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Regular article

Glucuronidation of bavachinin by human tissues and expressed UGT enzymes: Identification of UGT1A1 and UGT1A8 as the major contributing enzymes

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ABSTRACT

Bavachinin (BCI), a major bioactive compound in Chinese herbal *Psoralea corylifolia*, possesses a wide range of biological activities. In this study, the glucuronidation pathway of BCI was characterized for the first time, by using pooled human liver microsomes (HLM), pooled human intestine microsomes (HIM) and recombinant human UDP-glucosyltransferases (UGTs). One mono-glucuronide was detected in HLM in the presence of uridine-diphosphate glucuronic acid (UDPGA), and it was biosynthesized and well-characterized as BCI-4′-O-glucuronide (BCIG). Reaction phenotyping assay showed that UGT1A1, UGT1A3 and UGT1A8 were involved in BCI-4′-O-glucuronidation, while UGT1A1 and UGT1A8 displayed the higher catalytic ability among all tested UGT isoforms. Kinetic analysis demonstrated that BCI-4′-O-glucuronidation in both HLM and UGT1A1 followed sigmoidal kinetic behaviors and displayed much close K_m values (12.4 μM in HLM & 9.7 μM in UGT1A1). Both chemical inhibition assays and correlation analysis demonstrated that UGT1A1 displayed a predominant role in BCI-4′-O-glucuronidation in HLM. Both HIM and UGT1A8 exhibited substrate inhibition at high concentrations, and K_m values of HIM and UGT1A8 were 3.6 and 2.3 μM, respectively. Similar catalytic efficiencies were observed for HIM (199.3 μL/min/mg) and UGT1A8 (216.2 μL/min/mg). These findings suggested that UGT1A1 and UGT1A8 were the primary isoforms involved in BCI-4′-O-glucuronidation in HLM, and HIM, respectively.

1. Introduction

Fructus Psoraleae (Chinese name: *Buguzhi*), the dried ripe fruit of *Psoralea corylifolia Linn* (*Fabaceae*), is a famous traditional Chinese medicine (TCM) used in China and Southeast Asia [1]. As an edible herbal, *Fructus Psoraleae* (FP) possesses many beneficial activities, and has been widely used for the treatment of vitiligo, osteoporosis and various skin diseases with long history [2,3]. Bavachinin (BCI, Fig. 1), one of the most abundant bioactive components in FP, possesses a wide range of biological activities, including anti-inflammatory, antipyretic, and mild analgesic properties, has the potential as a potent anti-asthma drug, and thus has attracted much attention in recent years [4–6].

In contrast to the extensive studies in pharmacology activities, the metabolic pathways of BCI in humans and experimental animals have not been well characterized. To date, only one literature has been reported regarding the pharmacokinetics (PK) properties of BCI in mice, in which BCI can be rapidly absorbed but it can be extensively converted to a more polar metabolite [7]. Unfortunately, neither the metabolic pathway nor the major metabolite(s) of BCI in both mice and humans have been identified to date. It is well-known that the elucidation of metabolic pathway(s) and identification of the key drug-metabolizing enzymes will be very helpful for the understanding of the disposition of a given drug, thus provides essential information about the variation in drug efficacy and toxicity and potential drug-herbal interactions [8–12]. Therefore, it is of great importance to investigate the metabolic profiles and identify the major enzymes responsible for BCI metabolism in human tissues.

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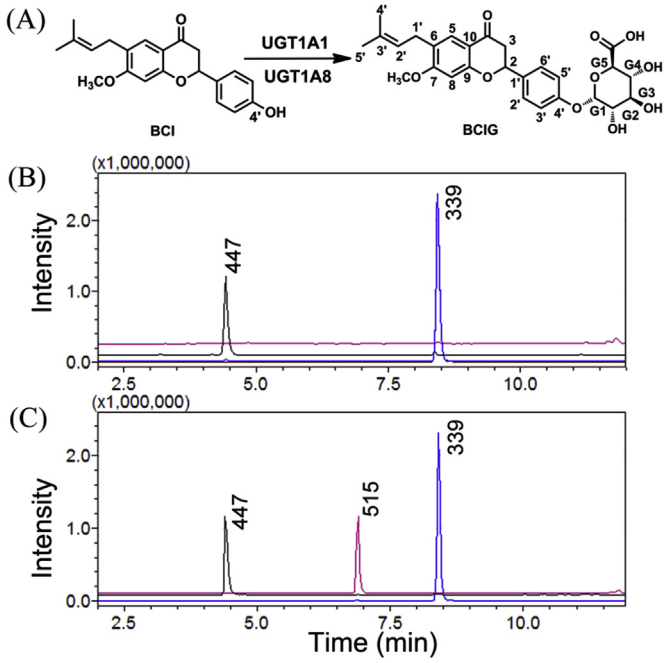

Fig. 1. Glucuronidation pathway of BCI (A). Representative selected ion chromatograms of *m/z* 515 (BCIG) and *m/z* 447 (Baicalin) and *m/z* 339 (BCI) in HLM: BCI was incubated at 37 °C for 60 min with (B) or without (C) UDPGA.

From the view of chemical structure, BCI is a natural phenolic compound with flavonoid skeleton coupled with one labile metabolic site (the phenolic group at the C-4′ site) for phase II metabolism. It is well known that most of flavonoids are subjected to rapid and extensive phase II metabolism (such as glucuronidation and sulfation), thus resulting in a poor oral bioavailability [13]. Previous studies have shown that UDP-glucuronosyltransferase 1A1 (UGT1A1) displays the specific ability of regioselective glucuronidation of 4′-OH group of flavonoids, such as 3,3′,4′-trihydroxyflavone (3,3′,4′-THF) and 3,6,4′-trihydroxyflavone (3,6,4′-THF) [14]. Our preliminary study on BCI metabolism also found that a mono-glucuronide could be rapidly formed in liver microsomes from various species including mouse and human, and the same metabolite could be easily detected following intravenous administration of BCI to mice. These findings implied that BCI was a good substrate for mammals UGTs and prompted us to investigate the glucuronidation pathway(s) of BCI in human tissues.

The main objective of this study is to characterize the glucuronidation pathway(s) of BCI, and to identify the major enzymes involved in BCI glucuronidation using human tissues and recombinant UGTs. To this end, reaction phenotyping, chemical inhibition assays, kinetic analysis and correlation analysis are used to assign the major enzyme(s) involved in BCI glucuronidation, as well as to characterize the glucuronidation pathway in human tissues.

2. Materials and methods

2.1. Chemicals and reagents

Bavachinin (purity > 98%), baicalin (purity > 98%) and glycyrrhetinic acid (purity > 98%) were purchased from Chengdu Pufei De Biotech Co., Ltd. (Chengdu, Sichuan, China), chenodeoxycholic acid (CDCA) was obtained from Dalian Meilun Biotech Co., Ltd. (Dalian, Liaoning, China), bavachinin-4′-O-glucuronide (BCIG) was biosynthesized by the author (Xia Lv) and fully characterized. 17β-estradiol, magnolol, 4-methylumbelliferyl-β-D-glucuronide hydrate (4-MUG), 3′-azido-3′-deoxy-thymidine (AZT), fluconazole, uridine diphosphoglucuronic acid (UDPGA) as triammonium salt, Brij58 and magnesium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials were of analytical grade. A panel of 12 recombinant human UGT isoforms (i.e., UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17) was purchased from BD Biosciences (Woburn, MA, USA). Pooled human liver microsomes (HLM, n = 50), pooled human intestine microsomes (HIM, n = 10) and a bank of individual human liver microsomes (n = 11, designated as HLM-1, HLM-2 …, HLM-11) were purchased from Research Institute for Liver Diseases (RILD, Shanghai, China).

2.2. Analytical instruments and conditions

BCI and its glucuronide were analyzed by a UFLC system (Shimadzu, Kyoto, Japan), equipped with a CBM-20A communications bus module, a DGU-20A3 vacuum degasser, two LC-20AD pumps, a SIL-20ACHT autosampler, a CTO-20AC column oven, an SPD-M 20A diode array detector, a mass detector (2010 EV) with an electrospray ionization (ESI) interface, and a computer equipped with UFLC-MS solution software (version 3.41; Shimadzu). A Shim-pack XR-ODS (2.2 μm, I.D. 2.0 mm × 75.0 mm; Kyoto, Japan, Shimadzu) analytical column with an ODS guard column (2.2 μm, I.D. 2.0 mm × 5 mm; Shimadzu) was used to analyze the parent compound and its corresponding glucuronide. Column temperature was kept at 40 °C. The mobile phase was consisted of CH_3CN (A) and water/0.2% formic acid (B) at a flow rate of 0.4 mL/min, with the following gradient: 0–1 min, 90% B to 75% B; 1–8 min, 75% B to 48% B; 8–11 min, 10% B; and 11–15 min, balanced to 90% B. BCI and its metabolite were detected at 275 nm.

Quantification of BCIG was performed by LC-ESI-MS in the positive ionization mode (ESI^+) with selective ion monitoring (SIM) detection. MS detection conditions were as follows: the detector voltage was set at +1.55 kV for positive ion detection; the curved desolvation line (CDL) temperature and the block heater temperature were both set at 200 °C, whereas the CDL voltage was set at + 40 V; interface voltage, + 4.0 kV for positive ion detection; nebulizing gas (N_2) flow, 1.5 L/min; and drying gas (N_2) pressure, 0.06 MPa. Data processing was performed using the LC-MS solution software (version 3.41; Shimadzu).

2.3. Estradiol-3-O-glucuronidation assays

Estradiol (10 μM) was incubated with individual HLM for 30 min, with the final protein concentrations of 0.2 mg/mL. UFLC analysis methods of estradiol glucuronidation samples were the same as the analysis of BCI with the detector wavelength set at 280 nm. Quantification of metabolites was performed by LC-ESI-MS in the negative ionization mode (ESI^-) with SIM detection. The other MS detection conditions were the same as the analysis of BCI glucuronidation.

2.4. Biosynthesis of BCIG and NMR analysis

The metabolite was biosynthesized using liver microsomes from rat, meanwhile isolation and purification was completed for structure elucidation and quantitative analysis. Briefly, the reaction system consisted of 500 μM BCI was incubated with 0.25 mg protein/mL liver microsomes, where 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, Brij 58 (0.1 mg/mg protein), and 2 mM UDPGA was also included for 5h-incubations at 37 °C. The reaction was terminated by adding of 200 μL acetonitrile. The SPE cartridge (C18 and anion exchange resin, 1000 mg; Dalian Sipore, Dalian, China) was