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Da-Bu-Yin-Wan and Qian-Zheng-San, two traditional Chinese herbal formulas, up-regulate the expression of mitochondrial subunit NADH dehydrogenase 1 synergistically in the mice model of Parkinson's disease

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ABSTRACT

Ethnopharmacological relevance: Da-Bu-Yin-Wan (DBYW) and Qian-Zheng-San (QZS), two traditional Chinese herbal formulas, were clinically employed to treat Parkinson's disease (PD) for decades.
Aim of the study: Our previous studies demonstrated neuroprotective effects of DBYW and QZS on mitochondrial function in mice model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In present research, we aimed to investigate the possible neuroprotective mechanisms of DBYW and QZS.
Materials and methods: The effects of DBYW and QZS on the behavioral changes (pole test), expression of tyrosine hydroxylase (TH) of substantia nigra by immunohistochemistry, monoaminergic contents and activity of striatum by high performance liquid chromatography, neuronal ultrastructure changes by transmission electron microscopy, mitochondrial DNA (mtDNA) damage by long-extension polymerase chain reaction (PCR), and mRNA expression of mitochondrial subunit NADH dehydrogenase 1(ND1) by qualitative real-time PCR were investigated.
Results: Present study demonstrated that DBYW and QZS not only ameliorated the behavior induced by the administration of MPTP and synergistically prevented the decreasing of TH expression, but also increased monoaminergic contents and activity, improved the ultrastructural changes, decreased the mtDNA damage, and synergistically up-regulated the expression of ND1 in mRNA level.
Conclusions: These results suggest that DBYW and QZS possess anti-parkinsonism and neuroprotective properties.

1. Introduction

Parkinson's disease (PD; *Online Mendelian Inheritance in Man*, OMIM ID: 168600) is a chronic, progressive neurodegenerative disorder, unilateral presentation with asymmetrical signs, characterized by resting tremor, bradykinesia, rigidity, and postural instability (Lees et al., 2009; Zhang et al., 2012a; 2012b). Pathologically, it is characterized by the extensive and progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) as well as an accumulation of intraneuronal inclusions (Lewy bodies) in the surviving neurons (Braak et al., 2004). However, in the majority of cases, the etiology and pathogenesis of PD remain unknown. Notably, pathophysiology of PD includes oxidative stress and impairment of mitochondrial energy metabolism, in particular a specific dysfunction of mitochondrial complex I (Schapira et al., 1990).

Complex I (NADH: ubiquinone oxidoreductase; EC 1.6.5.3) is the first and largest enzyme complex of the mitochondrial respiratory chain, oxidizing NADH to liberate electrons that facilitate the translocation of protons across the inner membrane to generate a proton gradient (Hirst, 2010; Vogel et al., 2004). Complex I derangement causes an enhanced production of reactive oxygen species that, in turn, further impairs mitochondrial function in a vicious cycle, triggering the downstream mechanisms leading to neuronal death (Tretter et al., 2004). Loss of complex I activity probably contributes to the cell loss and dysfunction seen in PD as evidenced by the fact that inhibition of this enzyme causes PD in humans and animal (Betarbet et al., 2000; Nicklas et al., 1985; Zhang et al., 2002). In addition, significant and consistent deficits in subunits and activity of complex I was reported in the SN of idiopathic PD (Henchcliffe and Beal, 2008).These findings suggest that complex I may play a pathogenically important role in PD.

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Complex I is assembled from 45 polypeptides, 7 (mitochondrial subunit NADH dehydrogenase 1–4, 4L, 5, and 6) encoded by the mitochondrial DNA (mtDNA) (Wallace, 2007). Furthermore, mitochondrial subunit NADH dehydrogenase 1 (ND1), which has a molecular mass of 36 kDa, is the most conserved among the rapidly diverging mitochondrially encoded subunits of complex I (Weiss et al., 1991). ND1 is synthesized within mitochondria using mitochondrial translation machinery, involved in the first step of the electron transport chain of oxidative phosphorylation (Carroll et al., 2003). In situ hybridization studies of the ND1 have demonstrated that expression of this mRNA is the strongest in nigral regions (Ruberg et al., 1997). Moreover, reduction in mitochondrial respiratory chain activity is likely to be attributable to a decrease in transcription of mRNA encoding the ND1 subunit (Kingsbury et al., 2001).

Da-Bu-Yin-Wan (DBYW, *Great Yin Tonic Pill*), a classic formula of traditional Chinese medicine (TCM), has been described in a famous TCM canon *Danxi Xin Fa* (*Danxi's Experiential Therapy*) written by Dan-Xi Zhu, a distinguished physicians in China Yuan Dynasty (A.D.1206–1368). According to clinical experiences and therapeutic principles, Dan-Xi Zhu summarized that "Yang frequently in excess, Yin frequently in deficiency" in human body. Therefore, he used to prescribe the Yin-tonic formula in the therapy for various diseases. DBYW is famous for its efficiency in tonifying the Yin, clinically employed to treat PD (Zai and Gu, 2009). Qian-Zheng-San (QZS, *Symmetry Leading Powder*) was described in another TCM monograph *Yang Shi Jia Chang Fang* (*Yang's Family Collecting Prescriptions*) by Dan Yang in China Song Dynasty (A.D.960–1279) and was also described in *Yi Xin Fang* (*Ishinhō*) by Tamba Yasuyori in Japan A.D.982. QZS has been extensively used for asymmetrical signs improvement and neurological functional recovery (Zhang, 1991).

In our previous study, DBYW and QZS showed neuroprotective effects with a significant improvement in complex I activity in mice of PD model (He et al., 2010a, 2010b). However, the underlying mechanism for the neuroprotective effects of DBYW or QZS remains unelucidated. Therefore, in present research, we investigated the possible neuroprotective mechanisms of DBYW and QZS.

2. Methodology

2.1. Chemicals and reagents

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and pepsin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade potassium chloride, sodium dihydrogen orthophosphate, 1-octane sulfonic acid sodium salt and ethylenediaminetetraacetic acid (EDTA) were purchased from the Alfa Aesar (Ward Hill, MA, USA). High performance liquid chromatography (HPLC) - grade methanol was obtained from Thermo-Fisher (Waltham, MA, USA). HPLC reference standards: dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were purchased at HPLC-grade purity from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used in the research were of analytical grade, obtained from Beijing Chemical Factory (Beijing, China).

2.2. Preparation of decoctions

The composition of DBYW and QZS was listed in Table 1. All TCM herbs were purchased from Tong-Ren-Tang Drugstore in Beijing City, originated from different regions in China and authenticated by experts in pharmacognosy. The voucher specimens are available in our department. In preparing the decoctions, extract amounts of component herbs were weighed according to the classic percentage and mixed well. The mixture was soaked in distilled water for 30 min and then boiled in 8 volumes of water (v/w) for 1 h and extracted twice; this preparation method followed the ancient method as described previously (Zhang and Li, 2009, 2010). The extract was condensed to final concentration (for DBYW, 7.74 g/ml; for QZS, 3.51 g/ml, respectively). The concentration was expressed in total dry weight of the crude herbs per milliliter in decoction.

2.3. Animal models and treatment

Male C57BL/6 mice (Vital River, Beijing, China), 8 weeks of age, 20 ± 1 g, were habituated for 1 week to the animal colony and had ad libitum access to food and water. Animals were housed in a colony room under a 12–12 light–dark cycle (lights on at 7 am) with room temperature at 21 ± 1 °C. All procedures involving animals and their care were conducted in conformity with National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985) and were approved by Animal Care and Use Committee of Beijing University of Chinese Medicine. The minimum number of animals was used and we made every effort to reduce animal suffering.

Mice were randomly divided into five groups: saline-injected group (control group), MPTP-injected group (model group), MPTP-injected plus DBYW-treated group (DBYW group), MPTP-injected plus QZS-treated group (QZS group), and MPTP-injected plus combined decoction (CD, DBYW+QZS)-treated group (CD group). Either saline or MPTP (20 mg/kg of free base) was injected intraperitoneally during 5 consecutive days (Doo et al., 2010) before decoction treatment. For various decoction-treated groups, DBYW (7.74 g/kg), QZS (3.51 g/kg), and CD (DBYW, 3.87 g/kg; QZS, 1.76 g/kg) were administered intragastrically everyday for 51 days after 5-day MPTP injection, respectively. For the control and model groups, an equal volume of distilled water was administered intragastrically instead of decoction. Experimental design was shown in Fig. 1.

Table 1
Constituents of Da-Bu-Yin-Wan and Qian-Zheng-San.

Formula	Chinese name	Botanical name	Common name	Weight (g)	Voucher numbers
Da-Bu-Yin-Wan (DBYW)	Huang-Bai	*Phellodendron chinense* Schneid.	Amur cork tree bark	60	DBYW01-091207
	Zhi-Mu	*Anemarrhena asphodeloides* Bunge.	Common anemarrhena	60	DBYW02-091207
	Shu-Di-Huang	*Schisandra chinensis* (Turcz.) Baill.	Prepared rehmannia root	90	DBYW03-091207
	Gui-Ban	*Paeonia lactiflora* Pall.	Tortoise plastron	90	DBYW04-091207
Qian-Zheng-San (QZS)	Bai-Fu-Zi	*Rhizoma Typhonii Gigantei*	Giant typhonium tuber	60	QZS01-091207
	Jiang-Chan	*Bombyx Batryticatus*	Stiff silkworm	60	QZS02-091207
	Quan-Xie	*Scorpio*	Chinese scorpion (detoxicated)	60	QZS03-091207