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Short communication

Distribution and metabolism of gastrodin in rat brain

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Abstract

Gastrodin is the major and bioactive component in Tianma (*Gastrodia elata* Bl.) and has sedative, anticonvulsive and neuroprotective effects. Since little is known about its neuropharmacokinetics and brain metabolism, this study was undertaken to investigate the kinetic inter-relationship of gastrodin in rat plasma, cerebrospinal fluid (CSF) and brain microdialysate (frontal cortex, hippocampus, thalamus and cerebellum). Gastrodin was administered via the femoral vein at a dose of 200 mg/kg, and blood, CSF and brain microdialysate were collected at timed intervals for the measurement of gastrodin concentrations by high-performance liquid chromatography. The samples were analyzed on a Diamonsil C18 column (5 μm, 250 mm × 4.6 mm i.d.) with a mobile phase consisting of acetonitrile–water (5% acetonitrile for brain microdialysate, 2.5% acetonitrile for plasma and CSF), and detected with a UV detector at 221 nm. The distribution of gastrodin in rat showed that levels of gastrodin declined rapidly after drug administration, and the entry of gastrodin into the brain was rapid. However, the ratios of AUC_{brain}/AUC_{plasma} were not high. The individual ratios of the AUC in the CSF, frontal cortex, hippocampus, thalamus and cerebellum to the AUC in the plasma were 4.8 ± 2.4%, 3.3 ± 1.2%, 3.0 ± 0.7%, 3.3 ± 1.3% and 6.1 ± 1.9%, respectively. The AUC in the cerebellum was significantly higher than that in other brain regions ($P < 0.05$). The concentrations of *p*-hydroxybenzyl alcohol, the main metabolite of gastrodin, were very low both in the CSF and plasma.

Keywords: Gastrodin; *p*-Hydroxybenzyl alcohol; Microdialysis; Cerebrospinal fluid; Brain distribution; Metabolism

1. Introduction

Gastrodin (Gas, Fig. 1) is one of the major and bioactive components in Tianma (*Gastrodia elata* Bl.) and has shown sedative, anticonvulsive and neuroprotective effects [1–6]. It has been approved as a drug for the treatment of neurasthenia, dizziness, headache and as adjunctive therapy for epilepsy in China. The results of recent clinical trials showed that it is efficient in the treatment of patients with vascular dementia.

p-Hydroxybenzyl alcohol (HBA, Fig. 1) is the main metabolite of Gas, and is also one of the components in Tianma (*G. elata* Bl.) and has pharmacological effects similar to Gas [1,7,8].

There have been some reports about the quantitative methods for measuring Gas and its metabolite HBA in biological samples; these methods require the use of extraction steps, many organic solvents and an internal standard [9–13]. However, little is known about its neuropharmacokinetics and brain metabolism, and it is important to quantify Gas in rat brain.

To measure Gas in the brain, previous studies [14,15] reported using ^{3}H-Gas, which involved sacrificing the rats or mice at each sampling point, so that many animals were needed and the results may have been prone to more error. Microdialysis has economical and ethical advantages, making it a powerful sampling technique for the study of the local actions of drugs in different tissues, especially in brain regions. In a recent study [16], microdialysis was used to measure Gas in the brain striatum and blood. However, the limited recovery achieved using microdialysis may be a potential disadvantage. The plasma protein-binding ratio of Gas is 4.3%, while that of HBA is 69.3% [14]. Only unbound drug can permeate the microdialysis membrane, and the concentration of HBA may be too low to be detected; therefore the levels of Gas and HBA in the plasma were determined. The concentration of drug in the cerebrospinal fluid (CSF) can reflect that in the brain, so the concentrations of Gas and HBA in the CSF were also determined. In the present study, microdialysate and CSF were analyzed directly. Based on our previous study [17],

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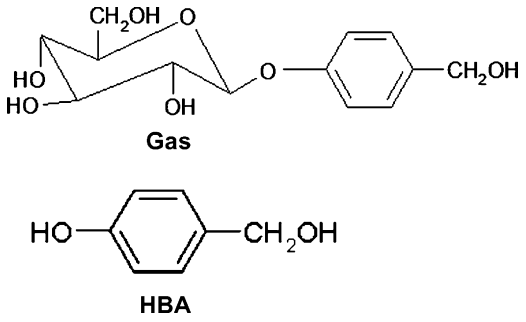

Fig. 1. Structure of Gas and HBA.

the plasma supernatant after precipitation of protein by perchloric acid can also be analyzed directly, thereby simplifying the pre-processing of biosamples.

The aim of this study was to establish a simple and sensitive method for the simultaneous determination of Gas and its metabolite HBA, by which to investigate the distribution and metabolism of Gas in the rat brain and to find out which brain region is most influenced by Gas.

2. Experimental

2.1. Chemicals and reagents

Gas (>99.0%) was supplied by Huizhou Orient Plant Health Care Sci.&Tech. Co. (Guangdong, China), and dissolved in water at a concentration of 100 mg/ml for intravenous use. HBA (>99.5%) was supplied by Yizheng Dixin Chemical Co., Ltd. (Jiangsu, China). Acetonitrile of high-performance liquid chromatography (HPLC) grade was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade and commercially available.

2.2. Chromatography

The HPLC system consisted of an LC-10A pump, SPD-10A UV detector, SCL-10A system controller (Shimadzu, Japan), N2000 chromatographic workstation (Intelligent Information Engineer Ltd. of Zhejiang University), and a Diamonsil C18 column (4.6 mm × 250 mm, 5 μm, Dikma). A mixture of acetonitrile–water was employed as a mobile phase, with a flow-rate of 1.0 ml/min (5% acetonitrile for the microdialysate, 2.5% acetonitrile for the plasma and CSF). The wavelength of the UV detector was set at 221 nm and the temperature of the column oven was maintained at 33 °C.

2.3. Analytical method validation and sample preparation

A stock solution containing 2.287 mg/ml Gas was prepared in water. This solution was diluted with water to prepare the working solutions. Linearity was assessed by analyzing 12 standards in plasma (0.28–571.70 μg/ml), nine standards in CSF (0.16–40.02 μg/ml) and nine standards in brain microdialysate (0.07–17.86 μg/ml). Similarly, a stock solution containing 2.360 mg/ml HBA was prepared in methanol. It was diluted with water to prepare the working solutions. Linearity was assessed by analyzing five standards in plasma (0.15–2.36 μg/ml), five standards in CSF (0.07–1.18 μg/ml) and five standards in brain microdialysate (0.04–0.59 μg/ml).

The calibration curve was based on drug peak area and was analyzed by weighted linear regression using the DAS 2.0 (Drug and Statistics for Windows) Program (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The lower limit of quantification (LLOQ) was defined as the lowest drug concentration producing at least five times the response compared to the blank response and with acceptable precision (R.S.D. < 20%) and accuracy (80–120% of nominal concentration). Intra- and inter-day precision and accuracy were determined by analyzing spiked samples at three different concentrations on five different days. Each concentration experiment was conducted in six replicates from sample preparation to chromatographic analysis. Three aliquots at each of the low and high concentrations of the spiked samples were stored at the intended storage temperature and time to study the stability of Gas and HBA according to the FDA guidance [18].

Aliquots of 50 μl of 6% perchloric acid were added into the 50 μl blank, control or plasma sample to precipitate protein. The mixture was vortexed for 1 min and centrifuged at 9000 rpm for 10 min. The supernatant was immediately injected into the HPLC system with a 20 μl fixed loop. The CSF and brain microdialysate samples were analyzed directly.

2.4. Animal experiment

Male Sprague–Dawley rats (250–300 g) were obtained from the Zhejiang Laboratory Animal Center (Hangzhou, China). The rats were anesthetized with an intraperitoneal dose of 1% (w/v) sodium pentobarbital (45 mg/kg). Intracerebral guide probes (BAS/MD-2251, USA) were implanted in the frontal cortex (coordinates: AP 2.1, ML 2.0, DV 1.0) and hippocampus (AP −6.0, ML −4.6, DV 3.0), or thalamus (AP −3.0, ML 1.0, DV 4.5) and cerebellum (AP −11.0, ML −1.3, DV 2.0) according to the Paxinos and Watson atlas [19]. After surgery, the rat was placed in a single cage for 7 days for recovery. The positions of the probes were verified by a standard histological procedure at the end of the experiments.

On the day of the *in vivo* experiment, the rat was maintained anesthetized with an intraperitoneal dose of 20% (w/v) urethane (1 g/kg), and the body temperature was kept at 37 °C by a heating pad. Collection of CSF was performed using the cistern puncture, in accordance with our previous study [17]. For brain microdialysis, the rat was perfused with Ringer's solution (144 mM Na^+, 4 mM K^+, 1.3 mM Ca^+; pH 7.2), which was prepared in ultra pure deionized water and filtered through a 0.45-μm nylon filter before use. Brain microdialysis systems consisted of a MD-1020K microinjection pump (BAS, USA) and microdialysis probes (MD-2204, membrane length 4 mm, BAS, USA). Each probe was subjected to *in vitro* recovery studies before *in vivo* experiments for validation. After being washed with Ringer's solution at a flow-rate of 2.5 μl/min, two microdialysis probes were each inserted into one of two different brain