

Probe substrate and enzyme source-dependent inhibition of UDP-glucuronosyltransferase (UGT) 1A9 by wogonin

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Abstract

Background: Drug-metabolizing enzymes (DMEs) inhibition based drug-drug interaction and herb-drug interaction severely challenge the R&D process of drugs or herbal ingredients.

Objective: To evaluate the inhibition potential of wogonin (an important flavonoid isolated from the root of *Scutellaria baicalensis*) towards one of the most important phase II DMEs, UDP-glucuronosyltransferase (UGT) 1A9.

Methods: Both recombinant UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation reaction and human liver microsomes (HLMs)-catalyzed propofol glucuronidation reaction were used as two different probe reactions.

Results: Wogonin noncompetitively inhibited recombinant UGT1A9-catalyzed 4-MU glucuronidation, and exerted competitive inhibition towards HLMs-catalyzed propofol glucuronidation. The inhibition kinetic parameters (K_i) were calculated to be 3.2 μ M and 52.0 μ M, respectively.

Conclusion: Necessary monitoring was needed when wogonin was co-administered with the clinical drugs mainly undergoing UGT1A9-mediated glucuronidation elimination. Additionally, probe reactions-dependent inhibition of wogonin towards the activity of UGT1A9 should be paid attention when translating these *in vitro* data into *in vivo* situation.

Keywords: wogonin, drug-drug interaction (DDI), UDP-glucuronosyltransferases (UGTs)

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Introduction

Since the beginning of the herbs' utilization from ancient times, the utilization of herbal medicines has a history of about 4000 years¹. With the increasing utilization of herbal remedies, more and more cases on herbal adverse effects are reported. Herb-drug interaction has been frequently reported, and the co-administered herbs might affect the pharmacokinetic behaviours of many important clinical drugs, including warfarin, aspirin, digoxin and cisplatin². The drugs can be efficiently eliminated by phase I and phase II drug metabolizing enzymes (DMEs)-mediated elimination system. Herbal components can change the activity of all these DMEs through the good interaction with them. When these herbs are co-administered with the drugs mainly undergoing the DMEs inhibited by herbal components, the exposed concentration of drugs might exceed the

minimal toxicity dose in the therapeutic window, and then induce severe toxicity.

The glucuronidation through the conjugation with glucuronic acid represents the main elimination pathway of clinical drugs, and the inhibition of this important pathway might induce the severe drug-drug interaction. For example, the exposed concentration of zidovudine (the probe substrate of UGT2B7) can increase by 31% and 74% due to the inhibition of glucuronidation pathway by atovaquone and fluconazole, respectively^{3,4}. Some previous literatures have indicated that some herbal components can exhibit the strong inhibition towards UGT isoforms. For example, the most abundant flavonoid component quercetin inhibited the activity of UGT1A1 and UGT1A9⁵. *In vitro* experiment showed that the activity of UGT1A1, 1A6, 1A9, 2B7 and 2B15 can be inhibited by silybin⁶. The herbal components deoxyschizandrin and schisantherin A have been demonstrated to have the potential to induce herb-drug interaction through the strong inhibition towards UGT1A3⁷. Fang *et al.* investigated the structure-activity relationship for the ginsenosides' inhibition towards UGT isoforms, elucidating the potential UGTs-inhibition based ginseng-clinical drugs interactions⁸.

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Wogonin, an important flavonoid isolated from the root of *Scutellaria baicalensis* Georgi, has been reported to exhibit multiple biochemical and pharmacological activities, including antioxidant⁹, anti-viral¹⁰, antithrombotic¹¹, and anti-inflammatory activities¹². The present study aims to study the inhibition of wogonin towards one of the most important UGT isoforms, UGT1A9. *In vitro* incubation system was performed. Given that the probe reactions-dependent behaviour for the inhibition of DMEs by compounds exists, two kinds of probe reactions were carried out, including recombinant UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation and human liver microsomes (HLMs)-catalyzed glucuronidation.

Methods

Chemicals and reagents

Wogonin (purity >98%), propofol, alamethicin, 4-methylumbelliferone (4-MU), 4-methylumbelliferone- α -D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). The glucuronide of propofol was purchased from Toronto Research Chemicals (North York, Ontario, Canada). Recombinant human UGT1A9 expressed in baculovirus-infected insect cells, and pooled human liver microsomes (HLMs) from 50 donors were obtained from BD Gentest Corp. (Woburn, MA, USA). All other reagents were of HPLC grade or of the highest grade commercially available.

Evaluation of wogonin's inhibition towards UGT1A9-catalyzed 4-MU glucuronidation

The inhibition of wogonin towards recombinant UGT1A9-catalyzed 4-MU glucuronidation was evaluated as previously described¹³. The mixture (200 μ l total volume) contained recombinant 0.05 mg/ml of UGT1A9, 5 mM UDPGA, 5 mM MgCl₂, 50 mM Tris-HCl buffer (pH 7.4), and 4-MU (30 μ M) in the absence or presence of different concentrations of wogonin. Wogonin was dissolved in DMSO and the final concentration of DMSO was 0.5% (v/v). After 5 min pre-incubation at 37°C, the UDPGA was added in the mixture to initiate the reaction. Incubation time was 30 min for UGT1A9. The reactions were quenched by adding 100 μ l acetonitrile with 7-hydroxycoumarin (100 μ M) as internal standard. The mixture was centrifuged at

20,000 \times g for 10 min and an aliquot of supernatant was transferred to an auto-injector vial for HPLC analysis as previously described⁸.

Investigation of wogonin's inhibition towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation

The inhibition of wogonin towards the propofol glucuronidation catalyzed by human liver microsomes (HLMs) was evaluated as previously described¹⁴. In brief, the incubation system contained 0.5 mg/ml human liver microsomes (HLMs), 5mM UDPGA, 5mM MgCl₂, 25 μ g/ml alamethicin, 10mM D-saccharic acid 1,4-lactone, propofol (approximately K_m value) and Tris-HCl buffer (pH=7.4).

The ultra-fast liquid chromatography (UFLC)-mass (MS) method was utilized to detect the glucuronide of propofol. The mobile phase contained CH₃CN (A) and water with 0.5% acetic acid (B) with the following gradient condition: 0–2 min, 95–82% B; 2–7 min, 82–20% B; 7–9.5 min, 10% B; 9.5–12.5 min, 95% B. Column temperature was kept at 37 °C. The flow rate was set at 0.3 ml/min and the injection volume was 10 μ l. The negative mode was used, and molecular ions ([M-H]⁻) 353 and 351 were employed for propofol glucuronide and the internal standard 4-MU glucuronide.

Determination of inhibition kinetic parameters

Inhibition kinetic parameters (K_i) were determined utilizing various concentrations of 4-MU (or propofol) in the presence of different concentrations of wogonin. Dixon and Lineweaver-Burk (L-B) plots were adapted to determine the inhibition type, and second plot of slopes from Lineweaver-Burk plot vs. wogonin concentrations was utilized to calculate K_i value.

Results

The inhibition of wogonin towards recombinant UGT1A9-catalyzed 4-MU glucuronidation reaction was evaluated, and the results were shown in figure 1. Wogonin exhibited dose-dependent inhibition towards 4-MU glucuronidation (figure 1A), and both Dixon plot (figure 1B) and Lineweaver-Burk plot (figure 1C) showed that the inhibition type was best fit to the noncompetitive inhibition. The second plot (figure 1D) was used to calculate the inhibition kinetic parameter (K_i) to be 3.2 μ M. Furthermore, human liver microsomes (HLMs)-catalyzed propofol glucuronidation reaction was employed to evaluate

the wogonin's inhibition towards UGT1A9 activity. Similarly, the concentration-dependent inhibition behaviour was also observed (figure 2A). The competitive inhibition type was demonstrated for wogonin's inhibition towards

HLMs-catalyzed propofol glucuronidation through Dixon plot (figure 2B) and Lineweaver-Burk plot (figure 2C). The inhibition kinetic parameter (K_i) was calculated to be 52.0 μM (figure 2D).

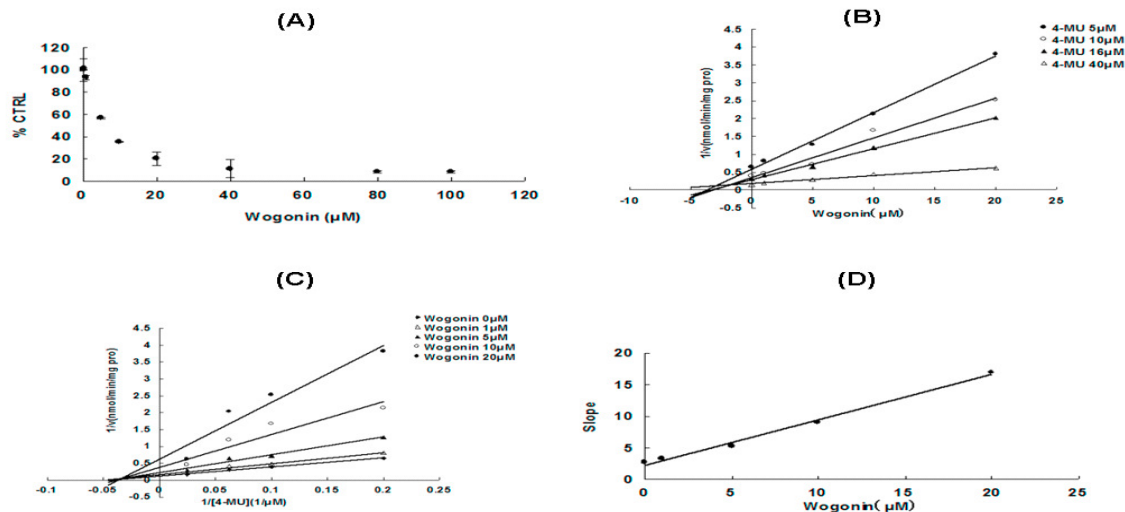


Fig. 1

Figure 1: Wogonin noncompetitively inhibits recombinant UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation.

(A) Dose-dependent inhibition of wogonin towards UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation; (B) Dixon plot of wogonin's inhibition towards UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation; (C) Lineweaver-Burk plot of wogonin's inhibition towards UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation; (D) Second plot of wogonin's inhibition towards UGT1A9-catalyzed 4-methylumbelliferone (4-MU) glucuronidation. The experiments were performed in duplicate, and data points were given as mean of the duplicate.

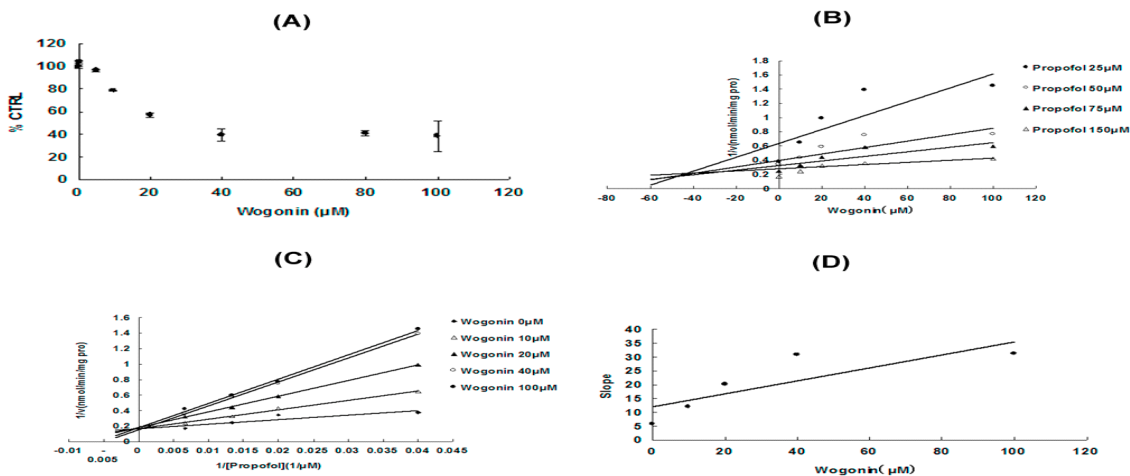


Fig. 2

Figure 2: Wogonin competitively inhibits human liver microsomes (HLMs)-catalyzed propofol glucuronidation.

(A) Dose-dependent inhibition of wogonin towards HLMs-catalyzed propofol glucuronidation; (B) Dixon plot of wogonin's inhibition towards HLMs-catalyzed propofol glucuronidation; (C) Lineweaver-Burk plot of wogonin's inhibition towards HLMs-catalyzed propofol glucuronidation; (D) Second plot of wogonin's inhibition towards HLMs-catalyzed propofol glucuronidation. The experiments were performed in duplicate, and data points were given as mean of the duplicate.

Discussion

Several phenolic hydroxyl groups exist in the structure of wogonin, and previous studies have indicated that the phenolic hydroxyl groups-containing compounds are the good substrates of UGT isoforms, including arbidol¹⁵ and magnolol¹⁶. The UGT isoforms involved in the metabolic elimination of wogonin have been identified. UGT1A9 was regarded as the main UGT isoform involved in the glucuronidation of wogonin in the liver, and UGT1A10 was the major UGT isoform responsible for wogonin's glucuronidation in the intestine¹⁷. All these information indicated the possibly good interaction between wogonin and UGT1A9.

In this study, wogonin showed inhibitory potential towards the activity of UGT1A9, regardless of the enzyme sources and probe substrates. However, the inhibition type and potential showed the difference when selecting different enzyme sources and probe substrates. Wogonin noncompetitively inhibited recombinant UGT1A9-catalyzed 4-MU glucuronidation, and competitively inhibited human liver microsomes (HLMs)-catalyzed propofol glucuronidation reaction. When choosing UGT1A9-catalyzed 4-MU glucuronidation reaction as the probe reaction, the inhibition potential was stronger. This kind of enzyme sources and probe substrates-dependent inhibition behaviour has been observed in the previous literatures¹⁸. UGT1A9 is one of the most important UGT isoforms in liver, kidney and intestine, and take part in the metabolism of many xenobiotics. For example, UGT1A9 is the major UGT isoform involved in the ethanol glucuronidation¹⁹. UGT1A9 showed extensive activity towards the glucuronidation of acetaminophen²⁰. Additionally, UGT1A9 has been demonstrated to be responsible for the glucuronidation of many clinical drugs or drug candidates, including noscapine and sorafenib^{21,22}. Therefore, the potential drug-drug interaction exists for the co-administration of wogonin and the clinical drugs mainly undergoing UGT1A9-catalyzed metabolic elimination.

Conclusion

Necessary monitoring was needed when wogonin was co-administered with the clinical drugs mainly undergoing UGT1A9-mediated glucuronidation elimination. Additionally, probe reactions-dependent inhibition of wogonin towards the activity of UGT1A9 should be paid attention when translating these *in vitro* data into *in vivo* situation.

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