Original Article

Effects of gastrodin on the dopamine system of Tourette's syndrome rat models

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Summary **Gastrodin is used in traditional Chinese medicine to treat Tourette's syndrome (TS). This study evaluated the effects of gastrodin on the dopamine system. TS rat models were established by intraperitoneal injection of apomorphine. After intervention by gastrodin, stereotyped behaviors of TS rats were significantly inhibited and levels of homovanillic acid (HVA) were significantly increased. We conclude that gastrodin effectively inhibited stereotyped behaviors and controlled TS symptoms by promoting dopamine metabolism, thereby increasing levels of HVA in sera.**

Keywords: Gastrodin, Tourette's syndrome rat model, homovanillic acid, dopamine D2 receptor

1. Introduction

Tourette's syndrome (TS) is a neuropsychiatric disorder characterized by stereotypic, involuntary, purposeless, and repetitive movements. These motor tics include headshakes, violent clonic tics consisting of thrusting head jerks and orofacial tics such as facial grimacing, eye blinking, and throat clearing (*1*). The prevalence of this syndrome is estimated to be between four and six per 1,000 children and adolescents (*2*). Initial symptoms of TS often occur around the age of 7 years. It occurs three to four times more frequently in males than in females (*3*).

The pathophysiology and etiology of TS are unclear. It is widely believed that dopamine system abnormalities play a primary role in the pathophysiology of TS (*4*). Dopamine modulates striatal neuron activity by stimulating dopamine receptors (*5*). There are two families of dopamine receptors, called D1-like (DRD1) and D2-like (DRD2) receptors (*6*). DRD2 receptors have been found in increased densities in the frontal cortex of TS patients, compared to matched controls (*7*). Fachinetto's study showed that the densities of prefrontal DRD2 were greater than 140% of their matched control (*8*). Autopsies also indicate that DRD2 activity was increased in TS patients (*9*). As the main metabolite of dopamine in the central nervous system, homovanillic acid (HVA) and its levels are generally regarded as a major indicator of dopamine activity (*10*). HVA levels were obviously lower in the patients than in the control group (*11*). All these facts indicate that TS is associated with dopamine activity and DRD2 density and sensitivity in striatum.

Haloperidol is a U.S. Food and Drug Administration-approved treatment for the symptoms of TS and other tic disorders. It can selectively curb the activity of postsynaptic dopamine receptor and inhibit the excitability of cortical motor areas through restraining activity on the dopamine receptor, alleviating TS symptoms (*12*). Although haloperidol is efficacious for the treatment of TS, a very high proportion of patients eventually discontinue the therapy because of the side effects, which include sedation, weight gain, extrapyramidal symptoms, and prolongation on the electrocardiogram of the QT interval (*13*). Therefore, development of new drugs for treatment of TS is urgently needed.

Traditional Chinese medicine has been developed and refined by the Chinese people over the course

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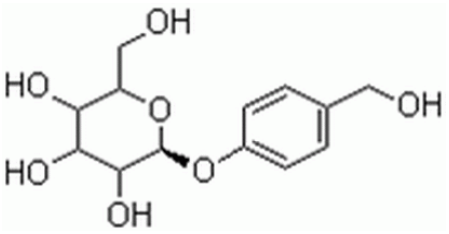

Figure 1. The chemical structure of gastrodin.

of thousands of years for use in the prevention and treatment of disease. Gastrodin (Gas, Figure 1), extracted from the Chinese herb *Gastrodia elata Blume*, has been shown to promote dopamine metabolism and to have obviously sedative, anticonvulsive, and antiepileptic properties (*14*). Our aim in this study was to explore the effect of gastrodin on TS and analyze the possible mechanisms.

2. Materials and Methods

2.1. *Materials*

Male wistar rats were purchased from Shandong Experimental Animal Center, Jinan, China. Apomorphine (Apo) was purchased from Sigma-Aldrich, St Louis, MO, USA. Haloperidol (Hal) was purchased from Shanghai Pharmaceutical Group Co., Ltd., Shanghai, China. Gastrodin was purchsed from Youcare Pharmaceutical Group Co., Ltd., Beijing, China. Trizol was purchased from Invitrogen, Carlsbad, CA, USA. RevertAid™ First Strand cDNA Synthesis Kit was purchased from Fermentas UAB, Vilnius, Lithuania, USA. TaKaRa TaqTM Hot Start Version was purchased from Takara Biotechnology (Dalian) Co., Ltd., Dalian, China, and Enzyme Immunoassay Kit from Adlitteram Diagnostic Laboratories, San Diego, CA, USA.

2.2. *Animals and experimental groups*

Rats (male, 4 weeks old, 100 ± 20 g) were housed in an air-conditioned animal room with a 12 h light/dark cycle, at a temperature of 22 ± 2°C and a humidity of 50 ± 10%. Rats were provided with a laboratory diet and water ad libitum, and maintained for 1 week before the start of the experiment. After 1 week, the rats were randomly divided into four groups: Control group (n = 16), Apo group (n = 16), Gas+Apo group (n = 16) and Hal+Apo group (n = 16). TS rat models were established by intraperitoneal injection of apomorphine (2 mg/kg) in rats in the Apo, Hal+Apo, and Gas+Apo group. Rats in the control group were intraperitoneally injected with normal saline (0.9%) (5 mL/kg). After injection, rats were intragastrically injected with gastrodin at 20 mg/kg (Gas+Apo group), haloperidol at 1.0 mg/kg (Hal+Apo), and normal saline (0.9%) at 10 mL/kg (control group and Apo group), respectively, once a day for 12 weeks. The rats' behaviors were observed by people who were familiar with the stereotypy actions, but were blind to the group. The stereotypy action included sniffing, body raising, licking, hyper-locomotion not associated with grooming, walking, eating, *etc*. Immediately following the treatment, the rats were observed for 1 min every 10 min for 60 min (a total of 6 observation periods). The standard was as follows (*15*): 0, asleep, resting or normal activity in place; 1, increased sniffing and head raising associated with hyper-locomotion; 2, discontinuous increased sniffing with body raising with hyper-locomotion; 3, discontinuous increased sniffing with head bobbing and body raising primarily in one place, with occasional rapid burst of locomotor activity (2-5 steps); 4, continuous sniffing, head bobbing and repetitive body raising/wall climbing; 5, continuous sniffing, licking, head bobbing, and continuous body raising/wall climbing. After 12 weeks, the rats were sacrificed. The blood was collected and striatum was isolated according to Paxinos and Watson's stereotaxic atlas of the rat brain (*16*). Animal handling for the experiments was in accordance with the American Physiological Society's "Guiding Principles in the Care and Use of Animals."

2.3. *HVA levels*

The levels of HVA in sera were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Briefly, dispensed antigen standards and sera were added to each well of 96-well plates pre-coated with first antibodies. After adding Biotin Conjugate Reagent and Enzyme Conjugate Reagent into each well, the plates were incubated at 37°C for 60 min. Then the plates were rinsed 5 times with distilled water. After chromogenic reaction, the absorbance was measured at 405 nm by Microtiter plate reader within 30 min.

2.4. *DRD2 mRNA expression*

Expression of DRD2 mRNA in striatum in each rat was determined by reverse transcriptase-polymerase chain reaction (RT-PCR). The striatum samples were homogenized, and total RNA was extracted with Trizol reagent according to the manufacturer's instructions. The primers for DRD2 were forward primer 5'-CTG GTA ATG CCG TGG GTT-3' and reverse primer 5'-CAG GGT GGG TAC AGT TGC-3' (487 bp) (AJ347728). The primers for β-actin were forward primer 5'-CCT GTG GCA TCC ATG AAA CTAC-3' and reverse primer 5'-CTT CTG CAT CCT GTC AGC AAT-3' (134 bp) (NM031144). The PCR protocol consisted of an initial denaturation step at 94°C for 30 sec, followed by 30 cycles of amplification. For amplification of cDNA, the cycles consisted of denaturation at 94°C for 30 sec,

annealing at 58°C for 30 sec, and elongation at 72°C for 30 sec. For amplification of β-actin cDNA, the cycles included denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and elongation at 72°C for 30 sec. To verify the specificity of amplification, PCR products were subjected to electrophoresis on a 1.5% agarose gel, which was then stained with ethidium bromide and examined with AlphaImager 2200 image analysis system (Alphaimager 2200 Pharmacia Biotech Co., San Francisco, CA, USA). β-Actin was used as an internal control in each sample.

2.5. *Statistical analysis*

Data were expressed as mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance. All analyses were performed using the SPSS statistical software package (Version 13.0, SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. *Behavior study*

As shown in Figure 2, treatment with gastrodin and haloperidol can inhibit the stereotyped behavior induced by apomorphine in TS rats. Apomorphine continued to induce stereotyped behavior without intervention and at the start of treatment ($p > 0.05$). Comparison of the three groups at the end of the experiment showed that the scores were conspicuously lower in the Gas+Apo group and the Hal+Apo group than in the Apo group ($p < 0.05$), while gastrodin had the same effect as haloperidol on stereotyped behavior.

3.2. *HVA levels in sera*

As shown in Figure 3, the HVA content was significantly lower in the Apo group than in the control group (3.49 ± 0.66 mg/mL *vs*. 4.76 ± 0.85 mg/mL, $p < 0.01$). The HVA content was significantly higher in the Gas+Apo group and the Hal+Apo group than in the Apo group (4.69 ± 1.30 mg/mL *vs*. 3.49 ± 0.66 mg/mL, 4.86 ± 1.48 mg/mL *vs*. 3.49 ± 0.66 mg/mL, $p < 0.05$). There was no significant difference between the Hal+Apo group and Gas+Apo group ($p > 0.05$).

3.3. *DRD2 mRNA expression*

Striatum DRD2 mRNA expression was analysed by RT-PCR. As shown in Figure 4, DRD2 mRNA expression in striatum of the Apo group was significantly increased compared with the control group ($p < 0.01$). In the Hal+Apo group, a significant decrease of DRD2 mRNA expression was observed compared with that of the Apo group ($p < 0.05$). Gastrodin had no effect on DRD2 mRNA expression ($p > 0.05$). DRD2 mRNA expression was significantly lower in the Hal+Apo group than in the Gas+Apo group ($p < 0.05$).

4. Discussion

Hyperfunction of the dopamine system plays an important role in the etiopathogenesis of TS. Dopamine

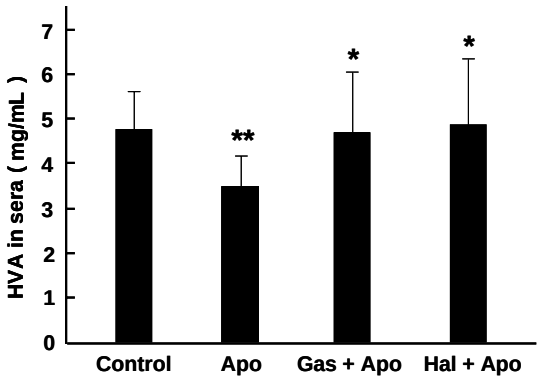

Figure 3. Effect of gastrodin on HVA levels in sera. Data are expressed as the mean ± S.D. ($n = 16$ rats/group). ** $p < 0.01$ versus the control group. * $p < 0.05$ versus the Apo group.

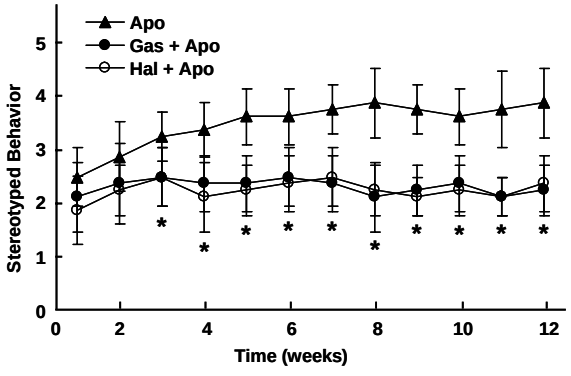

Figure 2. Stereotyped behaviors of rats in the three experimental groups during a 12-week period. The behaviors were recorded the last day of each week. Data were expressed as the mean ± S.D. ($n = 16$ rats/group). * $p < 0.05$ versus the Apo group.

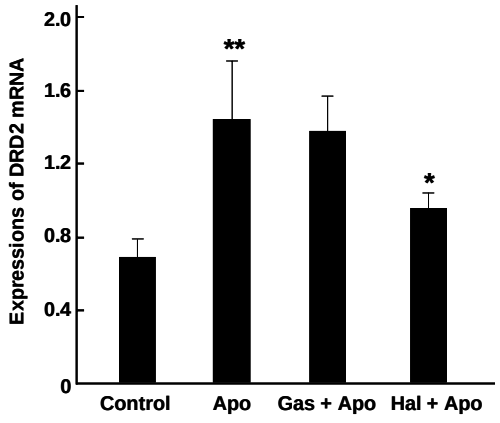

Figure 4. Effect of gastrodin on the expression of DRD2 mRNA in striatum. ** $p < 0.01$ versus the control group. * $p < 0.05$ versus the Apo group.

produced a remarkable effect only after combining with DRD2. After reuptake by dopamine transporters, the dopamine was transformed into HVA in neurons and released into the blood. Evidence shows that DRD2 activity is increased and HVA levels are decreased in TS patients (*9,11*). Promoting dopamine metaboly and inhibiting DRD2 activity may control tics.

Gastrodia elata Blume (Orchidaceae) is a medicinal plant and used in China as a crude drug for the treatment of epilepsy, infantile convulsions, headache, and dizziness. Gastrodin is one major active component of this herb. Gastrodin was found to penetrate the blood-brain barrier, enter the central nervous system, and protect from nerve lesions (*17*). Gastrodin reduces the seizure score in seizure-prone gerbils, modifies noradrenergic, dopaminergic, and serotonergic nervons in the rat brain, and have mitigative, antidepressant, anticonvulsive, and neuroprotective effects (*14,18*). Ju reported that gastrodin could inhibit activity of dopaminergic neurons, decrease dopamine reuptake, promote dopamine metaboly, and accommodate catecholamine in the nervous system (*19*). In the present study, we used gastrodin-treated TS rats and compared its effects with the effects of haloperidol. Confirming previous reports, our findings demonstrated that gastrodin significantly increases dopamine metabolism in TS rats and promotes its transformation into HVA, alleviating TS symptoms.

Haloperidol, a common medication for TS, reduces tic frequency and severity and elevates levels of HVA (*20*). In this study, we found that gastrodin also increases the levels of HVA and alleviates the stereotyped behaviors of apomorphine-induced TS rats, and that gastrodin has effects on HVA and on stereotyped behaviors similar to the effects of haloperidol. Both promote dopamine transformation into HVA. This observation indicates that gastrodin and haloperidol have the same effect on dopamine metabolism. DRD2 is a G-protein-coupled receptor. Excitable activity occurred after dopamine combined with DRD2. High DRD2 activity is associated with tics. In this study, we found the level of DRD2 mRNA in striatum was significantly lower in the Hal group than in the Apo group. Our data are consistent with other studies (*21*). However, we failed to observe any effect of gastrodin on DRD2 mRNA expression; this might indicate that gastrodin cannot act on DRD2 mRNA expression.

In summary, the results of this study provide evidence that gastrodin effectively inhibits the stereotyped behaviors and controls the symptoms of TS by promoting dopamine metabolism and increasing levels of the metabolic product HVA in sera.

Acknowledgements

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