Drug Metabolism and Pharmacokinetics 32 (2017) 218–223

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

Inhibition of UDP-glucuronosyltransferase (UGT)-mediated glycyrrhetinic acid 3-*O*-glucuronidation by polyphenols and triterpenoids

Mayuko Koyama [a], Tatsuya Shirahata [b], Rika Hirashima [a], Yoshinori Kobayashi [b], Tomoo Itoh [a], Ryoichi Fujiwara [a, *]

[a] Department of Pharmaceutics, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[b] Department of Pharmacognosy, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

ARTICLE INFO

Article history:
Received 12 January 2017
Received in revised form
6 April 2017
Accepted 24 April 2017
Available online 4 May 2017

Keywords:
UDP-Glucuronosyltransferase
UGT
Licorice
Glycyrrhetinic acid
Glycyrrhizin
Glucuronidation
Glucuronide
Natural medicine
Adverse reaction
Pseudohyperaldosteronism

ABSTRACT

Glycyrrhetinic acid (GA) is an active metabolite of glycyrrhizin, which is a main constituent in licorice (*Glycyrrhiza glabra*). While GA exhibits a wide variety of pharmacological activities in the body, it is converted to a toxic metabolite GA 3-*O*-glucuronide by hepatic UDP-glucuronosyltransferases (UGTs). To avoid the development of the toxic metabolite-induced pseudohyperaldosteronism (pseudoaldosteronism), there is a limitation in maximum daily dosage of licorice and in combined usage of other glycyrrhizin-containing natural medicine. In this study, we investigated the inhibitory effects of various polyphenols and triterpenoids on the UGT-mediated GA 3-*O*-glucuronidation. In human liver microsomes, UGT-mediated GA glucuronidation was significantly inhibited by protopanaxadiol with an IC_{50} value of 59.2 μM. Isoliquiritigenin, rosmarinic acid, alisol B, alisol acetate, and catechin moderately inhibited the GA glucuronidation with IC_{50} values of 96.4 μM, 125 μM, 160 μM, 163 μM, and 164 μM. Other tested 19 polyphenols and triterpenoids, including liquiritigenin, did not inhibit UGT-mediated GA glucuronidation in human liver microsomes. Our data indicate that relatively higher dosage of licorice can be used without a risk of developing pseudohyperaldosteronism in combination of natural medicine containing protopanaxadiol such as *Panax ginseng*. Furthermore, supplemental protopanaxadiol and isoliquiritigenin might be useful in preventing licorice-inducing pseudoaldosteronism.

1. Introduction

Licorice is the root of *Glycyrrhiza glabra*, which is one of traditional Chinese medicine used as an anti-inflammatory agent [1]. The main constituent of licorice, glycyrrhizin, is converted to Glycyrrhetinic acid (GA) by intestinal enterobacteria [2]. As shown in Fig. 1, GA is efficiently absorbed from small intestine into blood circulation due to its high hydrophobic property. In the liver, GA is further converted to GA 3-*O*-glucuronide by UDP-glucuronosyltransferases (UGTs; EC 2.4.1.17) [3], which are a family of membrane-bound enzymes that catalyze glucuronidation of endogenous and exogenous compounds by transferring the glucuronic acid moiety of UDP-glucuronic acid to the substrates [4,5]. Human UGTs are mainly divided into two distinct families, UGT1 and UGT2, on the basis of evolutionary divergence and homology [6]. It was previously reported that UGT1A1, UGT1A3, UGT2B4, and UGT2B7 were involved in the formation of GA glucuronide [3]. Although glucuronides are usually less toxic than the parent compounds, GA glucuronide has a potency to induce pseudohyperaldosteronism (pseudoaldosteronism) [7]. To avoid the onset of pseudohyperaldosteronism, therefore, there is a limitation in maximum daily dosage of licorice.

Combination of traditional Chinese medicine can increase or promote the therapeutic effectiveness. However, since various natural medicines other than licorice also contain glycyrrhizin and GA, combined usage of licorice with glycyrrhizin- and GA-containing natural medicine is not preferred. An important question here is whether this assumption is true. The enzymes involved in the metabolic activation of GA – UGTs – can be inhibited by a wide variety of administered xenobiotics [8–10]. Indeed, it was previously reported that UGT2B7 was significantly inhibited by

Abbreviations: DMSO, dimethyl sulfoxide; GA, Glycyrrhetinic acid; HPLC, high-performance liquid chromatography; UDPGA, UDP-glucuronic acid; UGTs, UDP-glucuronosyltransferases.

* Corresponding author.
E-mail address: fujiwarar@pharm.kitasato-u.ac.jp (R. Fujiwara).

http://dx.doi.org/10.1016/j.dmpk.2017.04.003
1347-4367/

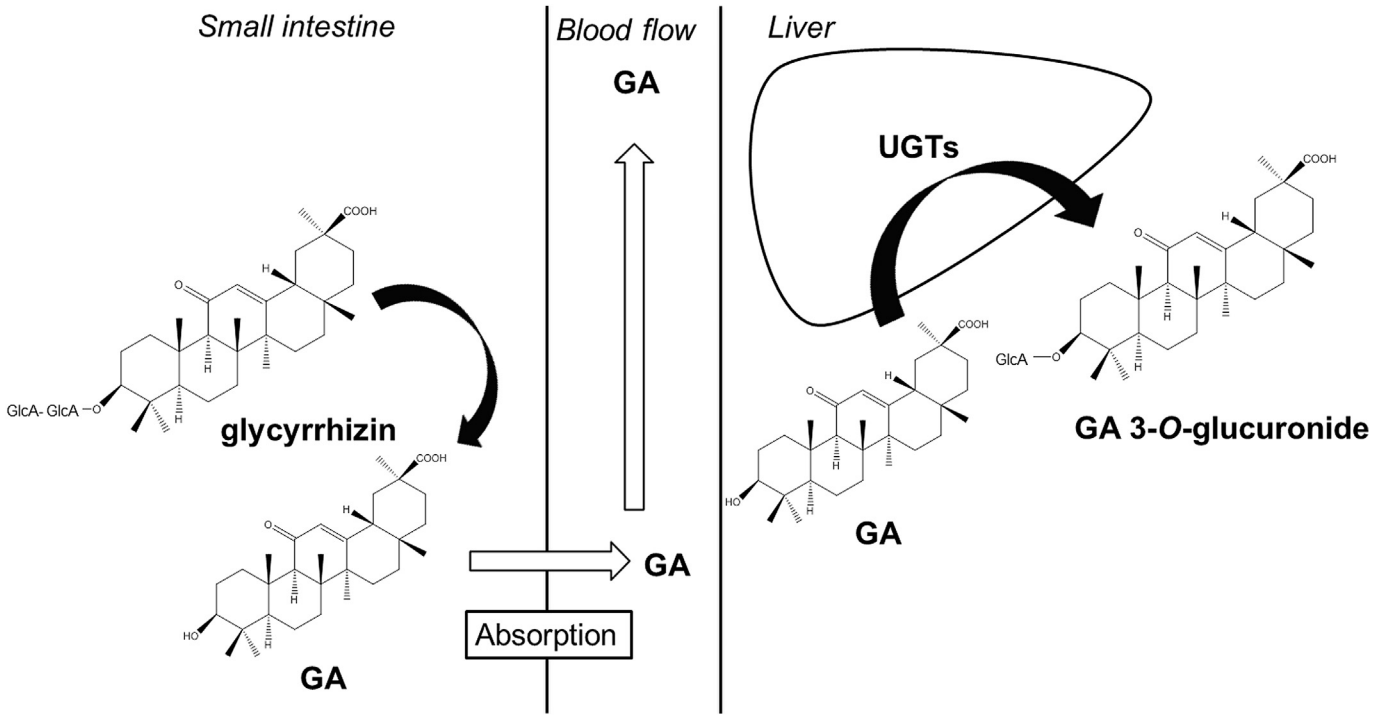

Fig. 1. The metabolic pathway of glycyrrhizin. Glycyrrhizin is first converted to its hydrolyzed form glycyrrhetinic acid (GA) in the small and large intestine. GA is absorbed into the circulating blood flow and it's metabolized to mono-glucuronide in the liver.

andrographolide, which is a main constituent of herbal *Andrographis paniculata* [11]. Deoxyschizandrin and schisantherin A, which are major bioactive lignans isolated from *Fructus schisandrae*, have also been identified as potent UGT inhibitors [12]. These observations indicate that inhibition of GA 3-*O*-glucuronidation by co-administered natural medicine might lead to an establishment of safe combined usage of licorice with other Chinese medicine.

In this study, inhibitory effects of 25 natural polyphenols and triterpenoids on UGT-mediated GA 3-*O*-glucuronidation were determined in human liver microsomes. This is the first study showing the potent inhibition of UGT-mediated GA 3-*O*-glucuronidation by protopanaxadiol and isoliquiritigenin. Combined usage of licorice with Chinese medicine that highly contains protopanaxadiol and/or isoliquiritigenin will maximize the therapeutic effectiveness of licorice without increasing a risk of developing pseudoaldosteronism.

2. Materials and methods

2.1. Chemicals

Human liver microsomes were purchased from BD Gentest (Woburn, MA). Alamethicin, estradiol, serotonin, licochalcone A, and liquiritigenin were obtained from Sigma–Aldrich (St Louis, MO). UDP-glucuronic acid (UDPGA) was purchased from Nacalai Tesque (Kyoto, Japan). GA, alisol A, alisol, alisol B acetate, (*E*)-asarone, astragaloside IV, ergosterol, β-eudesmol, ginsenoside Rb1, glabridin, liquiritin, perillaldehyde, rosmarinic acid, and saikosaponin were obtained from Wako Pure Chemical (Osaka, Japan). Apigenin 7-*O*-glucoside, linarin, luteolin, and luteolin 7-*O*-glucoside were purchased from Extrasynthese (Lyon-Nord, France). Betulinic acid, isoliquiritigenin, and oleanolic acid hydrate were from Tokyo Chemical Industry (TCI, Tokyo, Japan). Catechin mixture and protopanaxadiol were obtained from Matsuura Yakugyo Co., Ltd. (Nagoya Japan). Isoliquiritin was purchased from Kanto Chemicals (Tokyo, Japan). All other chemicals and solvents were of analytical grade or the highest grade commercially available.

2.2. Glucuronidation of GA and inhibition assays

Glucuronidation of GA was determined according to the method of Lu et al. [3] with slight modifications. Briefly, a typical incubation mixture (200 μl of total volume) contained 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 2.5 mM UDPGA, 50 μg/ml alamethicin, 0.25 mg/ml microsomes, 5 mM saccharolactone, 40 μM substrate (GA), and inhibitors. The reaction was initiated by the addition of UDPGA after a 5-min preincubation at 37 °C. After incubation at 37 °C for 15, 30, 45, 60, and 90 min, the reaction was terminated by addition of 200 μl of cold acetonitrile containing 5% glacial acetic acid. After removal of the protein by centrifugation at 12,000 g for 5 min, supernatant was subjected to high-performance liquid chromatography (HPLC).

In this study, GA and inhibitors were dissolved in dimethyl sulfoxide (DMSO). The concentrations of inhibitors used in our inhibition assays were listed in Table 1. The inhibitors were added into the reaction mixtures prior to the incubation at 37 °C. The final concentration of the organic solvent (DMSO) in the incubation mixture was 1% (v/v). IC_{50} values were directly determined from linear regression.

To obtain a K_i value, different concentrations of substrate (5 μM, 20 μM, and 40 μM GA) and inhibitor (25 μM and 100 μM protopanaxadiol) were used. K_i value was obtained from the Dixon plot.

2.3. HPLC conditions

GA 3-*O*-glucuronide was determined by the following HPLC condition. The HPLC systems used in the present study were a LC-10AD pump (Shimadzu, Kyoto, Japan), a SPD-10A UV detector (Shimadzu), a SIL-10A autosampler (Shimadzu), a SLC-10A system controller (Shimadzu) and a Mightysil RP-18 GP column