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Isoliquiritigenin showed strong inhibitory effects towards multiple UDP-glucuronosyltransferase (UGT) isoform-catalyzed 4-methylumbelliferone (4-MU) glucuronidation

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ABSTRACT

Isoliquiritigenin, a herbal ingredient with chalcone structure, has been speculated to be able to inhibit one of the most drug-metabolizing enzymes (DMEs) UDP-glucuronosyltransferase (UGT). Therefore, the aim of the present study was to investigate the inhibition of isoliquiritigenin towards important UGT isoforms in the liver and intestine, including UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9 and 1A10. The recombinant UGT-catalyzed 4-methylumbelliferone (4-MU) glucuronidation was used as probe reactions. The results showed that 100 μM of isoliquiritigenin inhibited the activity of UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10 by 95.2%, 76.1%, 78.9%, 87.2%, 67.2%, 94.8%, and 91.7%, respectively. The data fitting using Dixon plot and Lineweaver–Burk plot showed that the inhibition of UGT1A1, UGT1A9 and UGT1A10 by isoliquiritigenin was all best fit to the competitive inhibition, and the second plot using the slopes from the Lineweaver–Burk plot versus isoliquiritigenin concentrations was used to calculate the inhibition kinetic parameter (K_i) to be 0.7 μM, 0.3 μM, and 18.3 μM for UGT1A1, UGT1A9, and UGT1A10, respectively. All these results indicated the risk of clinical application of isoliquiritigenin on the drug–drug interaction and other possible diseases induced by the inhibition of isoliquiritigenin towards these UGT isoforms.

1. Introduction

Isoliquiritigenin, a herbal ingredient with chalcone structure, has been found in *Glycyrrhiza uralensis* (licorice), *Allium ascalonicum*, *Sinofranchetia chinensis*, *Dalbergia odorifera*, and *Glycine max* L. [1–5]. Various biological and pharmacological activities have been demonstrated for isoliquiritigenin, including inflammation-prevention [6] and anti-tumor activity towards various cancers, such as hepatocellular carcinoma, lung cancer, cervical carcinoma, and prostate cancer [7–9].

Drug metabolizing enzyme (DME)-catalyzed metabolic elimination significantly affects the concentration of drugs in plasma and therapeutic targets [10]. Inhibition of the DMEs' activity can significantly increase the exposure of drugs, possibly resulting in the adverse effects of drugs, especially for the drugs with narrow therapeutic index [11]. In the past decades, the cytochrome P450 (CYP) inhibition by xenobiotics (drugs, herbal ingredients, etc.) has been widely studied and regarded as the most important influencing factor for clinical drug–drug interaction (DDI) and herb–drug interaction (HDI) [12].

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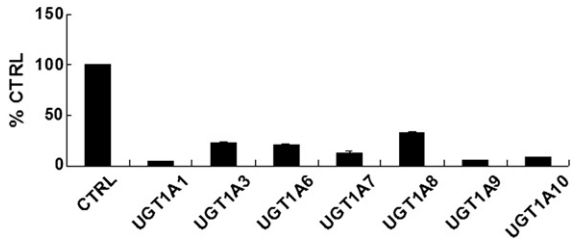

Fig. 1. Screening inhibition of isoliquiritigenin (100 μM) towards important UDP-glucuronosyltransferase (UGT) isoforms. Recombinant UGT enzyme-catalyzed 4-methylumbelliferone (4-MU) glucuronidation was used.

Glucuronidation reaction plays a crucial role in the metabolic elimination of clinical drugs, and is drawing more and more attention in recent years [13]. Inhibition of UGT activity can not only induce drug–drug interaction, but also cause other clinically significant results. For example, decreased activity of UGT1A8 and UGT2B4 has good correlation with high risk of esophageal cancer [14]. The inhibition of UGT1A1 can result in the decreased activity of bilirubin conjugation, and then might induce hyperbilirubinemia [15].

Licorice–drugs interaction has been considered to be a severe safety problem limiting the utilization of licorice, and the influence of its major ingredients towards drug-metabolizing enzymes might be an important reason. The present study focused on the evaluation of isoliquiritigenin's inhibition towards important UGT isoforms in the liver and intestine. Furthermore, the inhibition kinetic type and parameters (K_i) were determined. Extrapolation of in vivo inhibition magnitude was also carried out using the in vivo plasma concentration of isoliquiritigenin in rat.

2. Materials and methods

2.1. Chemicals and reagents

Isoliquiritigenin, 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris–HCl, and 7-hydroxycoumarin and uridine-5′-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St Louis, MO). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10) expressed in baculovirus-infected insect cells were obtained from BD Gentest Corp. (Woburn, MA, USA). All other reagents were of the HPLC grade or of the highest grade commercially available.

2.2. Incubation and analysis methods for inhibition evaluation

Recombinant UGT isoform-catalyzed 4-MU glucuronidation reaction was employed to evaluate the inhibition potential of isoliquiritigenin towards various UGT isoforms as previously described [16,17]. The incubation mixture (200 μL) contained recombinant UGT isoforms (final concentration: 0.25, 0.05, 0.025, 0.05, 0.05, 0.05, 0.05 mg/ml for UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, respectively), 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris–HCl buffer

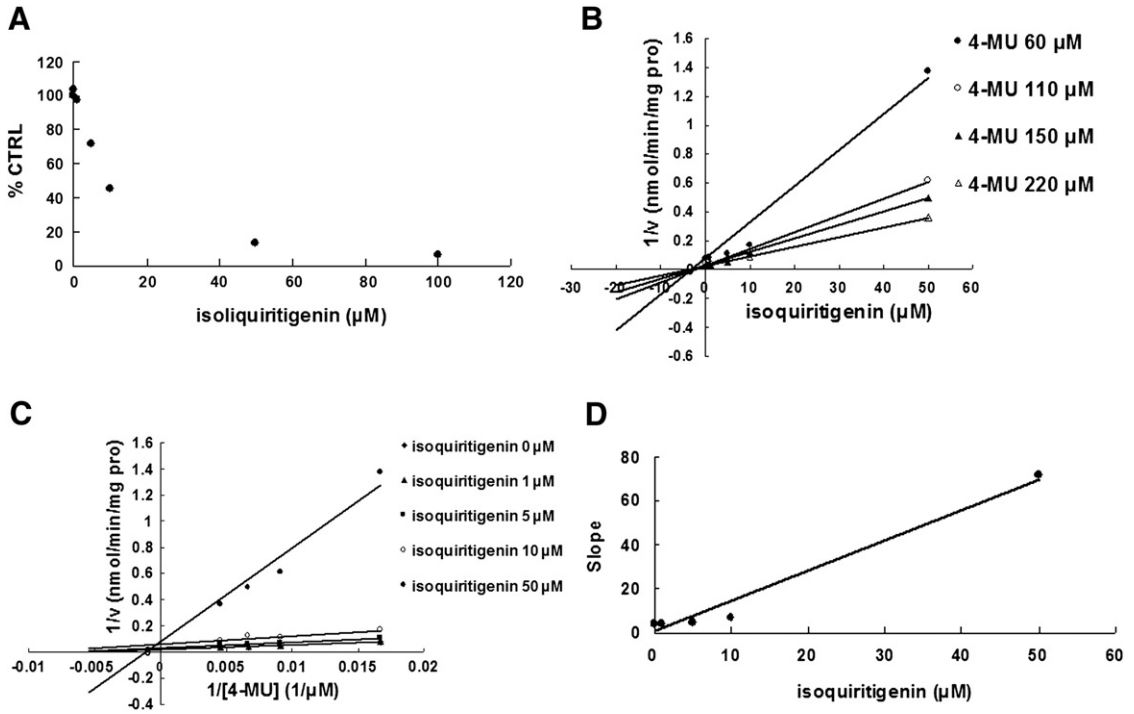

Fig. 2. Determination of inhibition kinetic type and parameters (K_i) for the inhibition of UGT1A1 by isoliquiritigenin. (A) Isoliquiritigenin exerted a dose-dependent inhibition towards UGT1A1-catalyzed 4-MU glucuronidation reaction. (B) Dixon plot of inhibitory effects of isoliquiritigenin towards recombinant UGT1A1-catalyzed 4-MU glucuronidation. (C) Lineweaver–Burk plot of inhibitory effects of isoliquiritigenin towards recombinant UGT1A1-catalyzed 4-MU glucuronidation. (D) Second plot of slopes from Lineweaver–Burk plot versus isoliquiritigenin concentrations. Every data point represents the mean of two replicates.