Journal of Ethnopharmacology 176 (2015) 49–54

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Comparative pharmacokinetics of gastrodin in rats after intragastric administration of free gastrodin, parishin and *Gastrodia elata* extract

Chunlan Tang [a,b], Li Wang [a], Xinxin Liu [a], Mengchun Cheng [a], Yang Qu [a], Hongbin Xiao [c,*]

[a] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian 116023, China
[b] University of Chinese Academy of Science, Beijing 100049, China
[c] Beijing University of Chinese Medicine, Beijing 100029, China

ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form
14 September 2015
Accepted 2 October 2015
Available online 22 October 2015

Keywords:
Gastrodin
parishin
Gastrodia elata extract
UHPLC-FLD
Comparative pharmacokinetics

Chemical compounds studied in this article:
Gastrodin (PubChem CID: 115067)
Parishin (PubChem CID: 44421666)
Parishin B (PubChem CID: 44715528)
Parishin C (PubChem CID: 46173915)

ABSTRACT

Ethnopharmacological relevance: Gastrodia elata Blume, a traditional Chinese herb, was widely used against convulsant, vertigo, paralysis, epilepsy, tetanus, asthma and immune dysfunctions. Gastrodin is one of the major bioactive components of *G. elata* and it is known for its anticonvulsive, anti-inflammatory, antiepileptic and neuroprotective effects.

Materials and methods: An ultra high performance liquid chromatography-fluorescence detection (UHPLC-FLD) method was developed to determine gastrodin in rat plasma. Gastrodin and Thiamphenicol (internal standard, IS) were extracted from rat plasma by immediately protein precipitation. The pharmacokinetics of gastrodin in rats by following differently administered types was studies: intragastric administration of gastrodin (100 mg/kg), parishin (116 mg/kg, with the same mole of gastrodin moiety) and *G. elata* extract (2.3 g/kg, with the same mole of gastrodin moiety). Non-compartmental pharmacokinetic profiles were constructed using the software of WinNonlin (Phoenix, version 6.3), and the pharmacokinetic parameters were compared using unpaired Student's *t*-test.

Results: The results showed that the pharmacokinetic parameters, including C_{max}, T_{max}, $AUC_{0-\infty}$, $t_{1/2}$, MRT, V_d, CL, were quite different among the three types of gastrodin administration. The administration of parishin and *G. elata* extract, which either could convert to gastrodin in vivo or contained free gastrodin and abundant gastrodin conjugates, gave rise to higher elimination half-life ($t_{1/2}$) and mean residence time (MRT) values for gastrodin compared to free gastrodin administered.

Conclusion: The comparison of the pharmacokinetics of gastrodin among three different administered types of gastrodin in rats suggested that administration of parishin or *G. elata* extract in clinic may result in a longer duration time of action than that of the administration of free gastrodin. The results may provide some guidance for the clinical applications of parishin and *G. elata*.

1. Introduction

Gastrodin, one of the main active ingredients in *Gastrodia elata* (*G. elata*) Blume (known as Tianma in Chinese), has been used as a phytochemical marker for the quality control of *G. elata* in Chinese pharmacopeia (Sate Pharmacopoeia Committee, 2015). Pharmacological studies have exhibited that gastrodin has a wide range of therapeutic efficacy, including analgesic (Qiu et al., 2014), anticonvulsive (An et al., 2003), anti-inflammatory (Wang et al., 2014), antiepileptic (Ojemann et al., 2006), antiobesity (Sun et al., 2012), learning and memory improvements (Hsieh et al., 1997) and neuroprotective effects (Kumar et al., 2013; Zeng et al., 2006).

The pharmacokinetic study of gastrodin has been previously reported. The concentration-time profile in rats after intragastric administration of gastrodin was fitted to a one compartment open model (Cheng et al., 2003). But in human plasma, it was fitted to a two compartment open model (Ju et al., 2010). The half-lives of gastrodin in rat and human plasma were 10 min and 6 h, respectively (Ju et al., 2010; Wang et al., 2002), which indicated that gastrodin was quickly eliminated in vivo. Thus, multi-dose administration was required to prolong the action time, which greatly limited its clinical therapeutic efficacy.

Besides gastrodin, several other bioactive phenolic compounds, such as parishin, parishin B, parishin C, parishin E and parishin G have been isolated and identified from *G. elata* (Chae et al., 2008; Ku et al., 1995; Wang et al., 2012; Yang et al., 2007). These compounds were regarded as gastrodin conjugates, which formed by the substitution of a citric acid with gastrodin moieties varying from one to three. The ester linkages between citric acid and gastrodin moieties were readily broken in gastrointestinal tract to

* Corresponding author. Fax: + 86 10 64035275.
E-mail addresses: hbxiao@dicp.ac.cn, hbxiao69@163.com (H. Xiao).

http://dx.doi.org/10.1016/j.jep.2015.10.007
0378-8741/

release the active form, gastrodin. In addition, our previously study has also demonstrated that parishin was mainly metabolized to gastrodin in rat plasma (Tang et al., 2014, 2015). Therefore, these compounds might act as prodrugs and release gastrodin in vivo. In this way, the pharmacokinetic properties of gastrodin after administration of these gastrodin conjugates might be enhanced compared to that of administration of free gastrodin.

In addition, it is well known that *G. elata* extract contains free gastrodin and abundant gastrodin conjugates, like parishin, parishin B and so on. However, previous study (Zhang et al., 2008) just reported the pharmacokinetics of free gastrodin after oral administration of *G. elata* extract and ignored conversion in vivo from gastrodin conjugates. Although Zhao et al. (2014) has quantified gastrodin and parishin in rat plasma after administration of *G. elata* extract, the total content of gastrodin, including free gastrodin and that converted from gastrodin conjugates in *G. elata* extract, was still not analyzed. That is, the dose of *G. elata* extract administered to rats was determined mainly according to the content of free gastrodin instead of the total content of gastrodin, which might not comprehensively present the metabolic properties of gastrodin in *G. elata* extract. Therefore, the total content of gastrodin in *G. elata* extract need to be analyzed for further dosing design. Additionally, the conversion in vivo from gastrodin conjugates and other complicated components in *G. elata* extract may both affect the pharmacokinetic properties of gastrodin after administration of *G. elata* extract. Therefore, it is necessary to compare the pharmacokinetics of gastrodin after administration of free gastrodin, gastrodin conjugate and *G. elata* extract.

The aim of this study is to explore whether pharmacokinetics behaviors of gastrodin were enhanced after intragastric administration of parishin or *G. elata* extract compared to that of administration of free gastrodin to rats. Up to now, few researches on the pharmacokinetic of gastrodin were reported when it was in the form of gastrodin conjugate. It is expected that the results of this study would be useful to explain and predict a variety of events related to the efficacy of *G. elata* and to provide a firm basis for the dosage design in pharmacological experiments and clinical applications.

2. Methods and materials

2.1. Chemicals and materials

The rhizome of *G. elata* was collected from Guangyuan city, Sichuan Province, China, and identified by Professor SuiQing Chen (Henan College of Traditional Chinese Medicine). The voucher specimen (No. 713215) has been preserved in our laboratory. The reference standard of parishin (PA > 98%), parishin B (PB > 95%), parishin C (PC > 95%) and gastrodin (GAS > 98%) were isolated from the dried roots of *G. elata* and identified by LC–MS and NMR data in our laboratory (Wang et al., 2007). Thiamphenicol as internal standard (IS) was purchased from Meilun Biotech Co., Ltd. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) was purchased from Fluka. Pepsin was obtained from Solarbio (Beijing, China). Sodium hydroxide and hydrochloric acid were purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Methanol, ethanol, ethyl acetate and formic acid of chromatographic grade were obtained by Burdick & Jackson (USA). Ultrapure water was obtained by the Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Preparation and basic hydrolyzation of G. elata extract

The dried powder of *G. elata* was extracted thrice by refluxing with 70% ethanol (1:5, w/v) for 1.5 h and the extraction solution was combined to be filtered, concentrated under vacuum and then suspended in 400 mL distilled water. The suspension was then extracted with the same volume of ethyl acetate for 3 times. The aqueous layer was obtained and evaporated at low temperature under vacuum. The residue was suspended with water for administration as *G. elata* extract.

G. elata extract (1 mg) was added in 1 mL of sodium hydroxide (1 mol/L) to obtain a concentration of 1 mg/mL. The sample solution was maintained in an oven at 30 °C for 1 h. Then, the sample was neutralized with hydrochloric acid and diluted with initial mobile phase to obtain solutions with concentration at 10 μg/mL. All the samples were filtered through 0.22 μm membrane filter before analysis.

2.3. In vitro degradation of parishin in simulated gastric medium

The simulated gastric mediums were prepared as follows: Medium A was 0.1 mol/L hydrochloric acid solution (pH 1.0). And Medium B was 0.1 mol/L hydrochloric acid solution containing 10 g/L pepsin. Parishin (1 mg) was exposed to two mediums (1 mL) mentioned above, respectively ($n=3$). These sample solutions were maintained in an oven at 37 °C by continuous shaking. And then, samples (100 μL) were successively collected at 5 min, 30 min, 1 h, 4 h and 8 h, and neutralized with NaOH (0.1 mol/L). After that, samples were freeze-dried and 300 μL ice-cold methanol containing IS (1.5 μg/mL) was added for desalination or precipitation. Subsequently, the samples were centrifuged at 12,000 rpm for 10 min. The supernatants were transferred and evaporated to dryness under a stream of nitrogen at 50 °C. The residue was reconstituted using 100 μL of 8% methanol–water solution, 1 μL of the supernatant was injected for UPLC-FLD analysis.

2.4. Animals

Sprague–Dawley (SD) rats (200 ± 20 g) were purchased from the Laboratory Animal Center in Dalian Medical University (Dalian, China). Before administration of drugs, the rats were fasted for 24 h with free access of water. All experimental protocols on animals were carried out according to the Guidelines of the Committee on the Care and Use of Laboratory Animals of China.

2.5. Drug administration and blood sampling

Experiments were performed on 18 rats randomly divided into three groups: intragastric administration of free gastrodin, parishin, and *G. elata* extract. Dose of administration was adjusted as follows: 100 mg/kg free gastrodin, 116 mg/kg parishin (with the same mole of gastrodin moiety) and 2.3 g/kg *G. elata* extract (with the same mole of gastrodin moiety). Gastrodin, parishin and *G. elata* extract were dissolved in pure water for intragastric administration, and *G. elata* extract was ultrasonic dissolved to achieve suspension at concentration of 0.23 g/mL. After administration, blood samples were collected into heparin-containing tubes from ophthalmic artery plexus of rats using capillary tubes before drug administration (0 h) and at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 h after drug administration. Blood samples were promptly centrifuged at 3000 rpm for 10 min and plasma section was separated and stored at −80 °C until analyzed.

2.6. Sample preparation

The plasma samples were thawed to 4 °C before processing. An aliquot of 100 μL plasma sample was transferred to an Eppendorf tube and added 300 μL of iced-cold methanol containing IS before vortex-mixed for 5 min. The samples were centrifuged at