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Sulfation disposition of liquiritigenin in SULT1A3 overexpressing HEK293 cells: The role of breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 4 (MRP4) in sulfate efflux of liquiritigenin

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ABSTRACT

This study aimed to investigate the cellular disposition of liquiritigenin via the sulfonation pathway and the role of efflux transporters in liquiritigenin sulfate excretion. The sulfonation disposition of liquiritigenin was investigated using SULT1A3 overexpressed HEK293 cells (HEK-SULT1A3 cells). Liquiritigenin generated one mono-sulfate metabolite (7-O-sulfate) in HEK-SULT1A3 cell lysate. And the sulfonation followed the Michaelis-Menten kinetic (V_{max} = 0.84 nmol/min/mg and K_m = 7.12 μM). Expectedly, recombinant SULT1A3 (hSULT1A3) showed a highly similar kinetic profile with cell lysate. Furthermore, 7-O-sulfate was rapidly generated and excreted in HEK-SULT1A3 cells. Ko143 (a BCRP-selective inhibitor) at 20 μM significantly decreased the excretion rate of liquiritigenin sulfate (> 42.5%, $p < 0.001$). Moreover, the pan-MRPs inhibitor MK-571 at 20 μM essentially abolished the liquiritigenin sulfate effluxion, resulting in the marked reduction of excretion rate (> 97.4%, $p < 0.001$). Furthermore, knockdown of BCRP led to moderate reduction in sulfate excretion (15.9%–16.9%, $p < 0.05$). Silencing of MRP4 caused significant decreased in sulfate excretion (20.2%–32.5%, $p < 0.01$). In conclusion, one sulfate metabolite was generated from liquiritigenin in HEK-SULT1A3 cells. BCRP and MRP4 should be the key factors for the cellular excretion of liquiritigenin sulfate.

1. Introduction

Flavonoids are major nature phenolic compounds and are widely distributed in fruits, nuts, vegetables and herbs (Ahmed and Eun, 2017; Geng et al., 2017). Chemically, these compounds can be categorized into flavanones, flavonols, flavones, isoflavones, catechins, anthocyanidins, and chalcones (Ross and Kasum, 2002). Previous studies have demonstrated that flavonoids undergo extensive phase II metabolism (Ruefer et al., 2005). Liquiritigenin [(*S*)-7-Hydroxy-2-(4-hydroxyphenyl)chroman-4-one] belongs to flavanone and is the active ingredient of traditional Chinese medicine *Glycyrrhizae radix et rhizome* (Fu et al., 2005). Due to the sweetness and multiple-activities, liquiritigenin is also ubiquitously used as a food additive and/or nutritional supplement (Kao et al., 2014; Liu et al., 2000; Madak-Erdogan et al., 2016; Lecomte et al., 2017). Various studies have indicated that liquiritigenin also possesses many pharmacological activities, such as anti-inflammatory, anti-oxidant, anti-diabetic and anti-cancer effects (Kim et al., 2008; Suh et al., 2014; Gaur et al., 2014; Wang et al., 2014). Although showing favorable permeability, liquiritigenin is found to have low oral bioavailability (Wang et al., 2017; Kang et al., 2009a). One of the main reasons for the poor bioavailability of liquiritigenin is extensive phase II metabolism (i.e., glucuronidation and sulfonation) (Lee et al., 2013). Therefore, inhibition of metabolism seems to be an effective pathway to improve the bioavailability of liquiritigenin.

Sulfonation reaction is an important phase II metabolic pathway in addition to glucuronidation and catalyzed by sulfotransferases (SULTs) (Gamage et al., 2006; Coughtrie, 2016). Under the action of SULTs, a sulfonate group is transferred from 3′-phosphoadenosine-5′-phosphosulfate

Abbreviations: CL_{int}, intrinsic clearance; CYP, cytochrome P450; HPLC, high performance liquid chromatography; K_m, Michaelis-Menten constant; MS, mass spectroscopy; PAPS, 3′-Phosphoadenosine-5′-phosphosulfate; QTOF, Quadrupole time-of-flight; shRNA, short hairpin RNA; SULT, sulfotransferase; UGT, uridine diphosphoglucuronic-glucuronosyltransferase; UPLC, ultra-performance liquid chromatography; V_{max}, maximal velocity

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(PAPS) to the nucleophilic group of the substrates (i.e., hydroxyl and amino group) (Gamage et al., 2006; Coughtrie, 2016). Generally, Sulfonation reaction can enhance the water solubility of parent drug, thereby facilitating inactivation and elimination of numerous endobiotics (e.g. estrogen, thyroid hormones and catecholamines) and xenobiotics (e.g. drugs, environmental pollutants and dietary polyphenols) (Mueller et al., 2015; James and Ambadapadi, 2013). Human SULTs have divided into four families, containing SULT1, SULT2, SULT4 and SULT6 (Gamage et al., 2006). Due to wide distribution and abundant expression in human liver, intestine and other metabolic tissues or organs, SULT1 and SULT2 families are regarded as vital enzymes involved in the metabolism of endobiotics and/or xenobiotics (Lindsay et al., 2008).

ATP-binding cassette (ABC) transporters mediate the transmembrane transport of endobiotics and xenobiotics using the energy from the ATP hydrolysis (Higgins, 2001). P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance-associated Proteins (MRPs) belong to ABC transporter family and are connected with the absorption, distribution, elimination of drugs (Fromm, 2003; Mao and Unadkat, 2015; Nigam, 2015). Due to the polar nature, the excretion of sulfates is relies on efflux transporters. BCRP and MRPs, which mainly transport anions, have been demonstrated to contribute to the extracellular transport of sulfate metabolites (Li et al., 2015; Sun et al., 2015a; Zhou et al., 2015). In addition, the interplay between efflux transporters and metabolic enzymes (i.e., CYPs, UGTs and SULTs) has been confirmed (Zhang et al., 2015; Sun et al., 2015b; Zhao et al., 2016; Li et al., 2016). Zhang et al. (2015) and Sun et al. (2015b) have demonstrated that BCRP, MRP1, MRP3 and MRP4 regulated the total glucuronidation of genistein and apigenin. Furthermore, Zhao et al. (2016) has confirmed that MRP4 mediated the efflux of hesperetin sulfates. However, there is no information about the effect of efflux transporter on liquiritigenin sulfate excretion.

Because liquiritigenin has shown many pharmacological activities, its metabolism has been mainly studied on animal models, such as mice, rats, rabbits and dogs (Kang et al., 2009a; Shimamura et al., 1993; Kang et al., 2009b). After intravenous or oral administration, five glucuronides and sulfates metabolites of liquiritigenin, known as 4′-O-glucuronide (M1), 7-O-glucuronide (M2), 4′-O-glucuronide-7-O-sulfate (M4) and 7-O-glucuronide-4′-O-sulfate (M5), have been found in plasma or bile of mice, rats, rabbits and dogs (Kang et al., 2009a; Shimamura et al., 1993). Furthermore, Oxidative metabolism of liquiritigenin has been extensively studied in rats and human liver microsomes (Wang et al., 2011). However, the sulfonation of liquiritigenin in human is still unknown.

In our present study, we aimed to evaluate liquiritigenin sulfonation using human expressed SULT enzymes. Kinetic parameters were determined by fitting an appropriate model to the data. SULT1A3 was selected because 1) this enzyme has shown high activities in conjugating flavonoids in compare with other isoforms, such as SULT1A1 and SULT1E1 (Pai et al., 2001; Huang et al., 2009; Meng et al., 2012); 2) SULT1A3 was one of the two highly expressed SULTs in the intestine, which indicated that this enzyme has implications for the oral bioavailability of a number of drugs (Riches et al., 2009; James and Ambadapadi, 2013). Hence, the cDNA of SULT1A3 was introduced into HEK293 cells using lentiviral transfection approach. Furthermore, the contribution of BCRP and MRPs in sulfates transport was determined using SULT1A3 overexpressed human embryonic kidney 293 (HEK293) cells.

2. Methods and materials

2.1. Materials

Recominant human SULT1A3 enzyme (hSULT1A3) was purchased from Sekisui XenoTech LCC (Kansas, USA). Liquiritigenin (> 98% pure) was purchased from Baoji Herbest Bio-Tech Co., Ltd. (Baoji, China). Liquiritigenin-7-O-sulfate (or liquiritigenin sulfate) was prepared in our laboratory using HEK-SULT1A3 cells. Adenosine 3′-phosphate 5′-phosphosulfate (PAPS), Ko143 and MK-571 was purchased from Sigma-Aldrich (St Louis, MO). Primary antibodies of SULT1A3, BCRP, MRP1, MRP2, MR3, MRP4, MRP5 and β-actin were purchased from OriGene Technologies (Rockville, MD). All other materials (typically analytical grade or better) were used as received.

2.2. Sulfonation assay and kinetics evaluation

Liquiritigenin was incubated with recombinant human SULT1A3 (hSULT1A3) enzyme to determine the rate of sulfation as described in our previous publications (Sun et al., 2015a). In brief, the incubation medium contained hSULT1A3 enzyme (3.50 μg/ml), PAPS (20 μM) and different concentrations of liquiritigenin (0.63 μM–80 μM) in 50 mM potassium phosphate buffer (pH 7.4) to a total volume of 200 μl. Reaction was conducted at 37 °C for 60 min, then ice-cold acetonitrile was added to terminate reaction. After vortex and centrifugation (15,000*g*; 15 min), the supernatant was subjected to HPLC analysis for sulfate quantification. All incubations were performed in triplicates. Sulfation rates were calculated as nmol sulfate formed per reaction time per protein amount (or nmol/min/mg). Preliminary experiments were performed to ensure that the rates of sulfation were determined under linear conditions with respect to incubation time and protein concentration.

2.3. Enzyme kinetics modeling

The sulfation rates of liquiritigenin were collected according to the sulfation assay protocol. The kinetic model Michaelis–Menten (Eq. 1) was fitted to the date of sulfation velocity (V) and substrate concentrations. Eadie-Hofstee plot was the transform of Michaelis–Menten profile, and it was visible for the kinetic model selection. In the Eadie-Hofstee plot, V was plotted versus the ratio of the velocity to the substrate concentration (V/[S]). And kinetic parameters were evaluated using GraphPad Prism software (5.3 version).

$$V = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad (1)$$

where V_{max} is the maximal velocity, K_m is the Michaelis constant. The intrinsic clearance (CL_{int}) was derived by V_{max}/K_m.

2.4. Structure identification of liquiritigenin sulfate

The structure of liquiritigenin sulfate was identified using UPLC-QTOF/MS method as described (Sun et al., 2015c). Briefly, samples were separated using the Waters ACQUITY UPLC system and BEH column (2.1 × 50 mm, 1.7 μm; Waters, Milford, MA). Gradient elution was performed using formic acid (0.1%) in water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 0.45 ml/min. Elution conditions was 5% B at 0–1.0 min, 5%–95% B at 1.0–3.0 min, 95%B at 3.0–3.5 min, 95%–5% B at 3.5–4.0 min.

After chromatographic separation, samples were analyzed through Xevo G2 QTOF/MS (Waters) in the negative mode. The precursor and fragment ion information was collected using the electrospray ionization source (ESI) in MSMS mode. The capillary, sampling cone and extraction cone voltages were 3000, 40 and 4 V, respectively. The desolvation gas (nitrogen) and cone gas was set to 800 and 30 L/h, respectively. The desolvation and source temperature were 350 °C and 100 °C, respectively. The collision energy was 20 eV. Dates were analyzed using MassLynx version 4.1.

2.5. Cell culture and cell-lysate preparation

SULT1A3 overexpression HEK293 cells (HEK-SULT1A3 cells) were established and preserved in our lab as described previously (Sun et al., 2015b). For experiments, HEK-SULT1A3 cells were grown in Dulbecco's