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Effects of Ningdong granule on the dopamine system of Tourette's syndrome rat models☆

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ABSTRACT

Ethnopharmacological relevance: Ningdong Granula (NDG) is a traditional Chinese medicine (TCM) preparation for the treatment of Tourette's syndrome (TS).
Aim of the study: To explore the effects of NDG on stereotyped behavior, homovanillic acid (HVA) in sera, dopamin (DA) and dopamin D2 receptor (DRD2) in striatum in TS rats.
Materials and methods: Sixty-four rats were randomly divided into control group and three experimental groups. TS rat models were induced by intraperitoneal injection (*i.p.*) of Apomorphine (Apo, 2 mg/kg) in the experimental groups. After Apo *i.p.*, rats were intragastrically injected (*i.g.*) with NDG at 370 mg/kg (NDG + Apo group), haloperidol (Hal) at 1.0 mg/kg (Hal + Apo group), and normal saline (0.9%) at 10 ml/kg (control group and Apo group), respectively, once a day for 12 weeks. The behaviors of the rats were observed and recorded each day. After 12 weeks, all rats were sacrificed and sera and striatum were collected. The levels of HVA in sera, DA in striatum were examined by ELISA, and the expression of DRD2 mRNA in striatum was measured by RT-PCR.
Results: NDG could increase the HVA content in sera ($P < 0.05$), meanwhile downregulate the expression of DRD2 mRNA in striatum ($P < 0.05$), and inhibit the stereotyped behaviors induced by Apo ($P < 0.01$) in TS rats, the same effects with Hal. NDG could also reduce the DA content in striatum ($P < 0.01$), while Hal could not.
Conclusions: NDG could effectively inhibit the stereotyped behaviors in TS rats, and the mechanisms may be related to the suppression of DA system by increasing the content of HVA in sera, decrease the content of DA and repressing the expression of DRD2 mRNA in striatum.

1. Introduction

Tourette's syndrome (TS) is a neuropsychiatric disorder characterized by stereotypic, involuntary, purposeless and repetitive movements. The motor tics include headshakes, violent clonic tics consisting of thrusting head jerks and orofacial tics such as facial grimacing, eye blinking and throat clearing (Grimaldi, 2002). The prevalence of this syndrome is estimated to be between four and six per 1000 children and adolescents (Cortese et al., 2008). Initial symptoms of TS often occur around the age of 7 years (Grimaldi, 2002). It occurs three to four times more common in males than in females (Peterson and Leckman, 1998). For some individuals, tics can cause lifelong impairment and about 5% of TS patients have life-threatening symptoms, which were defined malignant TS (Liu et al., 2008).

The pathophysiology and etiology of TS are not clarified. It is widely believed that abnormalities of dopamin (DA) neurotransmission play a primary role in the pathophysiology of TS (Wong et al., 2008). DA, released by nerve terminals originating from midbrain neurons, modulates striatal neurons activities through stimulate DA receptors (Centonze et al., 2004). There are two families of DA receptors, called D1-like receptor (DRD1) and D2-like receptor (DRD2) (Emilien et al., 1999). The densities of prefrontal DRD2 were greater than 140% of their matched control (Minzer et al., 2004; Dustin et al., 2007). Besides, autopsy also indicated that the DRD2 activity in TS patients was increased (Singer et al., 1991). As the main metabolite of DA in central neural system, homovanillic acid (HVA) is generally regarded as the major indicator of DA activity (Dhir and Kulkarni, 2007). HVA in the patients was obviously lower than that in control group (Yao and Ma, 2005). All these indicate that TS is correlated with content and activity of DA, density and sensitivity of DRD2 in striatum.

Blockage of DA receptors especially DRD2 by some receptor antagonists could reduce the tic frequency and severity (Sandor, 2003). Haloperidol (Hal) is a treatment approved by Food and

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Drug Administration (USA) for the symptoms of TS and other tic disorders. It can selectively curb the activity of postsynaptic DA receptors, and inhibit the excitability of cortical motor area through restraining the activity of DA receptors, so as to weaken TS symptoms (Fachinetto et al., 2007). Although Hal is efficacious for the treatment of TS, a very high proportion of patients eventually discontinue the therapy because of the side effects including sedation, weight gain, extrapyramidal symptoms, and QT prolongation (Yoo et al., 2006). Therefore, development of novel drugs for treatment of TS is urgently needed.

Traditional Chinese medicine (TCM), developed and refined over the course of thousands of years by the Chinese people for use in the prevention and treatment of disease. Ningdong granule (NDG), a TCM preparation, has been used as an anti-tics agent for years in clinic. Our previous study showed that the total effective rate of NDG for TS was 73.3–83.3% (Li et al., 2008). In this study, we aimed at exploring the possible mechanism of NDG on the DA system of TS rats.

2. Materials and methods

2.1. Materials

Wistar rats (male, 4-week-old, 100 ± 20 g) were purchased from Shandong Experimental Animal Center (Jinan, China). Apomorphine (Apo) was purchased from Sigma (USA), Hal from Shanghai Pharmaceutical Group Co. Ltd. (Shanghai, China), Trizol from Invitrogen (USA), RevertAid^{TM} First Strand cDNA Synthesis Kit from Fermentas (USA), TaKaRa Taq^{TM} Hot Start Version from Takara Biotechnology (Dalian) Co. Ltd. (Dalian, China), and Enzyme Immunoassay Kit from Adlitteram Diagnostic Laboratories (USA). Gene amp^{R} PCR System was supplied by Applied Biosystems Co. Ltd. (USA), and microtiter plate reader was offered by Molecular Devices Co. Ltd. (USA).

2.2. Preparation of NDG

NDG formulation consists of 4 different plant species, 3 animal substances, and human placenta as shown in Table 1. All of these formulation were provided and prepared by 999 Modern Chinese Medicine Co. Ltd. (999 Co. Ltd., Shenzhen, China), and carefully authenticated by Dr. Wei-Zhou Li, Pharmaceutical Preparation Section, 999 Co. Ltd. Voucher specimens (numbers were listed in Table 1) were deposited at the Herbarium of 999 Co. Ltd. After being dried, they were mixed in proportion and then macerated for 1 h at room temperature with distilled water. After that, the whole mixture was decocted twice for 1 h each time. The filtrates were mixed and condensed and then dried by vacuum-drier at 60 °C. The yield granule was stored at 4 °C.

Table 1
Composition and active ingredients of Ningdong granule.

Components	Voucher specimens number	Part used	Amount used (g)
Uncaria rhynchophylla (Miq.) Jacks	0706011	Ramulus	20
Gastrodia elata Blume	0705081	Root	6
Ligusticum chuanxiong Hort	0704081	Rhizome	6
Buthus martensii Karsch	0704021	Dried body	3
Scolopendra subspinipes mutilans L. Koch.	0707021	Dried body	Single band
Haliotis diversicolor Reeve.	0701041	Shell	20
Dried human placenta	0708021	Dried placenta	3
Glycyrrhiza uralensis Fisch.	0706011	Rhizome	3

2.3. Experimental animals

Sixty-four wistar rats were housed in an air-conditioned animal room with 12 h light/dark cycle, temperature of 22 ± 2 °C and 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 control group (n = 16) and three experimental groups: Apo group (n = 16), NDG + Apo group (n = 16) and Hal + Apo group (n = 16). TS rat models were induced by intraperitoneal injection (*i.p.*) with Apo (2 mg/kg) in the experimental groups. Rats in the control group were injected with normal saline (0.9%) (5 ml/kg, *i.p.*). After the injection of Apo (*i.p.*), rats were treated by intragastric administration (*i.g.*) with NDG at 370 mg/kg (NDG + Apo group, equal to 15-folds of clinic dosage), Hal at 1.0 mg/kg (Hal + Apo group), and normal saline (0.9%) at 10 ml/kg (Apo group), respectively, once a day for 12 weeks. The rats' behaviors were observed by people who were familiar with the stereotyped behavior, but blind to the group. 10 min after administration, the observer observed the behavior of rats for 1 min every 10 min for 60 min (a total of 6 observation periods) and scored them. The stereotypy actions including bites (teeth touching the cage, wood chips, vacuous chewing), taffy pulling (raises of the forepaw), self-gnawing, licking not associated with grooming, head shaking, paw buffeting, quick aversion, episodic utterances, *etc*. Scores ranging from 0 to 5 were averaged for each group. The standard was: 0—Asleep, resting in place or normal activity in place; 1—Increased sniffing and head raising; 2—Discontinuous increased sniffing with body raising; 3—Discontinuous increased sniffing, licking with head and body raising primarily in one place, with occasional rapid burst of locomotor activity (2–5 steps); 4—Continuous sniffing, biting, head bobbing and repetitive body raising/wall climbing in place; 5—Continuous sniffing, biting, licking, head bobbing, and continuous body raising/wall climbing wherein forepaws do not touch cage floor (Napier and Istre, 2008). After 12 weeks, the rats were sacrificed. The blood was collected and striatum were isolated according to Paxinos and Watson's stereotaxic atlas of rat brain (Paxinos and Watson, 1996). Animal handling for the experiments was in accordance with the *American Physiological Society* "Guiding Principles in the Care and Use of Animals".

2.4. Levels of HVA in sera and DA in striatum

The levels of HVA in sera and DA in striatum were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer. Briefly, dispensed antigen standards and samples 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.

2.5. Expression of DRD2 mRNA in striatum

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 was comprised of an initial denaturation step at