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Wedelolactone metabolism in rats through regioselective glucuronidation catalyzed by uridine diphosphate-glucuronosyltransferases 1As (UGT1As)

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ABSTRACT

Background: Wedelolactone (WEL), a medicinal plant-derived coumestan, has been reported to exhibit a diverse range of pharmacological activities. However, the metabolism and disposition of WEL remain unexplored.

Purpose: The present study aims to investigate the metabolism of WEL in rats and identify the enzymes responsible for forming major WEL metabolites.

Methods: Plasma, urine, feces, and bile samples were collected before and after 50 mg/kg WEL was orally administered to rats. Metabolites were profiled by ultrahigh performance liquid chromatography/quadrupole time-of-flight mass spectrometry and identified by high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy. The *in vitro* WEL glucuronidation activities of human liver microsomes, human kidney microsomes, human intestine microsomes, and 12 recombinant human uridine diphosphate-glucuronosyltransferase (UGT) isoforms were screened. Molecular docking simulation of the interaction between WEL and UGT1A9 was conducted.

Results: WEL underwent extensive metabolism, and 17 metabolites were identified. The major metabolic pathways observed were glucuronidation and methylation. Glucuronic acid was preferentially introduced into 5-OH, whereas no obvious regioselectivity was observed in the methylation of 11-OH and 12-OH. Multiple UGTs, including UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, were involved in forming WEL glucuronides and *O*-methylated WEL glucuronides.

Conclusion: The extensive glucuronidation and methylation is responsible for the low oral bioavailability of WEL in rats. UGT1A1 and UGT1A9 were the major enzymes involved in the glucuronidation of WEL and *O*-methylated WEL. Molecular docking studies revealed that 5-OH was accessible to the catalytic domain of UGT1As; therefore, 5-OH exhibited a high probability of glucuronidation.

Introduction

WEL (7-methoxy-5,11,12-trihydroxy-coumestan, Fig. 1), a naturally occurring coumestan (Govindachari et al. 1956), exhibits a wide range of pharmacological activities, including antitumor (Hsieh et al. 2015), anti-inflammatory (Yuan et al. 2013), anti-osteoporosis (Liu et al. 2014), anti-hepatic fibrosis (Xia et al. 2013), anti-hepatitis C virus (Manvar et al. 2012), anti-adipogenesis (Lim et al. 2012), and trypsin inhibition (Syed et al. 2003). According to Wagner et al. (1986) WEL, which is the main active component of *Wedelia calendulaceae* and *Eclipta alba*, exhibits 5-lipoxygenase inhibitory, hepatoprotective, and antiphlogistic effects. Mors et al. (1989) demonstrated that WEL exhibited protective effects against rattlesnake venom toxicity in adult Swiss mice. In addition,

Abbreviations: WEL, wedelolactone; OH, hydroxyl group; UGT, uridine diphosphate-glucuronosyltransferase; HPLC, high-performance liquid chromatography; SPE, solid-phase extraction; NMR, nuclear magnetic resonance; UPLC, ultraperformance liquid chromatography; Q-TOF MS, quadruple time-of-flight mass spectrometry; HLM, human liver microsome; HIM, human intestine microsome; HKM, human kidney microsome; UDPGA, uridine 5'-diphosphate-glucuronic acid; UV, ultraviolet; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum correlation spectroscopy; HMBC, heteronuclear multibond correlation spectroscopy; ROESY, rotating frame nuclear Overhauser effect spectroscopy; IDA, information-dependent acquisition; DMSO, dimethyl sulfoxide.

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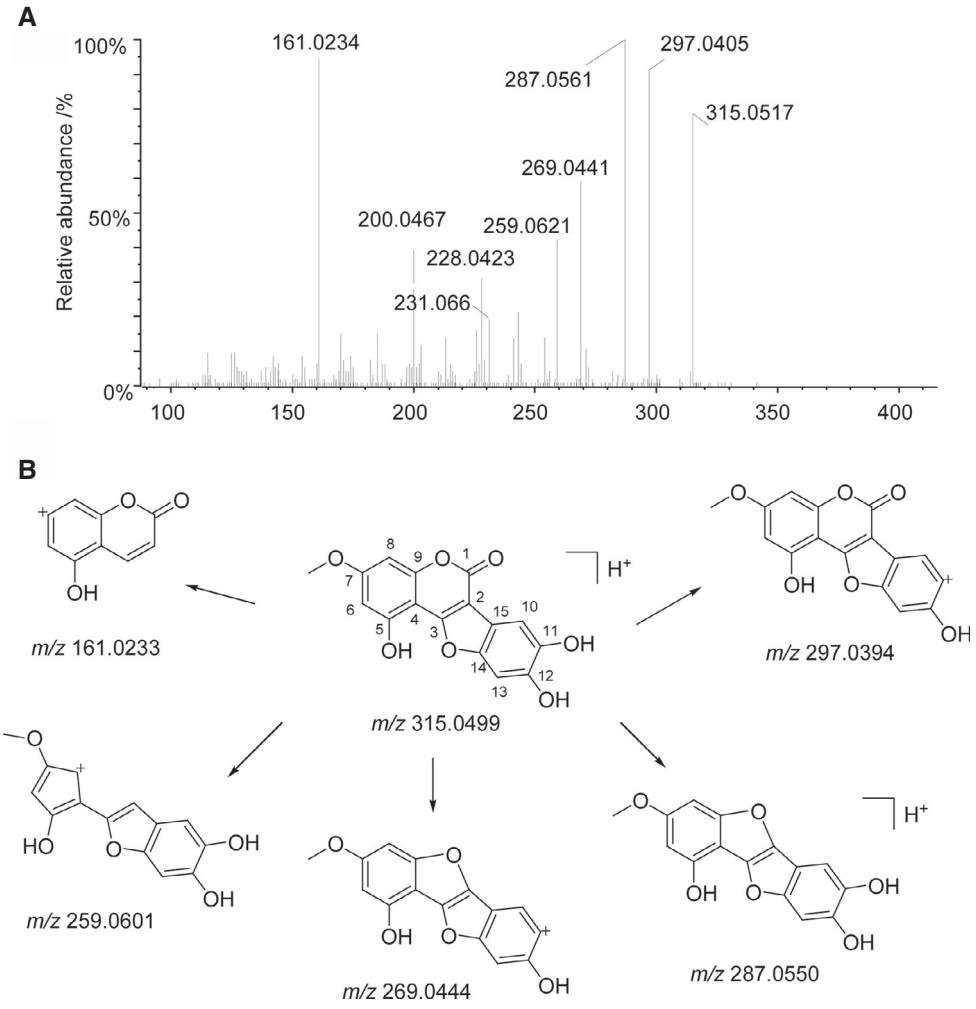

Fig. 1. Product ion spectrum of WEL as revealed by Q-TOF MS (A) and the proposed structures of fragment ions (B).

WEL-containing herbs, such as *Eclipta prostrata* L., *E. alba*, and *Wisteria sinensis*, have been routinely used in China and India to treat liver cirrhosis, infective hepatitis, septic shock, tuberculosis hemoptysis, seborrheic dermatitis, angina pectoris, and venom poisoning. In 2003, Li et al. (2003) successfully synthesized WEL. However, knowledge on the absorption, disposition, metabolism, and *in vivo* excretion of WEL remains lacking, which significantly hinders the comprehensive understanding of its efficacy and potential toxicity.

Our previous pharmacokinetic studies revealed the considerably low oral bioavailability (2%, 50 mg/kg, Table S1) of WEL in rats, which resulted from the extensive first-pass metabolism of WEL in the gastrointestinal tract. In rats, approximately 20% and 13% of WEL was recovered in unmodified form following an oral (50 mg/kg) and intravenous (5 mg/kg) administration, respectively. This result provided insight into the metabolic clearance of WEL in rats. Studies on *in vitro* metabolism have revealed that WEL undergoes glucuronidation, methylation, sulfation, and minimal oxidative metabolism after 3 h of incubation with primary rat hepatocyte. Despite the presence of three phenolic hydroxyl groups attached to C-5, C-11, and C-12 on the coumestan skeleton of WEL, a glucuronate conjugate was preferentially formed (Huang 2015).

The objectives of the present study included (1) to identify through ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) and high-performance liquid chromatography–solid-phase extraction–nuclear magnetic resonance spectroscopy (HPLC–SPE–NMR) the WEL metabolites formed in rats following an oral administration of 50 mg/kg WEL; (2) to determine the uridine diphosphate-glucuronosyltransferases (UGTs) responsible for WEL glucuronidation using human liver microsomes (HLMs), human intestine microsomes (HIMs), human kidney microsomes (HKMs), and recombinant human UGT enzymes; and (3) to explore the possible mechanisms of WEL regioselective glucuronidation.

Materials and methods

Chemicals and reagents

WEL (purity > 98%) was isolated from *E. prostrata*. Uridine 5'-diphosphate-glucuronic acid (UDPGA) trisodium salt and alamethicin were purchased from Sigma–Aldrich (St. Louis, MO). Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, UGT2B17, and pooled HLMs were obtained from BD Gentest (Woburn, MA). Pooled HKMs and HIMs were purchased from Xenotech (Lenexa, KS). All reagents and solvents were of HPLC grade or the commercially available reagents of the highest grade.

Animal study

Male Sprague Dawley rats that weighed 200–250 g were obtained from the SLRC Laboratory Animal Co., Ltd. (Shanghai, China). Three rats were placed in individual metabolism cages and dosed