Journal of Ethnopharmacology 152 (2014) 183–189

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Simulated gastrointestinal tract metabolism and pharmacological activities of water extract of *Scutellaria baicalensis* roots

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ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form
28 December 2013
Accepted 31 December 2013
Available online 8 January 2014

Keywords:
Gastrointestinal tract
Metabolism
Scutellaria baicalensis
Anticomplementary
Antibacterial

Chemical compounds studied in this article:
Baicalin (PubChem CID: 64982)
Wogonoside (PubChem CID: 29927693)
Baicalein (PubChem CID: 5281605)
Wogonin (PubChem CID: 5281703)
Oroxylin A (PubChem CID: 5320315)
Oroxyloside (PubChem CID: 38348319)
Norwogonin (PubChem CID: 5281674)
Norwogonoside (PubChem CID: 44258552)

ABSTRACT

Ethnopharmacological relevance:
Scutellaria baicalensis: Georgi (Labiatae) is a well-known traditional Chinese medicine to treat inflammation, cardiovascular diseases, respiratory and gastrointestinal infections, *etc.* The present study was to understand the metabolism of the root of *Scutellaria baicalensis* (a.k.a. Huangqin in Chinese) in the gastrointestinal tract and the correlation between the metabolites and their respective pharmacological activities.
Materials and methods: The water extract of the root of *Scutellaria baicalensis* (WESB) was incubated with simulated gastric and intestinal juices, and human fecal microflora for 24 h at 37 °C. The HPLC–DAD analysis was used to monitor the *in vitro* metabolic process and identify its metabolites by comparing their absorption spectrum and retention time with those of chemical references. The *in vitro* anticomplementary and antimicrobial activity was evaluated with hemolysis assay, agar disc-diffusion method and MIC value, respectively.
Results: Main constituents of WESB remain unchanged during the incubation with simulated gastric juice (pH=1.5) and intestinal juice (pH=6.8), whereas four flavones, baicalin, wogonoside, oroxyloside and norwogonoside were metabolized into their respective aglycons by human intestinal bacteria. All four metabolites were demonstrated to have higher anticomplementary and antimicrobial activity than those of WESB. The anticomplementary active metabolites were identified to be baicalein, oroxylin A and norwogonin, among them, norwogonin is the most active compound.
Conclusion: The presence of intestinal bacteria is demonstrated to play an important role in the gastrointestinal metabolism of WESB, and the pharmacological effects of *Scutellaria baicalensis* may be dependent on the intestinal bacteria metabolism.

1. Introduction

The root of *Scutellaria baicalensis* Georgi (Labiatae), also known as Huangqin, is a widely used herb in traditional Chinese medicine (TCM) with anticancer, antiviral, antibacterial and anti-inflammatory properties (Yoon et al., 2009). Traditionally, Huangqin has been prescribed as a diuretic, laxative, febrifuge, an antipyretic, and for hemoptysis, bloody stool, and nasal haemorrhage when prescribed in a compound recipe (The Pharmacopoeia Commission of PRC, 2010). Remarkably, Huangqin was recommended for the treatment and prevention of severe acute respiratory syndrome (SARS) by the State TCM Administration of the People's Republic of China (Miller et al., 2005; Zhang and Chen, 2008).

Huangqin was found to exert anti-inflammatory, antioxidant, anti-hepatitis B virus, anti-tumor, anti-allergic and anxiolytic properties (Li et al., 2009; Tong et al., 2012). The main active constituents of Huangqin are flavonoids, such as baicalin (baicalein-7-glucuronide), wogonoside (wogonin-7-glucuronide), baicalein, wogonin, oroxylin A and oroxyloside (oroxylin A-7-glucuronide) (Fig. 1). Among these flavonoids, baicalin is regarded as the most important determinants of the quality of Huangqin (Yuan et al., 2011). Due to its orally administered as decoctions in TCM, the metabolism of the constituents often occurs in the gastrointestinal tract caused by the low gastric pH conditions, as well as the presence of digestive enzymes or intestinal bacteria (Akao et al., 1994). It was found that baicalin was initially hydrolyzed to baicalein prior to the absorption, while baicalein could be readily absorbed with a fast and extensive first pass metabolism (Akao et al., 2000; Lu et al., 2007). Wogonoside was found to be metabolized to wogonin by human fecal microflora (Trinh et al., 2009). In addition, pharmacokinetic study of Huangqin-Tang Decoction indicated that the constituents of

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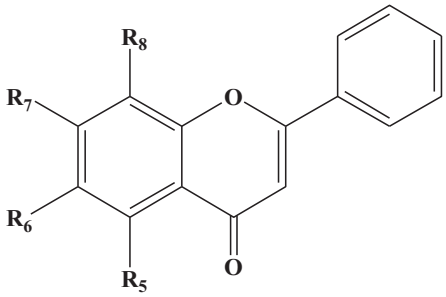
http://dx.doi.org/10.1016/j.jep.2013.12.056

wogonoside and oroxyloside had double-site absorption kinetics in rats which may due to the enteric circulation and enterohepatic circulation after oral dosing (Zuo et al., 2003). So far, there is no report on the metabolism of Huangqin in gastrointestinal tract. In addition, most studies on bioactivity of Huangqin were focused on antibacterial and anti-endotoxin activities but not anticomplementary activity.

In this study, the water extract of Huangqin, the root of *Scutellaria baicalensis* (WESB) was incubated with simulated gastric and intestinal juices, and human fecal microflora, their metabolites were identified and the *in vitro* anticomplementary activity was evaluated. The inhibitory activity of main compounds in Huangqin as well as their metabolites against the standard strains of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA), *Enterococcus faecalis* (gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeuroginosa* (gram-negative bacteria) was determined to understand the role of gastrointestinal tract conditions on pharmacological effects of Huangqin.



R_5=R_6=R_7=OH, R_8=H	baicalein
R_5=R_6=OH, R_7=O-GlcU, R_8=H	baicalin
R_5=R_7=OH, R_8=OCH_3, R_6=H	wogonin
R_5=OH, R_6=H, R_7=O-GlcU, R_8=OCH_3	wogonoside
R_5=R_7=OH, R_6=OCH_3, R_8=H	oroxylin A
R_5=OH, R_6=OCH_3, R_7=O-GlcU, R_8=H	oroxyloside
R_5=R_7=R_8=OH, R_6=H	norwogonin
R_5=R_8=OH, R_7=O-GlcU, R_8=H	norwogonoside

Fig. 1. Chemical structures of eight flavonoids.

2. Materials and methods

2.1. Material and reagents

The roots of *Scutellaria baicalensis* (Huangqin) were purchased from Leiyunshang drugstore (Shanghai) and authenticated by one of the authors Mengyue Wang. Voucher specimen (HN001) has been deposited at herbarium of School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China. Pepsin (1: 250) and pancreatin were purchased from Sangon Biotech Co., Ltd (Shanghai, China). General anaerobic medium broth (GAM broth) was purchased from Shanghai Kayon Biological Technology Co., Ltd (Shanghai, China). Sheep erythrocytes, rabbit erythrocytes and anti-sheep erythrocyte antibody were purchased from Shanghai Fortune Biological Technological Co., Ltd (Shanghai, China). Heparin (sodium salt, 160 IU/mg) and macroreticular resin (HZ-801) were purchased from China National Medicines Co., Ltd (Shanghai, China). Normal human serum was obtained from healthy adult donors. Guinea pigs serum was obtained from healthy guinea pigs from Laboratory Animal Research Center of Fudan University. MRSA (ATCC33591), MSSA (ATCC25923), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC8739), and *Pseudomonas aeuroginosa* (ATCC27853) provided by Dr. Xiuping Qian′s lab.

Reference substances: Baicalein, baicalin, wogonin, wogonoside, oroxylin A, oroxyloside were purchased from Shanghai Winherb Medical Technology Co., Ltd.; Norwogonin was purchased from J&K Chemical Ltd.; Norwogonoside was isolated from Huangqin in our laboratory. All lab-made compounds were characterized by their spectra data, which were in accordance with references. All other chemicals and reagents were of analytical grade.

2.2. Preparation of WESB

Dried Huangqin (30 g) was decocted twice with a ten-fold mass of water to boil 1 h. After filtrated, the two filtrates were combined and then concentrated to 30 ml (equivalent to 1 g (crude drug)/ml). The HPLC–DAD analysis of WESB was conducted (Fig. 2). Peaks 1–8 were confirmed by comparing the retention times and UV spectra with reference standards, and attributed to baicalin, norwogonoside, oroxyloside, wogonoside, norwogonin, baicalein, wogonin and oroxylin A, respectively. Noted that norwogonoside was isolated from Huangqin for the first time. The contents of main constituents in Huangqin, baicalin, norwogonoside, oroxyloside, wogonoside,

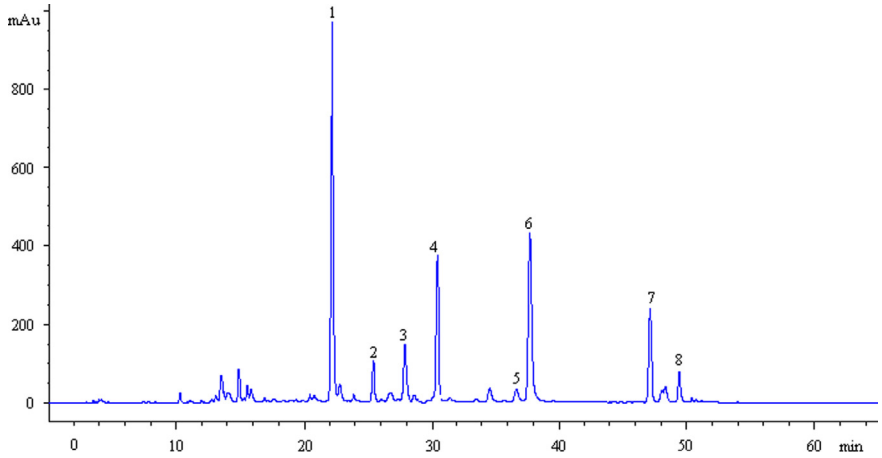


Fig. 2. HPLC chromatograms of WESB. (1) baicalin; (2) norwogonoside; (3) oroxyloside; (4) wogonoside; (5) norwogonin; (6) baicalein; (7) wogonin; (8) oroxylin A.