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Pharmacokinetics of Gastrodin in rat plasma and CSF after i.n. and i.v.

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Abstract

The pharmacokinetic behavior of Gastrodin in rat plasma and cerebrospinal fluid (CSF) after intranasal and intravenous administration (50 mg kg^{-1}) was investigated. Intranasal administration of Gastrodin provided a comparable AUC in CSF compared with the intravenous administration. But Gastrodin level in plasma was very low. The ratios of AUC values of intranasal to intravenous administration were 8.85% and 105.5% in plasma and CSF, respectively. The drug targeting index (DTI) was 12.34. In conclusion, intranasal administration of Gastrodin is a promising alternative to traditional administration. Olfactory mucosa did present another pathway for transport Gastrodin to the brain.
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Keywords: Gastrodin; Pharmacokinetics; Nasal administration; Cerebrospinal fluid; Plasma

1. Introduction

The blood–brain barrier (BBB) restricts the free diffusion of molecules across the endothelial membrane and prevents the transport of most substances from the systemic circulation into the central nervous system in order to maintain a stable environment. However, this barrier is also the main obstacle for the delivery of therapeutic substances to the brain, preventing many drugs from reaching there at therapeutic concentrations, especially for hydrophilic substances. Although some drugs with lipophlia and molecular weight less than 1000 can pass BBB, they can also be effluxed to blood by pump on membrane (e.g. glycoprotein) and the concentration in brain is too low. Therefore, brain-targeting delivery has been a great challenge in brain diseases therapy in recent years.

Intranasal administration has been increasingly paid attention to in the past decades because it is an attractive noninvasive route that can offer advantages such as rapid absorption, avoidance of first-pass metabolism, ease of convenience, and self-mediation (Illum, 2003). Recently increasing works have been reported in the possibility of circumventing the BBB for the delivery of drugs to the central nervous system by using the direct transport pathway from nose to brain via the olfactory region. It was shown that many substances, including tracer materials, heavy metals, low molecular weight drugs and peptides could reach the CSF, the olfactory bulb and other parts of the brain after nasal administration (Illum, 2000). Consequently, the nasal route may be important for drugs that are used in crisis treatments and for centrally acting drugs.

Gastrodin (Fig. 1) is one of the major and bioactive components in Tianma (*Gastrodia elata* Bl.) and has shown mitigative, anticonvulsive and neuroprotective effects (Hsieh et al., 1997; Kim et al., 2001; An et al., 2003; Liu et al., 2005; Ojemann et al., 2006) and has been approved as a drug for the treatment of neurasthenia, dizzy, headache and adjunctive therapy to epilepsy in China. The results of recent clinical trials showed that it was efficient in treatment of patients with vascular dementia. Currently, the marketed preparations of Gastrodin are intramuscularly, intravenously or orally administered, with injection as the most common dosage form. It showed species differences

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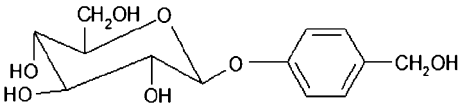

Fig. 1. Structure of Gastrodin.

in orally bioavailability (Liu et al., 1987; Cheng et al., 2003). Gastrodin is difficult to pass blood–brain barrier (BBB) because of water-solubility.

In present study, we have investigated the concentration profiles of Gastrodin in rat plasma and cerebrospinal fluid (CSF) after intranasal (i.n.) or intravenous (i.v.) administration. In order to find out whether the nasal route could be used to transport Gastrodin directly from the nasal cavity to the brain, bypassing the BBB, and also reveal the degree of drug targeting to the brain following Gastrodin i.n. delivery.

2. Materials and methods

2.1. Materials and chemicals

Gastrodin was obtained from Huizhou Orient Plant Health Care Sci. & Tech. Co. (Guangdong, China). Gastrodin was dissolved into water at a concentration of 250 mg ml^{-1} for nasal use, and was diluted 10 times for i.v. use. Acetonitrile of HPLC grade was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade and commercially available.

2.2. Animal experiments

Male Sprague-Dawley rats, weighed 250–300 g, were anesthetized with an intraperitoneal dose of 1% (w/v) sodium pentobarbital (45 mg kg^{-1}) and performed the cistern puncture according to the literature with some modifications (Van den Berg et al., 2002; Shi et al., 2005). Throughout the experiment, rats were kept under anaesthesia.

For the i.n. administration, an incision was made in rat neck and the trachea was cannulated with a polyethylene tubing to allow free breathing. To prevent drainage of nasally dosed solution and spirt out from nose when administration, the nasal cavity was isolated from the respiratory and gastrointestinal tracts by tying off the upper part of oesophagus. Then the animal was fixed onto a stereotaxic frame (MD3000, BAS, USA). The skin overlying the occipital bone was incised and the underlying muscle and tissue were bluntly dissected to expose the atlanto–occipital (a–o) membrane. A 25 gauge needle connected with a 5 cm silica gel tubing (I.D. 0.25 mm, O.D. 0.60 mm) was attached to a holder of the stereotaxic frame. The 5 cm silica gel tubing was attached to a 30 cm polyethylene tubing which was connected to a 1 ml syringe with water. During advancement of the needle through the muscles, the syringe plunger was pulled back to create negative pressure. Advancement of the needle was continued until air bubble moved into the tubing followed by CSF. Then the syringe and the 30-cm piece of polyethylene tubing were disconnected from the CSF collection system and mucilage was used to fasten the needle. Afterwards, the rat was placed in the supine -70° position according to the literature in order to let CSF drip out of the tubing by gravity. Before drug administration, a clamp was used to close the tubing.

Then, 25-30 μl of the nasal formulation (250 mg ml^{-1} Gastrodin in solution) was administered via micropipette into each nostril of the rats with a dose of 50 mg kg^{-1}. For the i.v. administration, the Gastrodin solution at a dose of 50 mg kg^{-1} was administered via femoral vein and the volumes were between 0.50 and 0.60 ml. At 5, 15, 30, 45, 60, 90, 120, 180 min for the i.n. or at 2, 10, 20, 40, 60, 90, 120, 180 min for the i.v. administration after dosing, CSF and blood sample were collected. The blood sample of 0.25 ml was taken from tail vein and placed into a heparinized PE conical tube, then centrifuged for 10 min at 9000 rpm and more than 100 μl plasma was obtained. The CSF sample of 30 μl was collected by connecting Hamilton syringe and the tubing was clamped after sampling. Then the CFS sample was placed in a PE tube without heparinization and stored at $-20\,^{\circ}C$ until analysis.

2.3. Analytical procedures

Gastrodin in plasma and CSF was assayed by the same HPLC system only with different pre-disposals. Aliquot of 100 μl plasma was mixed with 50 μl water or a standard solution of Gastrodin and 50 μl 8% perchloric acid (v/v) to precipitate protein. The mixture was vortexed for 2 min and centrifuged at 10,000 for 10 min. The supernatant was transferred to another PE conical tube, injected into the HPLC system with a 20 μl fixed loop. The CSF samples were analysed directly.

The HPLC system consisted of LC-10A pump, SPD-10A UV detector, SCL-10A system controller (Shimadzu, Japan), N2000 chromatographic workstation (Intelligent Information Engineer Ltd. of Zhejiang University), and C18 column (4.6 mm × 250 mm, 5 μm, Diamonsil, Dikma, USA). A mixture of acetonitrile–water (2.5:97.5, v/v) was employed as mobile phase with a flow rate of 1.0 ml min^{-1}. The wavelength of UV detector was set at 221 nm and the temperature of column oven was maintained at 33 °C.

Linearity was assessed by analyzing 9 standards with concentrations over the range of 0.390–99.85 μg ml^{-1} in plasma and 7 standards with concentrations over the range of 0.156–9.985 μg ml^{-1} in CSF ($n = 5$). The calibration curves were based on drug peak area and was analysed by weighted linear regression using DAS 2.0 program (Anhui, China).

2.4. Data analysis

Results obtained from the HPLC analysis were plotted as drug concentration–time curves in plasma and CSF. The area under the concentration–time curve (AUC) was calculated from the time zero to the last data point using trapezoidal method without extrapolation to infinity. Student's *t*-test was used to determine the statistical difference and a value of $P < 0.05$ was considered statistically significance (Computer program SAS version 6.12 for windows). The absolute nasal bioavailability of Gastrodin was calculated as the ratio of $AUC_{i.n.}/AUC_{i.v.}$. To evaluate the brain-targeting after nasal dosing, drug targeting index (DTI)

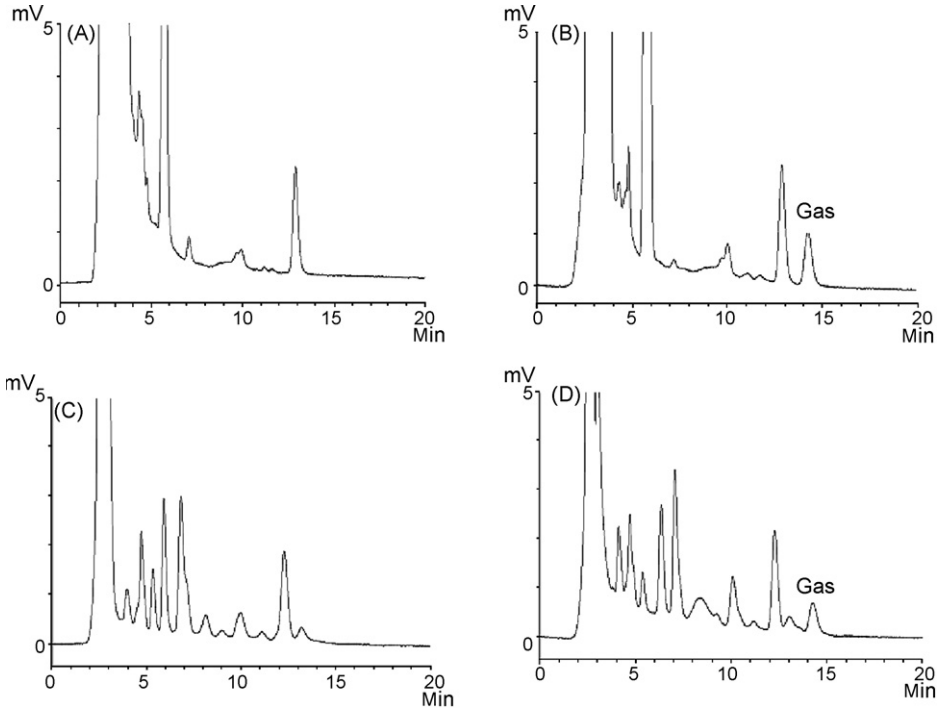

Fig. 2. Blank plasma (A) and sample (B) of plasma; Blank CSF (C) and sample (D) of CSF. Gas: Gastrodin.

(Wang et al., 2003; Wang et al., 2006) was described as the ratio of the value of AUC_{CSF}/AUC_{plasma} following i.n. administration to that following i.v. injection. The higher the DTI is, the further degree of Gastrodin targeting to the brain can be expected after i.n. administration.

$$DTI = \frac{(AUC_{CSF}/AUC_{plasma})_{i.n.}}{(AUC_{CSF}/AUC_{plasma})_{i.v.}}$$

3. Results

3.1. Validation of analytical method

The results showed in Fig. 2 indicated that the RP-HPLC method was specific for determination of Gastrodin under the chromatographic conditions employed. The retention time of Gastrodin was about 14.3 min, and not interfered by the compounds from plasma and CSF.

To assure the reliability of assay, the analytical method was validated using blank plasma and CSF samples in which different concentrations of Gastrodin were spiked. The RP-HPLC method established showed good linearity throughout the concentration range of 0.390–99.850 μg ml^{-1} in plasma and 0.156–9.985 μg ml^{-1} in CSF ($n = 5$). The regression equation was $Y = 8919.78X - 388.69$ and coefficient of correlation (r^2) was 0.9995 for plasma (weight = $1/X^2$), $Y = 17612.22X - 49.12$ and $r^2 = 0.9998$ for CSF (weight = 1). The average recovery of assay was 100.4% and 100.6% for CSF and plasma, respectively. Intra- and inter-day precision at three concentrations was < 7.0% with good accuracy (Table 1). The limit of detection (LOD) was 78 ng ml^{-1} based on S/N = 3. The limit of quantitation (LOQ) calculated from calibration curves was 0.156 μg ml^{-1} (accuracy 94.9%with R.S.D. < 5.4%) in CSF and 0.390 μg ml^{-1} (accuracy 101.8% with R.S.D. < 5.0%) in plasma ($n = 6$).

The stability of Gastrodin in bio-samples was also investigated. The blank plasma samples spiked with 3.120 and 49.925 μg ml^{-1} and blank CSF samples spiked with 0.624 and 4.992 μg ml^{-1} of Gastrodin were stored 10 h at room temperature and 1month at −20 °C (Table 2). During the storing period, three freeze–thaw (−20 °C/room temperature) cycles were carried out. The results indicate that Gastrodin in plasma and CSF was stable at room temperature for 10 h and at −20 °C for 1 month.

3.2. Disposition of Gastrodin on rats

The absorption of Gastrodin from nasal cavity to systemic circulation was slow and imcomplete. After 60 min, the concentration of Gastrodin in plasma was attained to steady state and

Table 1
precision and accuracy for the determination of Gastrodin in rat plasma and CSF (data are based on assay of six replicates on five different days)

Added concentration (μg ml^{-1})	R.S.D. (%)		Recoveries (%, $\bar{X} \pm$ S.D.)
	Intra-day	Inter-day	
CSF			
0.156	5.5	6.4	95.0 ± 5.3
0.624	5.3	6.0	102.1 ± 5.4
4.992	1.6	3.8	104.0 ± 1.6
Plasma			
0.390	5.1	4.2	101.8 ± 5.2
3.120	1.8	3.2	97.6 ± 1.7
49.925	1.9	2.5	102.5 ± 1.9

Table 2
Stability of Gastrodin in CSF and plasma ($\bar{X} \pm$ S.D., $n = 3$)

Concentration ($\mu g\ ml^{-1}$)	Percentage of initial value	
	Room temperature for 10 h	−20 °C for 1 month
CSF		
0.624	96.7 ± 1.1	93.2 ± 2.4
4.992	101.9 ± 1.0	98.1 ± 1.0
Plasma		
3.120	97.9 ± 3.3	96.1 ± 0.9
49.925	98.6 ± 1.8	99.4 ± 0.6

Table 3
AUC_{CSF}/AUC_{plasma} ratio of Gastrodin after intranasal delivery (i.n.) and intravenous infusion (i.v.) in rats ($\bar{X} \pm$ S.D., $n = 7$)

Parameter	i.n.	i.v.
AUC_{CSF} ($\mu g\ min\ ml^{-1}$)	150.94 ± 32.67	143.05 ± 33.57
AUC_{plasma} ($\mu g\ min\ ml^{-1}$)	222.22 ± 32.26	2597.87 ± 578.14
AUC_{CSF}/AUC_{plasma} (%)	67.93%	5.51%
$AUC_{CSF\ i.n.}/AUC_{CSF\ i.v.}$ (%)	105.52%	–
$AUC_{plasma\ i.n.}/AUC_{plasma\ i.v.}$ (%)	8.85%	–
DTI	12.34	–

still < 2 $\mu g\ ml^{-1}$ (Fig. 3a). The $AUC_{plasma\ i.n.}/AUC_{plasma\ i.v.}$ was only 8.85%.

At 10 min after intravenous administration, the CSF level of Gastrodin reached a high concentration and then was followed by an exponential decline. The transport of Gastrodin from nasal cavity to CSF was relatively slow. The maximum concentration appeared at 60 min (Fig. 3b). However, CSF concentrations of Gastrodin after 60 min following intranasal administration were always higher than those after intravenous administration until the last sampling point with statistical difference ($P < 0.05$) and $AUC_{CSF\ i.n.}/AUC_{CSF\ i.v.}$ was 105.5%. DTI was 12.34 (Table 3).

4. Discussion

Some researches have shown that low molecular weight and lipophilic compounds can be rapidly absorbed into systemic circulation after administration such as oxymorphone (Hussain and Aungst, 1997), a serotonin antagonist (Dahlin and BjÖrk, 2000), NX-066 (AchE inhibitor) (Dahlin and BjÖrk, 2001), estradiol and progesterone (Van den Berg et al., 2004a), melatonin (Van den Berg et al., 2004b), meptazinol (Shi et al., 2005). The results mentioned above suggested that there was no difference in concentrations of lipophilic molecules in plasma or CSF between i.v. and i.n. administration. The major cause of this phenomenon may be the compounds' lipophilicity, which makes them easily absorbed via respiratory mucosa to systemic circulation and later passed through BBB and diffused to CSF and brain tissues. Therefore, compounds with low molecular weight and high lipophilicity can be absorbed quickly and nearly completely after intranasal administration.

The high molecular weight and hydrophilic compounds such as vitamin B12 analogue hydroxocobalamin (Van den Berg et al., 2003) and fluorescein isothiocyanate-labelled dextran (FD3) (In't Veen et al., 2005) also present no difference in concentrations in plasma or CSF between i.v. and i.n. administration.

Nevertheless, the brain concentration of water-soluble and low molecular weight drugs (such as cephalexin (Sakane et al., 1991), dopamine (Dahlin et al., 2000), butyl ester prodrug of L-dopa (Kao et al., 2000), methotrexate (Wang et al., 2003), raltitrexed (Wang et al., 2006)) after i.n. administration was higher than that of i.v. This may be owing to their hydrophilicity, which resulted in their accumulation in the nasal cavity and continuous transport into the systemic circulation via the whole nasal mucosa or into the brain tissues via the olfactory mucosa, respectively.

Gastrodin is a water-soluble compound and whose saturated concentration is about 0.45 g ml^{-1} in water. Molecular weight of Gastrodin is 286 and its log P was calculated to be −1.36. Although $AUC_{CSF\ i.n.}$ was slightly higher than $AUC_{CSF\ i.v.}$, there was no significant statistics difference. While the Gastrodin level was very low in plasma after nasal administration, the DTI can be > 10.

The pharmacokinetic behavior of Gastrodin in CSF and plasma after i.n. administration showed there was obvious direct pathway to transport Gastrodin from nasal cavity to brain. The major reason may be the hydrophilicity of Gastrodin, making it not easy to be absorbed via respiratory mucosa to systemic circulation, thus detaining it at nasal and direct into CSF through olfactory passage. It was assumed that if drug concentration in the brain was significantly higher after intranasal administration than that of intravenous administration, or DTI > 1, a direct pathway from nasal olfactory area into the brain exists. The DTI of

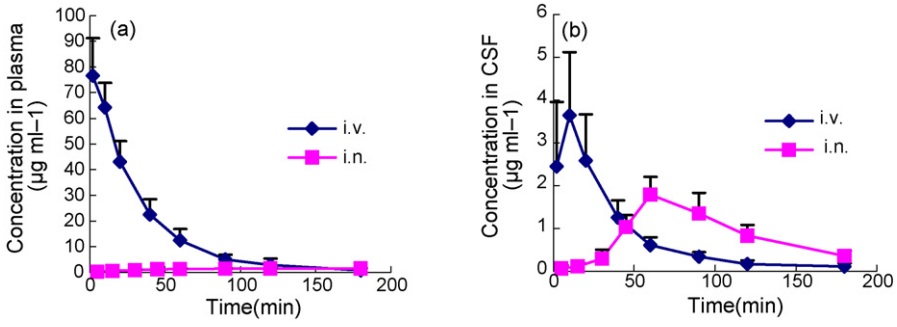

Fig. 3. (a) The concentration vs. time profile of Gastrodin in plasma after i.v. or i.n. administration on rats ($n = 7$). (b) The concentration vs. time profile of Gastrodin in CSF after i.v. or i.n. administration on rats ($n = 7$). The vertical bars represent S.D.

Gastrodin was > 10 although there was no difference in AUC for CSF between i.n. and i.v. administration. Therefore, we can conclude that, for drugs with hydrophilic and low molecular weight, it may be hopeful to obtain high DTI while administering nasally. In order to meet the low limit of detection for cerebrospinal fluid samples, the 250 mg ml^{-1} of Gastrodin was used for nasal formulation in our experiment, which was a little higher than that of 200 mg ml^{-1} in clinic intramuscular injection. Some studies revealed hyperosmotic stress was an important activator of aquaporin localized in nasal cytoplasmic vesicles and apical cell membranes, leading to significantly increased transepithelial water permeability (Pedersen et al., 2006). This maybe promotes the absorption of Gastrodin. But the osmotic pressure will decline quickly for Gastrodin solution being diluted by nasal mucus. So how effect of high osmotic pressure on transport Gastrodin from nose to brain needs to further study.

Therefore, our results supported Illum's conclusion (Illum, 2000) that the direct pathway from nose to brain may be significant for compounds which are poorly absorbed from nasal cavity to the systemic circulation or have low blood–brain barrier transport properties.

Nasally delivered drugs can reach the cerebrospinal fluid (CSF) which surrounds the brain and the actual brain tissue. The drug can cross the olfactory pathway by one or a combination of pathways (Illum, 2003). Firstly, the drug can be delivered by a transcellular pathway, which is especially suited for small lipophilic molecules or large molecules. Secondly, the drug can transport through the paracellular pathway by passing through the tight junctions or through open clefts in the membrane, which especially suited for smaller hydrophilic molecules. Thirdly, the drug can be transported through the olfactory neuron cells by intracellular axonal transport primarily to the olfactory bulb. For Gastrodin, which is water-solubility and small molecule, the second pathway may be predominated. The concentration of Gastrodin absorbed from nasal cavity in CSF and brain is not uniform. Gastrodin in olfactory nerve has a high concentration, which can diffuse into brain tissue and/or CSF, and flow from CSF to other place. There is a fast-diffusion equilibrium process between brain tissue and CSF, and the concentration tends to consistent. So the concentration of Gastrodin in CSF can reflect that of in brain. The higher concentration of Gastrodin in CSF takes advantage to enhance the pharmacological effect of drug in CNS.

In addition, the effect that the anesthesia may have on the drug absorption should be taken into account because the puncture method is the need for anesthetizing the animal. So, these can be explained why the T_{max} of Gastrodin in CSF after i.n. administration was slow and about 60 min. The concentration in CSF declined after 60 min, which suggested that it may relate to the transport saturation across the olfactory epithelium cells in olfactory area, and the mechanism needs to further study.

Different positions of the rat have an influence to the concentration of drug in CSF and the CSF sampling method using the supine −70° and 90° angle positions are suitable methods to study drug uptake into the CSF after nasal delivery in rats (Van den Berg et al., 2002). So the supine−70°was employed in the present study. To prevent drainage of nasally dosed solution, the nasal cavity was isolated from the respiratory and gastrointestinal tracts by tying off the upper part of esophagus. This operation can also prevent liquid of drug to spurt out from the nasal cavity when nasal administration because rat had some behavior reflex though it was kept under anesthesia.

It seems disadvantageous for the drug transport into CNS via the olfactory pathway when applied in human compared with rodents. In man, the olfactory mucosa is restricted to a small area in the roof of the nasal cavity of about 10 cm^2 (total surface area of the nasal cavity is about 150 cm^2). As a comparison, in the rat the olfactory mucosa constitutes 50% of the total nasal area (Illum, 2000, 2004). When it is used to man, the retention time of the drug over the olfactory areas should be prolonged by using viscous preparations and certain devices to apply the drug to the olfactory areas to overcome this disadvantage.

5. Conclusion

In summary, it is confirmed that the Gastrodin can be directly transported from the nasal cavity to CSF and there is a comparable AUC in CSF compared to the intravenous administration model. Gastrodin level in plasma was low and the DTI is high. The considerable uptake into CSF illustrates intranasal Gastrodin to be a promising alternative to traditional routes of administration in order to improve the therapeutic efficacy and reduce peripheral side effect.

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