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Anti-apoptotic effect of modified Chunsimyeolda-tang, a traditional Korean herbal formula, on MPTP-induced neuronal cell death in a Parkinson's disease mouse model

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ABSTRACT

Ethnopharmacological relevance: The modified-Chungsimyeolda-tang (DG) is an important traditional Korean herbal formula used in traditional oriental medicine for treatment of cerebrovascular disorders, including stroke. The formula is based on the book "Dongui Sasang Shinpyun".
Aim of the study: In the previous studies, the neuroprotective effect of DG is demonstrated in an *in vitro* Parkinson's disease (PD) model, and in this study, the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of PD is used to evaluate the behavioral effect of DG and possible mechanism through anti-apoptosis of DG. 6-Hydroxydopamine (6-OHDA) also is used to evaluate the anti-apoptosis effect of DG in SH-SY5Y cells.
Materials and methods: MPTP was used to evaluate the behavioral damage and neurotoxicity in mice. The bradykinesia symptom was measured by a Pole test and a Rota-rod test in mice. Also the loss of tyrosine hydroxylase (TH)-positive neurons induced by MPTP was examined by an immunohistochemical assay. The DG-mediated anti-apoptosis effect was measured using an immunoblotting assay with apoptosis-related markers such as Bax and cleaved caspase-3. DG and 1-methyl-4-phenylpyridinium (MPP^+) were co-treated with primary dopaminergic neurons to evaluate the protective effect of DG. The expression of caspase-3 and PARP was measured to detect the protective effect of DG from the damage by 6-OHDA.
Results and conclusions: The treatment with DG resulted in prophylactic effects on MPTP-induced Parkinsonian bradykinesia and the immunohistochemical analysis showed that DG provided the neuroprotection against the MPP^+-induced dopaminergic neurons loss through the anti-apoptosis effect. The present results suggested that it might be possible to use DG for the prevention of substantia nigra pars compacta (SNpc) degeneration induced by exposure to the toxic substances, such as MPTP/MPP^+, in PD mouse model.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disorder after Alzheimer's disease, is characterized by motor and behavioral disturbances that include a resting tremor, postural instability, and bradykinesia (Shimohama et al., 2003). The pathological hallmark of PD is degeneration of Dopaminergic neurons in the substantia nigra pars compacta (SNpc) and a subsequent loss of dopamine in the striatum (ST) (Bove et al., 2005; Shimohama et al., 2003).

It is widely agreed that oxidative stress plays a pivotal role in the neurodegeneration associated with PD (Chakraborty et al.,

Abbreviations: ABC, avidin-biotin peroxidase complex; DG, modified Chungsimyeolda-tang; FBS, fetal bovine serum; MYH, modified Youldahanso-tang; PD, Parkinson's disease; MEM, minimal essential medium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium; T-LA, time for locomotion activity; ROS, reactive oxygen species; ST, striatum; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; 6-OHDA, 6-hydroxydopamine

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2013; Subramaniam and Chesselet, 2013; Zuo and Motherwell, 2013). Oxidative stress is known to damage lipids, proteins, and DNA and these, along with decreased glutathione levels, have been observed in the postmortem ST and SNpc of PD (Schapira and Jenner, 2011). Data from human postmortem tissue also indicate that alterations in reactive oxygen species (ROS), nitric oxide, and mitochondrial complex I activity are important in the pathogenesis of sporadic PD (Banerjee et al., 2009).

As a highly lipophilic molecule, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) readily crosses the blood–brain barrier within minutes after systemic administration (Markey et al., 1984), and then converts into 1-methyl-4-phenylpyridinium (MPP^+), which is believed to elicit a specific intoxication of dopaminergic neurons (Dauer and Przedborski, 2003). MPTP can selectively damage dopaminergic neurons, which has been used extensively in various animal species. MPTP can induce behavioral, neuropathological and biochemical changes in primates and mice, which are similar to those that occur in idiopathic PD (Bergman and Deuschl, 2002; Przedborski et al., 2000). The MPTP mouse model is a common method used to study the neuroprotective effect of drugs and has provided considerable therapeutic insight into basal ganglia physiology and response to drug therapy (Smeyne and Jackson-Lewis, 2005). This model can recapitulate the primary pathological and biochemical features of Parkinson's disease, such as oxidative stress, mitochondrial dysfunction, and apoptosis (Schmidt and Ferger, 2001).

Cellular apoptotic dysfunction is one of the triggering events that lead to neuronal cell death with PD. It is now believed that apoptosis results from activated caspase proteolysis of various cellular components initiated by ROS generation (Serviddio et al., 2011). The mechanism of apoptosis is complex and involves a cascade of reactions; one of the key steps leading to apoptosis is the leakage of cytochrome C from the mitochondria and activation of caspase-3 (Wang et al., 2014). Hartmann et al. (2000) reported that using an antibody raised against activated caspase-3, the percentage of active caspase-3-positive neurons among dopaminergic neurons was significantly higher in PD patients than in-controls (Hartmann et al., 2000). In another human PD brain study, it is observed that a significantly higher percentage of dopaminergic SN neurons displayed caspase-8 activation in PD patients compared with controls (Hartmann et al., 2001). Two other studies found an increased expression of the anti-apoptotic factor Bcl-2 in the SNpc of PD patients compared to the cerebral cortex and age matched controls, probably as a compensatory mechanism (Marshall et al., 1997; Mogi et al., 1996). Therefore, suppressing the key elements related to the induction of apoptosis may propose a possible therapeutic approach in PD.

The modified Chungsimyeolda-tang (DG), a traditional Korean herbal formula, has been described in 'Dongui Sasang Shinpyun', a book that has a great effect on the clinical utilization of 'Sasang Constitutional Medicine' prescriptions. Sasang Constitutional Medicine, first introduced by Jema Lee in 1894, is an indispensable part of traditional Korean medicine (Leem and Park, 2007; Shim et al., 2008). The major ingredients of DG are *Polygala tenuifolia* (Willd.), *Angelica tenuissima* Nakai and *Dimocarpus longan* Lour mixed at 1:1:1 ratio (dry weight). The *Polygala tenuifolia* (Willd.) was used extensively in neurodegeneration diseases (Choi et al., 2011; Lv et al., 2009; More et al., 2013; Naito and Tohda, 2006). The *Angelica tenuissima* Nakai and *Dimocarpus longan* Lour were considered to have anti-oxidative, anti-cancer and anti-microbial activities (Jiang et al., 2014; Ka et al., 2005; Tseng et al., 2014) related with various diseases, including neurodegenerative disease (Barrett and Timothy Greenamyre, in press; Jazvinscak Jembrek et al., 2015; Romanucci and Della Salda, 2015). In our previous studies, we have used an *in vitro* PD model, exposure to toxic substances-MPP^+ and rotenone, to evaluate the neuro-protective effect of DG in SH-SY5Y cells (Bae et al., 2015), and also we have confirmed the protective effect of DG through autophagy induction in *in vitro*. The increasing evidence indicated that the autophagy has the emerging role in the Parkinson's disease, the pathogenesis of which appeared to be converging on oxidative stress, mitochondrial dysfunction and protein aggregation, all of which are tightly linked to autophagy (Lynch-Day et al., 2012). And the aim of the present study was to investigate the therapeutic potential of DG in experimental *in vivo* PD models. In this study, we evaluated the neuro-protective effects of DG against MPTP-induced neurotoxicity and the possible mechanisms of its effects *in vivo* were examined by performing behavioral impairment tests, brain tissue stereology, and measurements of apoptotic proteins in an experimental animal PD model. In addition, the neuro-protective effect of DG and its fractions was evaluated by exposure to 1-methyl-4-phenylpyridinium (MPP^+) to induce toxicity in primary dopaminergic neurons.

2. Materials and methods

2.1. Chemicals and reagents

Minimal essential medium (MEM), fetal bovine serum (FBS), horse serum and penicillin–streptomycin were purchased from Gibco (Carlsbad, CA, USA). Poly-L-lysine, MPTP, MPP^+, 3,3-diaminobenzidine (DAB), and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Hyclone (Logan, UT, USA). Rabbit anti-tyrosine hydroxylase (TH) antibody was purchased from Chemicon International Inc. (Temecula, CA, USA). Rabbit anti-cleaved caspase-3 antibody was obtained from Chemicon International Inc. (Temecula, CA, USA). Rabbit anti-Bax and mouse anti-β-actin antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Rabbit anti-caspase-3, anti-PARP and anti-GAPDH antibodies were purchased from Cell Signaling Technology Inc. (Boston, MA, USA). Normal goat serum and an avidin-biotin peroxidase complex (ABC) standard kit were purchased from Vector Laboratories Inc., (Burlingame, CA, USA). Anti-rabbit and anti-mouse horseradish peroxidase secondary antibodies were purchased from Assay Designs Inc. (Ann Arbor, MI, USA). All of the other chemicals were of analytical grade.

2.2. Preparation of DG

DG consisted of three individual herbs: *Polygala tenuifolia* (Willd.), *Angelica tenuissima* Nakai and *Dimocarpus longan* Lour. DG was prepared according to our previous study (Bae et al., 2015). The herbs were purchased by the Oriental Hospital at Daejeon University (Daejeon, Republic of Korea). The botany and drug department of Oriental Hospital assumed the identification and authentication of the herbs. The voucher specimens of the herbs (voucher number: *Polygala tenuifolia* (Willd.), KN00847A; *Angelica tenuissima* Nakai, KN00735A and *Dimocarpus longan* Lour, KN00804A) were deposited at the herbarium, Korea Institute of Science & Technology (Gangneung, Republic of Korea). Briefly, *Polygala tenuifolia* (Willd.), *Angelica tenuissima* Nakai and *Dimocarpus longan* Lour were air-dried, weighed 15 g (dry weight) of each herbs and mixed together, total 45 g of crude drugs. Distilled water was added at final 1 L, and boiled for twice. A final volume of 100 mL solution was left after evaporated, labeled as the DG extract, then, centrifuged the extract and collected the supernatant. The supernatant was filtered, freeze dried and obtained 2.925 g lyophilized extract (the yield was 6.5%, which was from 2.925 g lyophilized extract/45 g crude drug). And then the