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Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat

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Abstract

Gastrodin is a bioactive constituent of rhizome in *Gastrodia elata* Blume (Orchidaceae) The aim of this study is to develop a rapid and sensitive liquid chromatographic method coupled to microdialysis sampling system to measure the unbound of gastrodin in rat blood, brain and bile. Microdialysis probes were simultaneously inserted into the jugular vein, brain striatum and bile duct of each anesthetized rat for sampling after the administration of gastrodin (100 or 300 mg kg^{-1}) through the femoral vein. Separation of unbound gastrodin from various biological fluids was applied to an RP-select B column (250 mm × 4.6 mm i.d., 5 μm). The mobile phase consisted of acetonitrile–50 mM potassium dihydrogen phosphate buffer–triethylamine (5:95:0.1, v/v/v, adjusted to pH 2.5 with orthophosphoric acid) with a flow rate of 1 mL min^{-1}. The UV detector wavelength was set at 221 nm. Fifteen minutes after the administration, the gastrodin reached the peak concentration in brain and bile. In addition, the results indicate that gastrodin penetrates the blood-brain barrier (BBB) and goes through hepatobiliary excretion.

Keywords: *Gastrodia elata* Blume; Gastrodin; Hepato-biliary excretion; Microdialysis; Pharmacokinetics

1. Introduction

Gastrodia elata Blume (Orchidaceae) (Tienma in Chinese) is a traditional Chinese herbal medicine that has been used as an anti-convulsant in oriental countries for centuries. *G. elata* has also been used for the treatment of rheumatism, sedation, paralysis, hemiplegia, lumbago, headaches, and vertigo [1]. In addition, *G. elata* has been demonstrated to possess anti-convulsive and free radical scavenging activities [2] whereas the ether fraction of methanol extracts of *G. elata* has neuro-protective effect [3,4]. Chemical studies have shown that *G. elata* rhizome contains several phenolic compounds such as 4-hydroxybenzaldehyde, *p*-hydroxybenzyl alcohol, gastrodin, parishin and 4,4′-dihydroxybenzyl sulfoxide [5]. Among these components, gastrodin (a phenolic glucoside) (Fig. 1) is considered to be the most active component [1]. The content of gastrodin in *G. elata* is 1.97% [6]. It is found the seizure score and the immunoreactivities of GABA shunt (GABA degradation pathway) enzyme reduced in seizure-sensitive gerbils, which suggest that gastrodin may cause the elevation of GABA concentration by inhibiting the GABA shunt [7].

Microdialysis sampling is an important technique for the in vivo measurement of endogenous and exogenous substances in the extracellular fluid. This technique has been successfully applied to blood [8,9], bile [10,11], muscle [12], liver [11], kidney [13], tumor [14], and skin [15] samples. The microdialysis sampling technique is suitable to carry out pharmacokinetic studies because the microdialysis probes can be implanted in most organs or tissues whereby dialysate concentration will reflect extracellular in a distinct region. In addition, microdialysis sampling provides protein free drug, which is considered as the active determinant from a pharmacological point of view and does not need the clean up procedures.

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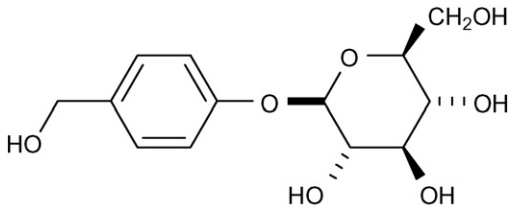

Fig. 1. Chemical structure of gastrodin.

With a significantly increasing use of plant and herbal medicine for health care worldwide in the recent decades, the development of a suitable analytical method for the analysis of bioactive compounds should be the major concern to investigate their safety and efficacy. Recently a LC-UV method was reported to measure the gastrodin in dog plasma [16]. However, a single blood sample should not enough to investigate the detail pharmacokinetic mechanism. In this paper we describe a simple liquid chromatographic method to measure gastrodin in various biological samples. To date, there are very few reports about detailed pharmacokinetic mechanism of gastrodin [16]. In this study, we develop a rapid and sensitive high-performance liquid chromatography coupled to microdialysis sampling system to simultaneously measure gastrodin in rat blood, brain and bile. The results provide the pharmacokinetic profile as well as the brain-to-blood and bile-to-blood distributions of gastrodin after the administration.

2. Experimental

2.1. Chemicals and reagents

Gastrodin was isolated from *G. elata*. Rhizomes of *G. elata* were purchased from drug store and identified by a botanist C.S. Shyu, Taipei, Taiwan, and a voucher specimen is deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan. Rhizomes of *G. elata* (3 Kg) were sliced and extracted with 60% ethanol (3 L × 2). The aqueous ethanolic extracts were combined and concentrated under vacuum to a volume of 1.5 L. The crude extract was then partitioned with ethyl acetate (1.5 L × 3) to give ethyl acetate layer and water layer. The water layer was subjected to column chromatography over Diaion HP-20 (Nippon Rensui, Tokyo, Japan) using water, 20% methanol and 40% methanol as elutents (each 5 L) to yield I–III fractions. Fr. III was rechromatographed over silica gel (25% methanol/chloroform) to give gastrodin. The structure of gastrodin was identified by spectroscopic analysis [^{1}H and ^{13}C NMR spectra (Varian Inova-500, Palo-Alto, CA, USA), and mass spectrometry (Finnigan LCQ, San Jose, CA, USA)]. Sodium dihydrogen phosphate monohydrate, triethylamine, orthophosphoric acid 85% and chloroform were purchased from Merck (Darmstadt, Germany). Analytical reagent grade ethanol, methanol and ethyl acetate were purchased from Mallinckrodt Baker (Paris, KY, USA). Liquid chromatographic grade acetonitrile was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Deionized water (Millipore, Bedford, MA, USA) was used throughout the experiment.

2.2. Instrumentation

The chromatographic system consisted of a chromatographic pump (Bioanalytical System, BAS PM-80, West Lafayette, IN, USA), an on-line injector (CMA 160, Stockholm, Sweden) equipped with a 20 μL sample loop, and a UV/vis detector (Varian, Walnut Creek, CA, USA). Gastrodin was separated from dialysate using a LiChrospher® 60 RP-select B column (Merck, Darmstadt, Germany) maintained at an ambient temperature to perform the ideal chromatographic phase. The detector wavelength was set at 221 nm. The mobile phase comprised acetonitrile, 50 mM sodium dihydrogen phosphate monohydrate, and triethylamine (5:95:0.1, v/v/v), which was adjusted to pH 2.5 with 85% of orthophosphoric acid. Analysis was run at a flow rate of 1 mL min^{-1} and the samples were quantified using peak area. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software version 6.7, Pleasanton, CA, USA).

2.3. Experimental animals

Adult, male Sprague–Dawley rats (280–350 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were specifically pathogen-free and were allowed to acclimate in their environmentally controlled quarters (24 ± 1 °C and 12:12 h light–dark cycle) for at least 5 days before experimentation. Food (Laboratory Rodent Diet no. 5P14, PMI Feeds Inc., Richmond, IN, USA) and water were available ad libitum. All experimental animal surgery procedures/protocols were approved by the institutional animal experimentation committee of the National Yang-Ming University. The rats were initially anesthetized using urethane (1 g mL^{-1}) and α-chloralose (0.1 g mL^{-1}) (1 mL kg^{-1}, i.p.), and then implanted with polyethylene tubing (PE-50; Clay Adams, NJ, USA) in the femoral vein for drug administration. The rats were remained anesthetized throughout the experimental period and their body temperature was maintained at 37 °C with a heating pad.

2.4. Microdialysis in the rat blood, brain and bile

A flexible microdialysis probe was applied to sample the unbound endogenous or exogenous substances in rat blood. A 10 mm dialyzing membrane was used for blood sample. The blood microdialysis probe was implanted into the jugular vein/right atrium and then perfused with ACD (anticoagulant citrate dextrose) solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow-rate of 2.5 μL min^{-1}, by a microinjection pump (CMA/100). Rigid microdialysis probe was applied to sample the unbound endogenous or exogenous substances in rat brain. A dialyzing membrane of 3 mm was used for brain sample. Under anesthesia, the rat was mounted on a Kopf stereotaxic frame. An incision was made in the scalp by drilling a small hole for the implantation of microdialysis probe. The brain microdialysis probe was perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^+; 4 mM K^+; pH 7.0) by a microinjection pump at a flow-rate of 2.5 μL min^{-1}.