarily to be 1, which made the prediction value maximum. Therefore, when the metabolic contribution of UGT2B7 towards the metabolism of drugs was less than 1, the *in vivo* inhibition magnitude will be lower. Additionally, many factors might influence the quantities of herbal components, including processing of herbs and environmental factors (soil, altitude, seasonal variation in temperature, length of daylight, rainfall pattern, shade and dew), which also affect the *IVIVE* process.

In conclusion, the inhibition capability of bakuchiol towards important UGT2B isoforms was evaluated in the present study. UGT2B7 was strongly inhibited among the tested UGT2B isoforms, and high possibility of *in vivo* inhibition was predicted through *in vitro-in vivo* extrapolation (IVIVE). Co-administration use of bakuchiol and drugs metabolized by UGT2B will need further evaluation

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