Deglycosylation of Glucoaurantio-Obtusin Affects its Inhibition Capability Towards Drug Metabolizing Enzymes (DMEs)

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SUMMARY. Intestinal bacteria play a key role to change the properties of drugs or herbal components, including therapeutic role, toxicity and pharmacokinetic behaviour. The present study aims to investigate the role of intestinal bacteria in changing the inhibitory potential of herbal components towards drug-metabolizing enzymes (DMEs) through comparing the inhibition of UDP-glucuronosyltransferase (UGT) 1A7 by glucoaurantio-obtusin and aurantio-obtusin. The results showed that the activity of UGT1A7-catalyzed 4-MU glucuronidation reaction was inhibited by 60.4% and 80.5% at 100 μM of glucoaurantio-obtusin and aurantio-obtusin, indicating the importance of deglycosylation process for strengthening the inhibitory effect of glucoaurantio-obtusin towards UGT1A7. Noncompetitive inhibition was demonstrated for the inhibition of aurantio-obtusin towards UGT1A7, with the inhibition kinetic parameter (Ki) to be 23.8 μM. Given that low activity of UGT1A7 was related with the occurrence of some diseases (e.g. cancer, etc.), the inhibition of aurantio-obtusin towards UGT1A7 should be given much attention in clinical application of glucoaurantio-obtusin and aurantio-obtusin.

INTRODUCTION

Drug metabolism, also known as xenobiotic metabolism, is defined as a biochemical modification of pharmaceutical substances. Drug metabolism can be divided into phase I and phase II metabolism, converting lipophilic chemical compounds into hydrophilic products 1. The enzymes involved in the drug metabolism have been called as drug-metabolizing enzymes, and contained cytochrome P450 monooxygenase system, flavin-containing monooxygenase system, alcohol dehydrogenase, monoamine oxidase, UDP-glucuronosyltransferases, glutathione S-transferases, and methyltransferase, sulfotransferases 2.

Patients often take several drugs to achieve the therapeutic role. Additionally, patients might be exposed to various food and herbal components. Therefore, potential drug-drug interaction, herb-drug interaction, and food-drug interaction might occur. The compounds orally administered will undergo the structural modification catalyzed by metabolism by intestinal bacteria 3, which might influence the efficiency, toxicity, and pharmacokinetic behaviour. Previous literatures have indicated that the deglycosylation of licoritlin strongly enhanced the inhibitory potential towards drug-metabolizing enzymes UDP-glucuronosyltransferases (UGTs) 4.

Glucoaurantio-Obtusin is an important ingredient isolated from Cassiae Semen. Oral administration of glucoaurantion-obtusin can undergo deglycosylation process to form aurantion-obtusin 5. The present study aims to compare the inhibition potential of glucoaurantio-obtusin and aurantio-obtusin towards one of the most important UGT isoforms UGT1A7, indicating whether the deglycosylation biotransformation...
of glucourantio-obtusin towards aurantio-obtusin can affect the inhibition potential towards UGT1A7.

MATERIALS AND METHODS

Chemicals

Glucoaurantio-obtusin (purity ≥ 98%) and aurantio-obtusin (purity ≥ 98%) were purchased from Sichuan Weikeqi Biological Technology Co., Ltd. 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine-5’-diphosphoglucuronic acid (UDPGA, trisodium salt) were purchased from Sigma-Aldrich (St Louis, MO). Recombinant human UGT1A7 was obtained from BD Gentest Corp. (Woburn, MA, USA). Acetonitrile and methanol with HPLC grade was purchased from Merck, and all aqueous solutions were prepared using ultrapure Milli-Q water (> 18 MΩ). All other reagents were of HPLC grade or of the highest grade commercially available.

Evaluation of the inhibition of glaucourantio-obtusin and aurantio-obtusin towards UGT1A7-catalyzed 4-MU glucuronidation reaction

The typical incubation system to evaluate the inhibition of UGT1A7 by glaucourantio-obtusin and aurantio-obtusin contains recombinant UGT1A7 (0.05 mg/mL), 5 mM UDPGA, 5 mM MgCl2, 50 mM Tris-HCl buffer (pH = 7.4), and 15 µM 4-MU. The co-factor UDPGA was added after 5 min pre-incubation, and the incubation time was 30 min. 100 µL acetonitrile (100 µM 7-hydroxycoumarin as internal standard) was used to terminate the incubation reaction. The samples were centrifuged at 20000 × g for 10 min, and an aliquot (10 µL) was injected for HPLC analysis as previously described.

To determine the inhibition type and kinetic parameters (Ki), the reaction velocity was determined at a variety of concentrations of 4-MU and aurantio-obtusin, and Dixon plot and Lineweaver-Burk plot were employed to determine the inhibition type. As shown in Fig. 2, the intersection point in Dixon plot (Fig. 2A) and Lineweaver-Burk (Fig. 2B) was located in horizontal axis, indicating the noncompetitive inhibition of aurantio-obtusin towards UGT1A7. The Ki value was calculated to be 23.8 µM through nonlinear regression using noncompetitive inhibition equation.

RESULTS

As shown in Fig. 1, the activity of UGT1A7-catalyzed 4-MU glucuronidation reaction was inhibited by 60.4 and 80.5% at 100 µM of glaucourantio-obtusin and aurantio-obtusin, respectively. Furthermore, multiple reaction velocity (v) was determined at various concentrations of 4-MU and aurantio-obtusin, and Dixon plot and Lineweaver-Burk plots were employed to determine the inhibition type. As shown in Fig. 2, the intersection point in Dixon plot (Fig. 2A) and Lineweaver-Burk (Fig. 2B) was located in horizontal axis, indicating the noncompetitive inhibition of aurantio-obtusin towards UGT1A7. The Ki value was calculated to be 23.8 µM through nonlinear regression using noncompetitive inhibition equation.

DISCUSSION

Herbs are taken to promote vitality, balance, and longevity. The strength of botanicals lies in their capacity to support and nurture the body’s innate healing capacity. Herb-drug interaction (HDI) is not avoidable because herbs are always administered in combination with western therapeutic drugs. For example, Scutellaria baicalensis Georgi (huangqin) ameliorated irinotecan-induced gastrointestinal toxicity in cancer patients. Piperine from black (Piper nigrum L.) and long (P. longum L.) peppers induced the elevation of the AUC of phenytoin, propranolol and theophylline in healthy volunteers and plasma con-
concentrations of rifamipicin (rifampin) in patients with pulmonary tuberculosis.

Human UGT1A7 belongs to a family of nine proteins encoded at the human UGT1A gene locus on chromosome 2q37, which is highly conserved in vertebrate organisms. UGT1A7 is not expressed in hepatic tissues and has been shown to be differentially regulated in gastrointestinal organs such as the oesophagus, stomach, small intestine, and colon. The low activity of UGT1A7 has been strongly correlated with the occurrence of colorectal cancer (CRC).

In the present study, the influence of deglycosylation process towards strengthening the inhibitory activity towards drug-metabolizing enzymes (DMEs) was demonstrated through comparison of the inhibition potential of glucourantio-obtusin and aurantio-obtusin towards UGT1A7, indicating the important role of intestinal bacteria in changing the pharmacokinetic factors of compounds. Additionally, the clinical monitoring for the application of glucourantio-obtusin and aurantio-obtusin should be performed due to the strong inhibition of aurantio-obtusin towards UGT1A7.

REFERENCES