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Oral pharmacokinetics of baicalin, wogonoside, oroxylin A 7-*O*-β-D-glucuronide and their aglycones from an aqueous extract of Scutellariae Radix in the rat

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ABSTRACT

Scutellariae Radix (SR) has been extensively prescribed in folk medicines due to its notable beneficial activities. The flavonoid glucuronides baicalin (BG), wogonoside (WG), oroxylin A 7-*O*-β-D-glucuronide (OG) and their aglycones baicalein, wogonin and oroxylin A, are the main components of the herb. So far, majority of previous studies failed to report the pharmacokinetics and none offered an explanation for the systemic exposures of these six flavonoids when the herbal extract was orally administered. In this study, when a SR extract was orally dosed to rats (800 mg/kg, equivalent to BG 324.80, WG 124.00, OG 43.04, baicalein 25.36, wogonin 24.40, and oroxylin A 5.79 mg/kg), all six flavonoids were detectable throughout the experimental period (48 h) using an LC–MS/MS method with the Cmax and $AUC_{0-48\,h}$ of the glucuronides 10–130 times that of respective aglycones. As the lowest among the three glucuronides in the herb, OG was the most abundant *in vivo*, while the systemic exposure of wogonin was the highest amongst the three aglycones. The dose-normalized $AUC_{0-48\,h}$ descended in orders of OG/oroxylin A, WG/wogonin and BG/baicalein. Two di-conjugates of baicalein (BG glucuronide and BG glucoside), two BG isomers (minor BM1 and major BM2), and one WG isomer (wogonin 5-*O*-glucuronide) were detected in rat plasma. Semi-quantitation of the isomers with peak area data revealed that the AUPs (area under peak area ratio-time curves) of BG isomers were ~3 times that of BG, yet the AUP of wogonin 5-*O*-glucuronide was only one seventh of WG. BM2, tentatively assigned as baicalein 6-*O*-glucuronide, was formed from both microbial isomerization of BG and hepatic glucuronidation of baicalein. Wogonin 5-*O*-glucuronide was only formed in hepatic glucuronidation of wogonin. Demethylated wogonin was observed in gut bacteria, offering an optional origin of BM1 apart from baicalein glucuronidation. Microbial isomerization of BG and extensive hepatic glucuronidation of baicalein to form BM2 as well as a poorer intestinal permeability of baicalein ($P_{app} \times 10^{-6}$ cm/s) should account for the lower systemic exposures of BG and baicalein. Faster microbial hydrolysis of WG, high intestinal permeability ($P_{app} \times 10^{-5}$ cm/s) and less hepatic glucuronidation of wogonin explain the relatively high systemic exposure of wogonin. Sole microbial deglycosylation of OG, high intestinal permeability ($P_{app} \times 10^{-5}$ cm/s) and extensive hepatic glucuronidation of oroxylin A supported the highest systemic exposure of OG. Taken together, the oral kinetics of six flavonoid glucuronides and aglycones in the SR extract were simultaneously obtained. Microbial conversion, intestinal epithelial permeability and hepatic glucuronidation are determinant factors for their systemic exposures.

1. Introduction

Scutellaria Radix (SR), known as *Huangqin* in Chinese, is the dried root of *Scutellaria baicalensis* Georgi (*Lamiaceae*). It is a well-known traditional herbal medicine used to treat inflammation, cardiovascular diseases, respiratory and gastrointestinal infections [1]. SR contains a variety of flavones, phenylethanoids, amino acids, sterols and essential oils. Baicalin (BG), wogonoside (WG), oroxylin A 7-*O*-β-D-glucuronide (OG), baicalein (B), wogonin (W) and oroxylin A (O) (structures shown in Fig. 1) are the major flavonoid glycosides and aglycones in SR and have demonstrated numerous pharmacological activities, such as anti-inflammatory [2], antivirus [3], anti-cancer [4], antimicrobial [5], neuroprotective [6], and

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antithrombotic [7] activities. SR and the flavonoid compounds from this herb have attracted extensive research interests [8].

To understand the underlying mechanism of the beneficial effects of SR observed in preclinical studies and clinical practice, extensive research efforts have been dedicated to characterize the *in vivo* dispositions of the main flavonoids, as single compound or in the mixture extracted from SR or SR-constituted compound formulas, on *in vivo* and *in vitro* models. Several studies have intended to measure the plasma levels of SR flavonoids after oral administration of SR or its medicinal preparations by using HPLC–UV [9–15], HPLC–ECD [16], UPLC–MS [17] and LC–MS/MS [18–21]. However, the majority of these studies was unable to obtain the kinetic profiles of the six main flavonoids in the rat plasma simultaneously after oral administration of the extract of the herb or compound formulas due to poor sensitivity of the methods employed [9,14], the design of the studies, or low contents of some flavonoids in the SR products used [21]. The absorption [10,13,22], distribution [23], metabolism [24–26] and excretion [12,27,28] of the pure compounds baicalein, baicalin, and wogonside were also extensively reported. Microbial metabolism, intestinal epithelial permeability, and first-pass intestinal and hepatic conjugation, in particular hepatic glucuronidation [29,30], have been demonstrated to be main factors determining the systemic exposure of flavonoid glycosides and aglycones, which is also true for the SR flavonoids and was taken into consideration when the *in vivo* dispositions of these compounds were interpreted. However, the metabolic and absorptive properties of the 3 pairs of glucuronides and aglycones have never been integrated into one study to assess the impact of structural factor on the *in vivo* dispositions and systemic exposures of the six flavonoid compounds.

Baicalein could be converted to the 7-glucuronide (BG) and the 6-glucuronide [25,31] and predominantly existed as glucuronides in circulation after orally administered alone or in the herbal extract [10,18,23]. The C-6 isomer showed higher systemic exposure than baicalin in rats receiving an oral dosage of baicalin or baicalein [32]. Cumulative excreted amounts of BG and the C-6 isomer in urine samples collected over 48 h were comparable when baicalin was orally administered to rats [33]. However, the sites of formation of BG C-6 isomer and its relative abundance to BG after the SR extract is orally administered were unclear. In a recent study [14], a WG isomer was reported and demonstrated to be originated from glucuronidation of wogonin *in vivo*, not oroxylin A, yet the site where the WG isomer was formed remains to be addressed. It is also unclear whether OG isomer(s) can be formed *in vivo* from co-existing flavonoids when the SR extract is orally administered. In traditional Chinese medicinal practice, most crude drugs or compound formulas are prepared as decoctions and taken orally. Presystemic interaction of flavonoid glycosides with gut microbiota plays an important role in generating more permeable metabolites. Gut microbiota can catalyze multiple reaction types of xenobiotics, among which hydrolysis or deglycosylation is usually the main reaction of glycosides and has been extensively studied. Hydrolysis of the flavonoid glucuronides from SR by gut bacteria has been evidenced from studies with pure compounds or Huangqin-Tang, the compound formula containing SR as the king herb [34,35]. Gut microbiota also catalyze isomerization of some flavonoids [36]. Whether microbial metabolism also contributes to the formation of SR flavonoid glucuronide isomers *in vivo* remains unclear.

Therefore, in this study three flavonoid glucuronides BG, WG, OG and their aglycones, as well as the glucuronide isomers formed *in vivo* were simultaneously determined in rat plasma after oral administration of a SR extract to rats by using a sensitive HPLC–MS/MS method. *In vitro* microbial metabolism of the glucuronides, transport properties of the glucuronides and the aglycones in the extract across Caco-2 cell monolayer, as well as the glucuronidation of the aglycones in rat liver microsomes, were also studied to elucidate the origins of the isomers found *in vivo* and to identify the factors determining the systemic exposures of the six SR flavonoids.

2. Experimental

2.1. Materials and reagents

Baicalin, baicalein, wogonin and wogonoside (>98%) were purchased from Weikeqi Biotechnology Company (Sichuan, China). Oroxylin A 7-O-β-D-glucuronide (>98%) was purchased from Shanghai Forever Biotech Co., Ltd. (Shanghai, China). Oroxylin A (>98%) was supplied by Yuanye Biotechnology Company (Shanghai, China). Formononetin (>98%) was purchased from Shanghai R&D Center for Standardization of Chinese Medicines (Shanghai, China) and used as the internal standard (IS).

Methanol, acetonitrile, formic acid and 1-butanol were HPLC-grade from Merck (Darmstadt, Germany). Deionized water was purified by a Milli-Q purification system (Millipore; Bedford, MA, USA). Heparin sodium salt was supplied by Sigma (St. Louis, MO, USA). BBL™ Brain Heart Infusion (BHI) medium, GasPak™ EZ Anaerobe Container System with Indicator and GasPak™ EZ Large Incubation Container were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). L-Cystine was from Research Organics, Inc. (Cleveland, Ohio, USA). Dimethyl sulfoxide (DMSO), hemin bovine and vitamin K1, uridine 5′-diphosphoglucuronic acid (UDPGA) and alamethicin were supplied by Sigma–Aldrich (St. Louis, MO, USA).

Rat liver microsomes (RLMs) were a pool of liver microsomes from 10 untreated male SD rats and prepared at Sun Yat-sen University (Guangzhou, China) according to the method described previously [37]. The concentration of protein was measured by Lowry's method [38].

2.2. Preparation of the extract of Scutellariae Radix

The Scutellaria Radix crude drug was purchased from Shaanxi Genuine Chinese Herbal Medicine Planting Co., LTD (Shaanxi Province, China) and identified to be the dried roots of *Scutellaria baicalensis* Georgi. by Prof. Qingwen Zhang from our institute. The specimens were stored at the Institute of Chinese Medical Sciences, University of Macau (Macao, China).

The SR extract was prepared following the method documented in China Pharmacopoeia [39]. Briefly, the dried roots were ground into powder (60-mesh) and 250 g was extracted twice with 10 volumes boiling water (Milli-Q water) for 1 h. The supernatants were combined and filtrated, and lyophilized. The extract (powder) was stored at 4 °C till use. The contents of the flavonoids in the extract were determined to be 406 mg (BG), 155 mg (WG), 53.8 mg (OG), 31.7 mg (B), 30.5 mg (W) and 7.24 mg (O) per gram of the extract using an HPLC-DAD method (Supplemental Table S1). The SR extract was suspended in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) solution to a concentration of 100 mg/mL for *in vivo* study or *in vitro* transport study.

2.3. Animals

Male Sprague-Dawley rats (SD rats) weighing 200–250 g were supplied by Sam Yao Hong Ltd. (Macao, China). The rats were maintained under controlled conditions of temperature (22 ± 2 °C) and relative humidity (50 ± 10%) with a 12-h light/dark cycle, with access to water and rat chow *ad libitum*. The animal experiments were conducted according to a protocol approved by the Animal Ethics Committee (file no.: ICMS-AEC-2013-05), Institute of Chinese Medical Sciences at University of Macau (Macao, China).