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The inhibitory effects of nor-oleanane triterpenoid saponins from *Stauntonia brachyanthera* towards UDP-glucuronosyltransferases

Dan Liu [a], Shuang Li [a], Jia-qi Qi [a], Da-li Meng [a,*], Yun-feng Cao [b,c]

[a] Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Key Laboratory of Contraceptives and Devices Research (NPFPC), Shanghai Engineer and Technology Research Center of Reproductive Health Drug and Devices, 200000, China
[c] Translational Medicine Center, The First Affiliated Hospital of Liaoning Medical University, Jing Zhou, China

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ABSTRACT

The inhibition of UDP-glucuronosyltransferases (UGTs) by herbal components might be an important reason for clinical herb-drug interaction (HDI). The inhibitory effects on UGTs via nor-oleanane triterpenoid saponins, which were the bioactive ingredients from *Stauntonia brachyanthera*, a traditional Chinese folk medicines with highly biological values, were evaluated comprehensively with recombinant UGT isoforms as enzyme source and a nonspecific substrate 4-methylumbelliferone (4-MU) as substrate. The results showed that there are seven compounds, **2**, **3**, **4**, **8**, **9**, **13** and **14**, respectively, exhibited potential inhibitions towards UGT1A1, UGT1A3 and UGT1A10 among all 23 compounds isolated from the plants. The IC_{50} values were 17.1 μM, 13.5 μM, 9.5 μM, 15.7 μM, 16.3 μM, 1.1 μM, and 0.3 μM, respectively. Data fitting using Dixon and Lineweaver-Burk plots demonstrated that the inhibition of UGT1A10, UGT1A1 and UGT1A3 was best fit to noncompetitive type and competitive, respectively. The inhibition kinetic parameter (K_i) was calculated to be 39 μM, 17 μM, 3.3 μM, 10 μM, 9.3 μM, 0.19 μM, and 0.016 μM, respectively. All these experimental data suggested that HDI might occur when compounds containing herbs were co-administered with drugs which mainly undergo UGTs-mediated metabolism.

1. Introduction

In the recent decades, with the development of modern industry, people pay more attention to the drug-drug interaction (DDI) and herb-drug interaction (HDI). This is an important concern in drug discovery and development research as well as in the evaluation of patient safety in clinical practice [1,2]. Drug metabolizing enzyme (DME)-catalyzed metabolic elimination significantly affects the concentration of drugs in plasma and therapeutic targets [3]. In the past years, 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 DDI and HDI [4]. For instance, it was observed that *Centella asiatica* (CA) and *Orthosiphon stamineus* (OS) inhibited CYP2C19 activity with varying potency. Thus, care should be taken when these CA and OS components are co-administered with CYP2C19 substrates (such as omeprazole, proguanil, barbiturates, citalopram, and diazepam) [5]. However, the studies on the inhibition of UDP-glucuronosyltransferases (UGTs) caused by herbal constituents remain to be limited. UGTs also play a crucial role in the metabolic elimination of clinical drugs, and could catalyze the metabolism of ~35% of all drugs metabolized by Phase II enzymes [6]. The ginsenoside Rg_3, for example, could competitively inhibit UGT1A7, 2B7 and 2B15-catalyzed 4-MU glucuronidation reaction, and exert noncompetitive inhibition towards UGT1A8-catalyzed 4-MU glucuronidation [7]. Inhibition of UGT isoforms by xenobiotics might not only induce severe drug–drug interaction, but also result in the metabolic disorders of endogenous substances. In conclusion, the investigation of the inhibitory effect of compounds on UGT-mediated metabolism is supposed to be given high attention from clinical point.

Stauntonia brachyanthera Hand.-Mazz., a kind of plant of *Stauntonia*, is mainly distributed in Hunan, Guizhou and Guangxi provinces of China [8]. The whole plant is demonstrated to possess definite medicinal values during the treatments of cancer, inflammation, pain, and also be used as diuretics [9] and anti-inflammation in China, especially in the area of Dong ethnic minority area. In the meanwhile, a series of rare nor-oleanane triterpenoid saponins, which were the main constituents of the plant [10], were also obtained from different parts of this medicinal plant. The nor-oleanane triterpenoids, which is a new kind of triterpenoids that is never being under investigated for its inhibitory effects on UDP-glucuronosyltransferase. Then, some people would doubt that saponins were generally stripped of their sugar decoration in the intestine, and why we didn't study the more meaningful activity

* Corresponding author at: School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China.
E-mail address: mengdl@163.com (D. Meng).

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data on the genins directly. It was true that some saponins would be absorbed after the hydrolysis of sugar moieties. But there were still some saponins that were absorbed into plasma in the form of prototype or secondary glycosides, such as glycyrrhetinic acid, ginsenosides [7], and so on. For instance, during the study on its analgesic effect of YM_{11}, a kind of nor-oleanane triterpenoid, it was found only the prototype could be absorbed into the plasma and play biological activities. But, once they were absorbed from the digestive system, they will firstly arrive at liver and inevitable acted with metabolic enzymes existing in the liver. So, it would be very important to evaluate the inhibitory activities of these nor-oleanane glycosides on metabolism enzymes. Although the potential bioactivities such as hepatoprotection [11] and antioxidants [12] were proved, the safeties of this kind of compounds were never under the investigation because of their novelty. The inhibitory effects of the other kinds of triterpenoids, such as tetracyclic triterpenoids and oleanane type triterpenoids have been reported previously. Therefore, it's very necessary to predict their potential possibilities on HDI, especially UGT, and guarantee their clinical safety.

There were various recombinant human UGTs in research, including UGT1A1, UGT1A3, UGT1A4, UGT2B4, UGT2B7, and so on. So far few completely specific substrates for all UGTs were found except 4-methylumbelliferone (4-MU), which can be catalyzed by at least 12 kinds of UGT subtypes and is widely used during the research of UGTs [13,14]. Although UGT1A4 is very important in glucuronidation of amines, it can only be catalyzed by trifluoperazine (TFP). Therefore, considering the complete evaluation in one research plan, only those including UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, which used 4-MU as nonspecific substrate were selected in our present study. While further related studies on UGT1A4 will be carried out afterwards in another test and reported separately.

Based on the above discussion, the inhibitory effects of the nor-oleanane triterpenoid saponins from *S. brachyanthera* will be evaluated in this paper. For these UGTs whose residual activities were lower than 20% after incubation with the compounds, the IC_{50} values and the parameters (K_i) were calculated in order to judge the inhibition kinetic type as well as guide rational drug usages of these bioactive compounds.

2. Materials and methods

2.1. Plant material

The plant of *S. brachyanthera* was collected in Hunan Province by Shumo Mei, Huaihua Medical College, and was identified by Pro. Jincai Lu, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University in October 2009. A voucher specimen (No.·HLG-0910) is deposited in School of Traditional Chinese Material Medica, Shenyang Pharmaceutical University.

2.2. Extraction, isolation and identification

The air-dried plants of *S. brachyanthera* (8.5 kg) were chopped into small pieces and were extracted with 70% aqueous EtOH (35 × 4 L) until reflux for 2 h. After evaporation of the combined EtOH extracts in vacuo, the resultant aqueous residues were suspended in water and passed through macroporous adsorptive resin (HPD-100, Cangzhou Bon Adsorber Technology Co., Ltd., Cangzhou, China), eluted sequentially with H_2O, 40% EtOH, 65% EtOH and 95% EtOH, respectively. The 65% EtOH eluates (200 g) were chromatographed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Corporation, Qingdao, China) column chromatography (CC), ODS (50 μm, YMC Co. Ltd., Kyoto, Japan) CC and preparative HPLC (Shimadzu LC-20AR, Kyoto, Japan) to give **3** (3.9 mg), **9** (3.5 mg), **5** (8.8 mg), **2** (4.4 mg), **6** (9.6 mg), **12** (10.5 mg), **7–8** (9.8 and 5.2 mg), **13** (4.8 mg), **15** (10.6 mg), respectively. The 40% EtOH eluates (120 g) were also chromatographed on silica gel CC, ODS CC, HPLC, as well as Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) CC to afford **1** (16.5 mg), **4** (5.0 mg), **10–11** (9.8 and 11.0 mg), and **14** (3.5 mg), respectively. The NMR spectra of these compounds were acquired using Bruker ARX-400 and ARX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany). Chemical shifts (δ ppm) are relative to TMS (tetramethylsilane) as an internal standard.

2.3. Chemicals

4-Methylumbelliferone-β-D-glucuronide (4-MUG), 5′-diphosphoglucuronic acid (UDPGA, trisodium salt), 4-methylumbelliferone (4-MU), Tris-HCl and 7-hydroxycoumarin were all purchased from Sigma-Aldrich (Sigma-Aldrich China, Shanghai, China). Recombinant human UGT supersomes (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B7) 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.

2.4. Investigation of inhibition of UGT isoforms by pentacyclic triterpenoids

Recombinant UGT isoform-catalyzed 4-MU glucuronidation reaction was employed to evaluate the inhibition potential of compounds towards various UGT isoforms as previously described [15,16]. The incubation mixture (200 μL) contained recombinant UGTs (final concentration: 0.125, 0.05, 0.025, 0.05, 0.025, 0.05, 0.05, 0.25, and 0.05 mg/mL for UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B7, respectively), 5 mM UDPGA, 5 mM MgCl2, 50 mM Tris-HCl buffer (pH 7.5), and 4-MU in the absence or presence of different concentrations of compounds. Incubation system and condition were carried out as prior recorded [15]. The concentrations of 4-MU were as follows: 110 μM for UGT1A1 and UGT1A6, 1200 μM for UGT1A3, 30 μM for UGT1A7, UGT1A9 and UGT1A10, 750 μM for UGT1A8, 1000 μM for UGT2B4 as well as 350 μM for UGT2B7. The compounds were dissolved in DMSO and final concentration of methanol was 0.5% (v/v). After 3 min pre-incubation at 37 °C, the UDPGA was added in the mixture to initiate the reaction. Incubation time was 120 min for UGT1A1, UGT1A3, UGT1A10, UGT2B4 and UGT2B7, 30 min for UGT1A6, UGT1A7, UGT1A8 and UGT1A9, respectively. The reactions were quenched by adding 100 μL acetonitrile with 7-hydroxycoummarin (100 μM) as internal standard. The mixture was centrifuged at 20,000 ×*g* for 20 min and an aliquot of supernatant was transferred to an auto-injector vial for UFLC analysis. The Shimadzu (Kyoto, Japan) prominence ultra-fast liquid chromatography (UFLC) system contained a CBM-20A communications bus module, a SIL-20ACHT auto sampler, two LC-20AD pumps, a DGU-20A3 vacuum degasser and a CTO-20AC column oven. A SPD-20AVP UV detector chromatographic separation was carried out using a Shim-pack XR-ODS column (75 mm × 2.0 mm, 2.2 μm, Shimadzu) at a flow rate of 0.4 mL/min and UV detector at 320 nm. The mobile phase consisted of acetonitrile (A) and H_2O containing 0.2% (v/v) formic acid (B). The following gradient condition was used: 0–4 min, 95–50% B; 4–7 min, 5% B; and 7–10 min, 95% B. The calculation curve was generated by peak area ratio (4-MUG/internal standard) over the concentration range of 4-MUG 0.1–100 mM. The curve was linear over this concentration range, with r^2 value >0.99. The limits of detection and quantification were determined at signal-to-noise ratios of 3 and 10, respectively. The accuracy and precision of the back-calculated values for each concentration were <5%.

2.5. Data fitting for the determination of inhibition type and parameters (K_i)

The reverse inhibition type can be categorized into competitive, uncompetitive, and noncompetitive inhibitions [17]. Inhibition kinetic parameters (K_i) were determined utilizing various concentrations of