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Dopamine transporter inhibitory and antiparkinsonian effect of common flowering quince extract

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ABSTRACT

Common flowering quince (FQ) is the fruit of *Chaenomeles speciosa* (Sweet) Nakai. FQ-containing cocktails have been applied to the treatment of neuralgia, migraine, and depression in traditional Chinese medicine. The present study assessed whether FQ is effective in dopamine transporter (DAT) regulation and antiparkinsonism by utilizing *in vitro* and *in vivo* assays, respectively. FQ at concentrations of 1–1000 μg/ml concentration-dependently inhibited dopamine uptake by Chinese hamster ovary (CHO) cells stably expressing DAT (D8 cells) and by synaptosomes. FQ had a slight inhibitory action on norepinephrine uptake by CHO cells expressing the norepinephrine transporter and no inhibitory effect on γ-aminobutyric acid (GABA) uptake by CHO cells expressing GABA transporter-1 or serotonin uptake by the serotonin transporter. A viability assay showed that FQ mitigated 1-methyl-4-phenylpyridinium-induced toxicity in D8 cells. Furthermore, in behavioral studies, FQ alleviated rotational behavior in 6-hydroxydopamine-treated rats and improved deficits in endurance performance in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. Furthermore, immunohistochemistry revealed that FQ markedly reduced the loss of tyrosine hydroxylase-positive neurons in the substantia nigra in MPTP-treated mice. In summary, FQ is a selective, potent DAT inhibitor and has antiparkinsonian-like effects that are mediated possibly by DAT suppression. FQ has the potential to be further developed for Parkinson's disease treatment.

1. Introduction

FQ is the dried, nearly ripe fruit of *Chaenomeles speciosa* (Sweet) Nakai (Family Rosaceae) that is mainly distributed in China, with centers of origin in Anhui, Yunnan, Shanxi, Gansu, Guizhou, Sichuan, and Guangdong Provinces. The nutritional and edible fruit has been widely used for its beneficial health effects for hundreds of years in China by processing it into preserved fruit, canned food, wine, vinegar, syrup, and hair- and skin-care products. Clinically, FQ is commonly used as a therapeutic agent for the treatment of rheumatism, cholera, enteritis, and beriberi. In addition, FQ-containing prescriptions (oral herbal cocktails) contain 9–30 g of FQ and have been widely used in folk medicine for the treatment of neuralgia, migraine (He and Jiang, 2006), stroke (Wang, 2006), depression (An and Cheng, 2007), and other diseases with symptoms of limb tremors or spasms (dyskinesias) resembling symptoms of Parkinson's disease. Such therapeutic applications suggest that FQ may provide significant antiparkinsonian benefit.

Parkinson's disease is a chronic neurodegenerative disorder characterized by massive and progressive degeneration of the nigrostriatal dopaminergic system (Park et al., 2003), resulting in abnormal motor behaviors such as muscular rigidity, postural abnormality, and tremor (Blum et al., 2001). However, the etiopathogenesis of dopaminergic loss in Parkinson's disease remains unclear (Sulzer, 2007; Rosner et al., 2008). Current evidence points to the presence of ongoing oxidative stress and the generation of radical oxygen species due to inhibition of complex I selectively in the substantia nigra leading to aggregation of α-synuclein and subsequent loss of dopamine neurons (Chiueh et al., 2000; Dawson and Dawson, 2003; Eberhardt and Schulz, 2003). L-DOPA administration is the most commonly employed treatment (Agnati et al., 2004), but it only alleviates clinical symptoms in the early stages of the disease and is less effective as the disease progresses (i.e., when side effects of on–off fluctuations and dyskinesias occur) (Ahlskog and Muenter, 2001; Hurtig, 1997). No therapy has yet been made available that slows or halts the neurodegeneration associated with Parkinson's disease (Ravina et al., 2003).

The dopamine transporter (DAT) is an important regulating protein involved in dopaminergic transmission. The membrane protein belongs

Abbreviations: FQ, common flowering quince; i.p., intraperitoneal; i.g., intragastric; s.c., subcutaneous; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter; GABA, γ-aminobutyric acid; GAT-1, γ-aminobutyric acid transporter; CHO, Chinese hamster ovary cells; 6-OHDA, 6-hydroxydopamine; GBR12,935, (1-[2-(diphenylmethoxy)ethyl]-4-[3-phenylpropyl]-piperazine); MTT, methyl thiazolyl tetrazolium acid; HBSS, Hank's balanced salt solution; PBS, phosphate-buffered saline; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium; TH, tyrosine hydroxylase.

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to Na^+/Cl^--dependent neurotransmitter transporters, is expressed mainly in the extrapyramidal system, and plays crucial roles in limiting dopamine activity in the central nervous system (Hersch et al., 1997; Nelson, 1998). Compounds that directly (Hansard et al., 2002b) or indirectly (Joyce et al., 2004) inhibit DAT activity may be useful in the treatment of the motor symptoms of Parkinson's disease. Some DAT inhibitors have been shown to have antiparkinsonian-like effects in animal models (Nutt et al., 2004) via binding to presynaptic DAT, and thereby blocking dopamine reuptake back into the synaptic cleft and enhancing dopaminergic neurotransmission by activating dopamine receptors (Torres et al., 2003). Previous studies have shown that DAT inhibitors such as amphetamine (Parkes et al., 1975), nomifensine (Teychenne et al., 1976; Park et al., 1981), bupropion (Goetz et al., 1984), and mazindol (Delwaide et al., 1983) have beneficial effects in patients with advanced Parkinson's disease.

We therefore hypothesized that FQ may have an inhibitory effect on DAT and a regulatory effect on the abnormal extrapyramidal system manifested in an animal model of Parkinson's disease. In this study, we screened hundreds of Chinese herbs by a transgenic cell-line screening system and discovered that FQ indeed had an inhibitory effect on DAT and an antiparkinsonian-like effect in two Parkinson's disease models.

2. Materials and methods

2.1. Preparation of aqueous extract of FQ

FQ, a dried fruit of *C. speciosa* (Sweet) Nakai, was bought from Anhui Province, China, and was identified and authenticated by an expert herbalist at the Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The voucher specimen (no. 20050708) was deposited at the Herbarium of Cell-Star Bio-Technologic Co., Ltd. (Shanghai, China). The specimen was washed, crushed, and homogenized. The crushed aggregate was diluted in distilled water (weight:volume, 1:10) and heated at 100 °C for 1 h. The supernatants were collected, and the remaining portion was diluted and heated further for 1 h to yield additional extract. The two extract solutions were mixed, filtered, and centrifuged (2000 ×*g* at 20 °C) for 10 min to remove any water-insoluble components. The supernatants were lyophilized in a freeze dryer. The dried powder (yield rate 20.9%) was collected and stored at −20 °C for experimental use. Prior to experimentation, FQ extract powder was redissolved in 0.9% normal saline or distilled water at different concentrations for *in vivo* or *in vitro* study, respectively.

2.2. Cell culture and DAT transfection

CHO cells expressing rat DAT, rat serotonin transporter (SERT), mouse GABA transporter (GAT-1), or human norepinephrine transporter (NET) were used in this study, and the transgenic methods have been described previously (Liu et al., 2001; Xu et al., 2006). Rat DAT-pCDNA3 was transferred to CHO cells through electroporation. Selection of the transfected cells was conducted in culture by the addition of Geniticin (G418). CHO cells stably expressing DAT then were subcloned by limiting dilution methods. Several subclones were selected using a [^{3}H]dopamine uptake assay. The cell clone with the highest uptake, designated as D8 cells, was chosen for further experimentation. Similarly, a clone highly expressing GAT-1, SERT, or NET (designated as G1 cells, S6 cells, and N1 cells, respectively) was obtained. Fig. 1 shows 20- to 30-fold enhancement of neurotransmitter uptake after the CHO cells were transfected with the corresponding neurotransmitter transporter. GBR12,935 (selective DAT reuptake inhibitor), desipramine (NET inhibitor), fluoxetine (SERT inhibitor), and tiagabine (GAT-1 inhibitor), at concentrations of 1, 10, 10, and 10 μM, respectively, significantly inhibited the enhanced uptake, thus confirming the validity of the screening model.

2.3. [^{3}H]Dopamine uptake inhibitory assay in vitro

D8 cells were grown in RMPI1640 medium (Gibco BRL Life Technologies) containing 10% fetal bovine serum (Gibco BRL Life Technologies) to near confluence in 48-well tissue culture plates (Costar) (approximately 60,000 cells per well). D8 cells then were rinsed once with phosphate-buffered saline (PBS) and pre-incubated in 100 μl Hank's balanced salt solution (HBSS) at room temperature for 10 min. [^{3}H] Dopamine (8.8 Ci/mmol, Amersham Pharmacia Biotech), ascorbic acid, and pargyline were added to final concentrations of 0.1, 100, and 100 μM, respectively. Cells then were incubated at room temperature for another 20 min. The reaction was terminated by aspiration of the HBSS, and the cells were washed three times rapidly (10 s/wash) with ice-cold PBS followed by solubilization in 2 N NaOH. An aliquot was measured by a liquid scintillation counting analyzer (TRI-CARB2900TR, Pakard) to quantify [^{3}H]dopamine uptake. For the study of the inhibitory effect of FQ, different concentrations of FQ and GBR12,935 solutions were added at the beginning of the uptake assay. For serotonin and norepinephrine uptake assays, the procedures were similar to that for dopamine uptake assay in D8 cells, with the exception that 50 nM [^{3}H]serotonin (111 Ci/mmol, Amersham Pharmacia Biotech) or 25 nM [^{3}H]norepinephrine (40 Ci/mmol, Amersham Pharmacia Biotech) was used instead of [^{3}H] dopamine for S6 cells or N1 cells, respectively. For the GABA uptake assay, 50 nM [^{3}H]GABA (88 Ci/mmol, Amersham Pharmacia Biotech) was used instead of [^{3}H]dopamine and ascorbic acid and pargyline in the system for G1 cells. The concentration–effect curve was established to measure IC_{50} values (concentration required to inhibit specific dopamine uptake by D8 control cells by 50%), and E_{max} values (maximal effect on dopamine uptake inhibition) and EC_{50} values (effective concentration to reach 50% of E_{max}) were analyzed by nonlinear aggression. Absolute inhibition (%)=[(DPM value of vehicle-treated D8 cells−DPM value of drug-treated D8 cells)/(DPM value of vehicle-treated D8 cells−DPM value of background)]×100%, where DPM indicates the disintegrations per minute.

2.4. Uptake of dopamine by striatal synaptosomes

The procedure has been described elsewhere (Kokoshka et al., 1998) with some modifications. Briefly, male Sprague–Dawley rats (200–250 g) were sacrificed by decapitation, and the striatum was dissected out. Fresh striatal tissues were homogenized with a glass homogenizer with 10 to 20 strokes in ice-cold 0.32 M phosphate-buffered sucrose and centrifuged (1000 ×*g* at 4 °C) for 10 min. The supernatants (S1) then were

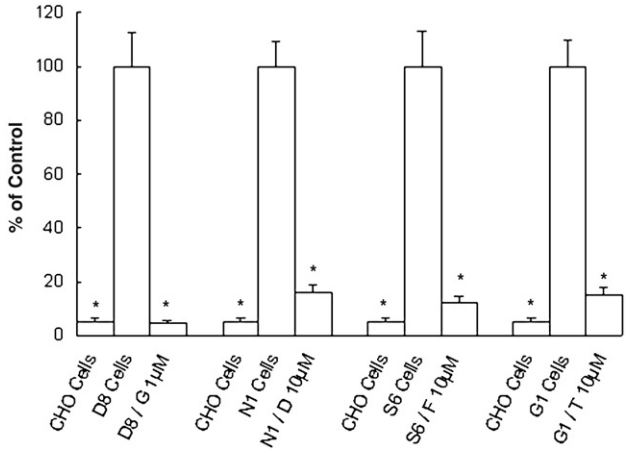

Fig. 1. Functional confirmation of *in vitro* screening systems. Dopamine, norepinephrine, serotonin, and GABA uptake assays were conducted in transgenic CHO cells expressing DAT (D8 cells), NET (N1 cells), SERT (S6 cells), or GAT-1 (G1 cells), respectively. G (GBR12,935), D (desipramine), F (fluoxetine), and T (tiagabine) are selective inhibitors of DAT, NET, SERT, and GAT-1, respectively. *$P<0.001$ compared with control group (D8, G1, S6, and N1 cell groups). Values are expressed as mean±SEM of triplicate cell samples.