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Effects of Gypenosides on Dopaminergic Neuronal Cell Death in 6-Hydroxydopamine-lesioned Rat Model of Parkinson's Disease with Long-term L-DOPA Treatment

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Abstract – The goal of this study was to determine whether gypenosides (GPS) exert protective effects against dopaminergic neuronal cell death in a 6-hydroxydopamine (OHDA)-lesioned rat model of Parkinson's disease (PD) with or without long-term 3,4-dihydroxyphenylalanine (L-DOPA) treatment. Rats were injected with 6-OHDA in the substantia nigra to induce PD-like symptoms; 14 days after injection, groups of 6-OHDA-lesioned animals were treated for 21 days with GPS (25 or 50 mg/kg) and/or L-DOPA (20 mg/kg). Dopaminergic neuronal cell death was assessed by counting tyrosine hydroxylase (TH)-immunopositive cells in the substantia nigra and measuring levels of dopamine, norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the striatum. Dopaminergic neuronal cell death induced by 6-OHDA lesions was ameliorated by GPS treatment (50 mg/kg). L-DOPA treatment exacerbated 6-OHDA-induced dopaminergic neuronal cell death; however, these effects were partially reversed by GPS treatment (25 and 50 mg/kg). These results suggest that GPS treatment is protective against dopaminergic neuronal cell death in a 6-OHDA-lesioned rat model of PD with long-term L-DOPA treatment. Therefore, GPS may be useful as a phytotherapeutic agent for the treatment of PD.
Keywords – Gypenosides, 6-Hydroxydopamine-lesioned rat, Parkinson's disease, Tyrosine hydroxylase immunohistochemistry, L-DOPA

Introduction

Parkinson's disease (PD) involves the degeneration of dopaminergic neurons in the substantia nigra pars compacta,[1] which leads to motor impairments including slowness of movement, rigidity, resting tremor, and postural instability.[2] L-3,4-Dihydroxyphenylalanine (L-DOPA), the natural precursor to dopamine, is currently the most effective therapy for PD.[3] However, chronic L-DOPA administration leads to the development of severe side effects, such as motor fluctuations and dyskinesia.[4] L-DOPA can also induce oxidative stress-related neurotoxicity by stimulating reactive oxygen species (ROS) production in both dopaminergic neurons and rat adrenal pheochromocytoma (PC12) cells.[5,6] In addition, low-dose L-DOPA treatment (10 mg/kg) elicits neuroprotective effects, whereas doses greater than 20 mg/kg are neurotoxic.[7]

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Neurotoxic and genetic animal models of PD, mainly rats and mice, are commonly used to examine the pathogenesis of PD and the efficacy of therapeutic agents. Neurotoxic models using 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat are models of end-stage PD.[8,9] However, PD is a multi-symptomatic disease, and no existing neurotoxic or genetic animal model displays all PD symptoms.[8-10] Thus, the use of combined genetic and neurotoxic models or double neurotoxic models has been suggested.[10]

Gynostemma pentaphyllum Makino (GP) is a commonly used medicinal plant in Southeast Asia that contains approximately 90 dammarane-type glycoside derivatives (gypenosides; GPS), flavonoids, polysaccharides, amino acids, and vitamins.[11] Numerous pharmacological properties of GP extract (GP-EX) and GPS have been reported.[11] GP-EX has shown anti-stress and anxiolytic effects in mice.[12,13] GP-EX treatment protects dopaminergic neurons in the 6-OHDA-lesioned rat model of PD.[14] GPS are protective against oxidative neurotoxicity in primary rat cortical cell cultures and prevent 1-methyl-4-phenylpyri-

dinium (MPP^+)-induced oxidative injury in primary dopaminergic cell cultures.[15,16] GPS have been shown to attenuate damage induced by chronic cerebral hypoperfusion in rats.[17] In addition, GPS show protective effects in the MPTP-lesioned mouse PD model.[18]

In this study, therefore, the effects of GPS on dopaminergic neuronal cell death in 6-OHDA-lesioned rat model of PD with or without long-term L-DOPA treatment were investigated in order to confirm the functions of GPS in addition to MPTP-induced animal model of PD.

Experimental

Materials – GPS were purchased from Ankang Dongke Maidisen Nature Pharmaceutical Co. (purity > 99%, confirmed by HPLC analysis) (Xi'an, China).[15,16] L-DOPA, 6-OHDA, norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), benserazide hydrochloride, apomorphine, Na_4EDTA, and L-ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). A tyrosine hydroxylase (TH) antibody was obtained from Millipore (AB152; Temecula, CA, USA). Anti-mouse IgG and Vectastain diaminobenzidine and avidin/biotin complex kits were purchased from Vector Laboratories, Inc. (Burlingame, CA, USA). All other chemicals were of analytical grade.

Animals – Male Sprague-Dawley rats (200 – 250 g) were purchased from Samtako Co. (Animal Breeding Center, Osan, Korea). Animals were housed at 23 ± 2 ºC with 60 ± 5% humidity under a 12-h light-dark cycle with *ad libitum* access to water and standard diet. All procedures were approved by the Animal Ethics Committee of Chungbuk National University Laboratory Animal Research Center (Approval No., CBNUA-528-13-02).

Unilateral 6-OHDA lesions – Unilateral 6-OHDA lesions were induced as described previously.[7,19] Rats were anesthetized with Zoletil 50 (100 mg/kg i.p.; Virbac, Carros, France) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The coordinates for the striatum were determined relative to the bregma (anteroposterior: -5.3 mm; lateral: +1.9 mm; dorsoventral: -7.5 mm),[19] and 6-OHDA (8 μg in 2 μL of saline solution containing 0.1% L-ascorbic acid) was injected into the left substantia nigra at a rate of 1 μL/min using a Hamilton syringe. Rats in the control group received 2 μL of saline containing 0.1% L-ascorbic acid. After the injection, the needle was left in place for 5 min before being retracted in order to allow complete diffusion of the solution. Fourteen days after surgery, rats were challenged with apomorphine (0.5 mg/kg s.c.), and contralateral rotation was monitored.[20,21]

Experimental design – Rats were divided into 7 groups (n = 8 – 10 per group), including the saline-injected control group, 14 days after surgery. Three groups of 6-OHDA-lesioned rats were treated with both L-DOPA (20 mg/kg, i.p.) and benserazide (15 mg/kg, i.p.) at 10:00 a.m. once per day for 21 days. Two groups each of 6-OHDA-lesioned rats with or without L-DOPA treatment were treated with GPS (25 or 50 mg/kg, orally) 2 h after each L-DOPA treatment. Following the final GPS and L-DOPA treatments, rats were sacrificed for immunohistochemical and biochemical analyses.

TH immunohistochemistry – Rats were intracardially perfused with saline and then with 4% paraformaldehyde. The brains were removed and placed in 30% sucrose solution. Sections were cut at 35 mm thick using a vibratome (Leica Microsystems GmbH, Wetzlar, Germany). Every 6th serial section was used for TH immunohistochemistry. The tissue sections were incubated overnight at 4 ºC with rabbit anti-TH antibody diluted at 1:200 in PBS containing 0.3% Triton X-100. The sections were then incubated with a 1:250 dilution of biotinylated anti-rabbit IgG (1:250), followed by avidin/biotin complex kit reagents. TH immunoreactivity was visualized using diaminobenzidine. Photomicrographs of TH and digitized bright-field images were captured at 100× magnification using a Zeiss Axiophot microscope (Carl Zeiss Micro-Imaging GmbH, Jena, Germany). TH-positive cells in the substantia nigra were counted using Axiovision software (Carl Zeiss MicroImaging GmbH). Cell counts obtained on the ipsilateral side (6-OHDA-lesioned side, L) to the 6-OHDA lesion were expressed as a percentage of those on the intact contralateral side (intact side, R).

Measurement of striatal dopamine, norepinephrine, DOPAC, and HVA content – The brains were rapidly removed, and the striatum was dissected in cold conditions and homogenized in 300 μL $HClO_4$. The homogenates were immediately centrifuged at 50,000 × *g* at 4 ºC for 20 min, and the supernatants were filtered through 0.45 μm Millex-GV filters (Waters, Milford, MA, USA). Levels of dopamine, norepinephrine, DOPAC, and HVA were measured using an HPLC system,[22] consisting of a solvent delivery pump (model 1525), an electrochemical detector (+0.85 V, Ag/AgCl reference electrode; model 2465), and a 120 ODS-BP column (5 μm, 50 × 4.6 mm; all from Waters). The mobile phase consisted of 10 mM citric acid, 0.13 mM Na_4EDTA, 0.58 mM SOS, and 10% methanol and had a flow rate of 1 mL/min. Results are expressed as ng/mg tissue.

Statistical analysis – All data were analyzed by one-

way ANOVA followed by Tukey's test. Results are expressed as mean ± S.E.M., with P values < 0.05 considered statistically significant.

Result and Discussion

Rats with 6-OHDA lesions showed symptoms of PD, which were confirmed by an excess of 150 – 300 contralateral rotations in a 60 min period after treatment with apomorphine (0.5 mg/kg).[20,23]

TH-immunopositive cells in the substantia nigra of 6-OHDA-lesioned rats were markedly reduced on the side ipsilateral to the lesion (left side; Fig. 1A). The number of TH-immunopositive cells on the 6-OHDA-lesioned side was expressed as a percentage of those in the intact contralateral side. Rats with 6-OHDA lesions showed a marked decrease in the number of TH-immunopositive cells in the 6-OHDA-lesioned side to 59.8% ($P < 0.05$) compared with the control group (Fig. 1B). TH-immunopositive cells on the 6-OHDA-lesioned side were small, with thinner processes and uneven color. However, 6-OHDA-lesioned rats treated with GPS (50 mg/kg) showed reduced losses of TH-immunopositive cells (Fig. 1A). The percentages of surviving TH-immunopositive cells in 6-OHDA-lesioned rats treated with 25 and 50 mg/kg GPS were 63.6% and 69.6% ($P < 0.05$), respectively, compared with that of the control group (Fig. 1B).

Reductions in TH-immunopositive cells in 6-OHDA-lesioned rats were exacerbated by L-DOPA treatment (Fig. 1A); 6-OHDA-lesioned rats treated with L-DOPA showed decreases in the numbers of TH-immunopositive cells to 48.4% ($P < 0.05$) compared with the 6-OHDA-lesioned group (Fig. 1B). However, treatment with GPS (25 and 50 mg/kg) partially alleviated these losses (Fig. 1A); 6-OHDA-lesioned rats treated with L-DOPA and 25 and 50 mg/kg GPS showed increases in the number of TH-immunopositive cells to 60.9% and 66.6%, respectively (both $P < 0.05$), compared with 6-OHDA-lesioned rats treated with L-DOPA alone (Fig. 1B).

Rats with 6-OHDA lesions showed significant decreases compared to controls in striatal levels of dopamine (43.2%, $P < 0.05$), norepinephrine (60.7%, $P < 0.05$), DOPAC (58.3%, $P < 0.05$), and HVA (56.1%, $P < 0.05$) on the 6-OHDA-lesioned side (Figs. 2 – 5). GPS treatment slightly reversed these decreases. In 6-OHDA-lesioned rats treated with GPS (25 and 50 mg/kg) for 21 days, levels of dopamine, norepinephrine, DOPAC and HVA increased to 48.1% and 51.9% ($P < 0.05$), 67.3% ($P < 0.05$) and 66.4% ($P < 0.05$), 65.7% and 72.2% ($P < 0.05$), and 59.5% and 62.4% ($P < 0.05$), respectively, compared with 6-OHDA-lesioned group (Figs. 2 – 5).

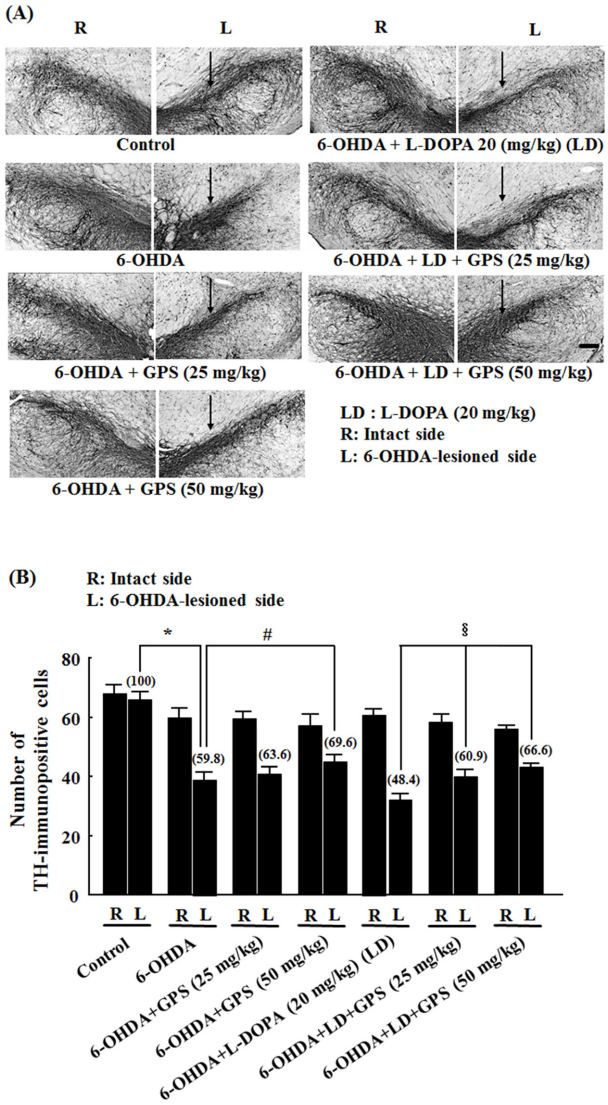

Fig. 1. Representative photomicrographs of tyrosine hydroxylase (TH) immunoreactivity (A) and numbers of surviving TH-immunopositive cells (B) in the substantia nigra. Fourteen days after lesions were induced by 6-hydroxydopamine (6-OHDA) injection into the substantia nigra, rats were treated for 21 days with gypenosides (GPS) and/or a combination of L-DOPA (LD) and benserazide. (A) Arrows indicate 6-OHDA-lesioned areas. Scale bar = 100 μm. (B) Numbers of TH-immunopositive cells on the lesioned side (L) were analyzed as a percentage of the intact side (R). Results are presented as mean ± S.E.M.; n = 8 – 10 animals per group. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-lesioned group; $^{\S}P < 0.05$ compared with 6-OHDA-lesioned group treated with L-DOPA alone.

Treatment of 6-OHDA-lesioned rats with L-DOPA further decreased the levels of dopamine, norepinephrine, DOPAC, and HVA to 49.3%, 52.9%, 49.8%, and 50.5% of levels in the control group, respectively (Figs. 2 – 5). GPS treatment (25 and 50 mg/kg) partially reversed these

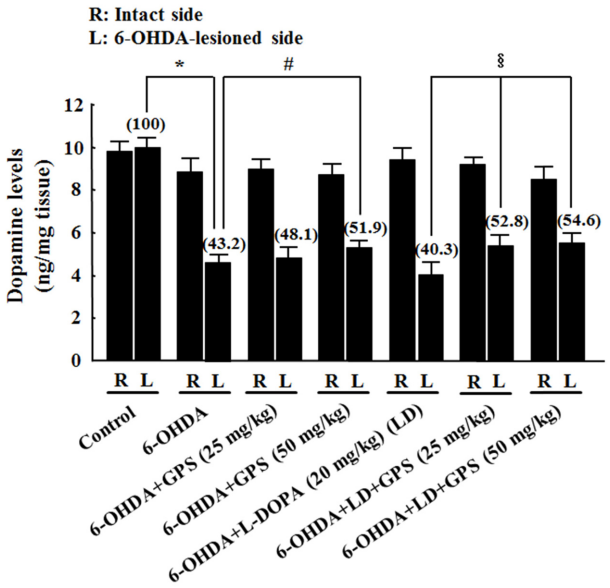

Fig. 2. Effects of GPS on striatal dopamine levels on the intact (R) and 6-OHDA-lesioned (L) sides. Following 6-OHDA lesion and L-DOPA and/or GPS treatment, levels of dopamine in the striatum were determined using HPLC. Numbers in parentheses represent dopamine levels expressed as percentages of the control group (L). Results are presented as mean ± S.E.M.; n = 8 – 10 animals per group. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-lesioned group; $^{\S}P < 0.05$ compared with 6-OHDA-lesioned group treated with L-DOPA alone.

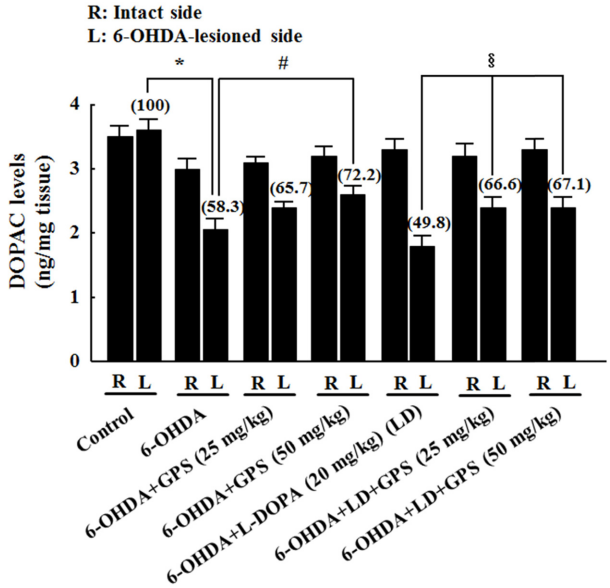

Fig. 4. Effects of GPS on striatal 3,4-dihydroxyphenylacetic acid (DOPAC) levels on the intact (R) and 6-OHDA-lesioned (L) sides. Numbers in parentheses represent DOPAC levels expressed as percentages of the control group (L). Results are presented as mean ± S.E.M.; n = 8 – 10 animals per group. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-lesioned group; $^{\S}P < 0.05$ compared with 6-OHDA-lesioned group treated with L-DOPA alone.

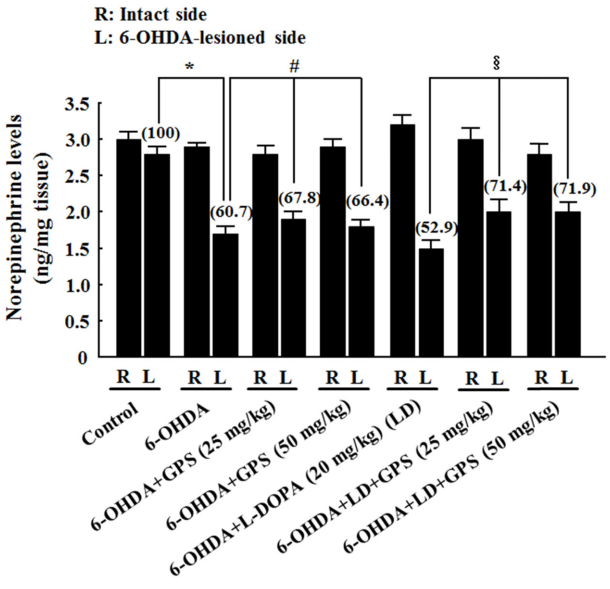

Fig. 3. Effects of GPS on striatal norepinephrine levels on the intact (R) and 6-OHDA-lesioned (L) sides. Numbers in parentheses represent norepinephrine levels expressed as percentages of the control group (L). Results are presented as mean ± S.E.M.; n = 8 – 10 animals per group. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-lesioned group; $^{\S}P < 0.05$ compared with 6-OHDA-lesioned group treated with L-DOPA alone.

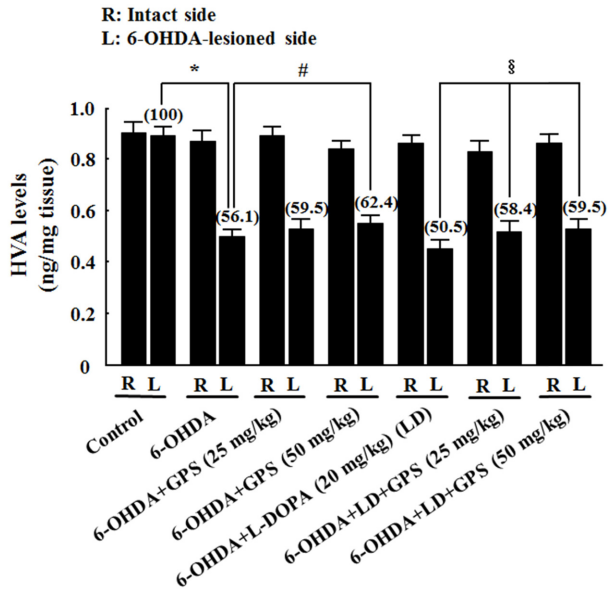

Fig. 5. Effects of GPS on striatal homovanillic acid (HVA) levels on the intact (R) and 6-OHDA-lesioned (L) sides. Numbers in parentheses represent HVA levels expressed as percentages of the control group (L). Results are presented as mean ± S.E.M.; n = 8 – 10 animals per group. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-lesioned group; $^{\S}P < 0.05$ compared with 6-OHDA-lesioned group treated with L-DOPA alone.

effects. In 6-OHDA-lesioned rats treated with L-DOPA and GPS (25 and 50 mg/kg), levels of dopamine, norepinephrine, DOPAC, and HVA increased to 52.8% and 54.6%, 71.4% and 71.9%, 66.6% and 67.1%, and 58.4% and 59.5%, respectively (all $P < 0.05$ compared with 6-OHDA-lesioned rats treated with L-DOPA alone) (Figs. 2 – 5).

Rats and mice are widely used as neurotoxic models of PD with lesions induced by 6-OHDA or MPTP.[8,24] Neurotoxic models show symptoms similar to those of moderate- or end-stage PD. Genetic models are created through gene overexpression or knockout methodology and occasionally through genetic knock-in or conditional expression methodology.[10] However, as neither neurotoxic nor genetic animal models display all of the symptoms of PD, recent studies have combined genetic models with neurotoxic models or used double neurotoxic models.[10]

GP-EX protects dopaminergic neuronal cell death in 6-OHDA-lesioned rat model of PD.[14] GPS show protective effects on dopaminergic neuronal cell death in MPTP-lesioned mouse model of PD[18] and on L-DOPA-induced dyskinesia in 6-OHDA-lesioned rat model of PD.[7] GPS have also been shown to alleviate symptoms of affective disorders in MPTP-lesioned mice.[25] In this study, the neuroprotective effects of GPS against dopaminergic neuronal cell death in the 6-OHDA-lesioned rat model of PD with or without long-term L-DOPA treatment were investigated in order to confirm the protective effects of GPS in multiple disease models.

L-DOPA shows dose-dependent dual functions in 6-OHDA-lesioned rat model of PD: treatment with L-DOPA (10 mg/kg) shows neuroprotective effects, however, L-DOPA at higher than 20 mg/kg exhibits neurotoxicity.[7,26] In this study, GPS treatment partially alleviated the loss of TH-immunopositive cells in the substantia nigra in both L-DOPA-treated (20 mg/kg) and -untreated 6-OHDA-lesioned rats. GPS treatment also significantly increased levels of dopamine, norepinephrine, DOPAC, and HVA in L-DOPA-treated and -untreated 6-OHDA-lesioned rats. These results indicate that GPS exhibit protective effects against dopaminergic neuronal cell death in the 6-OHDA-lesioned rat model of PD with long-term L-DOPA treatment.

Neurotoxic effects of 6-OHDA occur through oxidative stress, which is triggered by the formation of ROS after 6-OHDA enters the neuron via dopamine transporters.[27,28] Treatment with 6-OHDA also leads to reductions in glutathione content and superoxide dismutase and catalase activities in the striatum.[29] Stereotaxic injection of 6-OHDA into the substantia nigra, medial forebrain bundle and striatum of the brain in rat and mouse induces TH-containing dopaminergic neuronal cell death by ROS production, which leads to decrease in the level of dopamine and its metabolites in the TH-immunopositive terminals of the striatum.[30-32] The human and rat brains contain endogenous 6-OHDA,[33] which is produced by the high levels of dopamine, hydrogen peroxide, and free iron in dopaminergic neurons.[34] L-DOPA treatment alone and in combination with Fe^{2+} enhances 6-OHDA production in the rat brain.[35] In addition, L-DOPA and dopamine can directly cause neurotoxicity in PC12 cells by inducing oxidative stress.[5,36] Long-term L-DOPA treatment alleviates oxidative stress-induced neurotoxicity in both striatal dopaminergic neurons and PC12 cells.[6,36,37] Furthermore, daily L-DOPA administration increases nitric oxide production in the striatum through activation of neural nitric oxide synthase, which is associated with PD and L-DOPA-induced dyskinesia.[38,39] In MPTP-lesioned PD models, MPTP is metabolized to MPP^+ (Heikkila et al., 1984), which is taken up by dopaminergic neurons and blocks complex I of the mitochondrial electron chain, inducing oxidative stress, energy failure, and inflammation.[40] Thus, anti-oxidative agents may be key to the prevention and control of PD symptoms.[35,41]

GPS are potent free radical scavengers that significantly increase superoxide dismutase activity.[42] GPS show protective effects against oxidative damage in aortic endothelial cells[43] and primary rat cortical cell cultures[15] and prevent MPP^+-induced oxidative injury in cultured dopaminergic neurons.[16] In addition, GP-EX has demonstrated anti-stress and immunomodulatory effects in mice through modulation of c-Fos expression.[12,13] These studies suggest that the protective effects of GPS and GP-EX against dopaminergic neuronal cell death observed in this study, as well as in previous studies using 6-OHDA-and MPTP-lesioned PD models, may be due to the anti-oxidative properties of these compounds.

GPS dosages between 50 and 200 mg/kg and GP-EX dosages between 50 and 400 mg/kg are not associated with adverse effects such as weight loss, diarrhea, vomiting, and death.[13] Reported LD_{50} values of GPS are 755 – 838 mg/kg when injected into the abdominal cavity and 402 mg/kg i.p. in mice,[11] indicating that GPS are a low-toxicity compound.

Agents that enhance the bioavailability of dopamine or prevent dopaminergic neuronal cell death may provide protection against PD in humans and in animal models.[41] The protective effects of GPS against the pathophysiological effects of 6-OHDA lesions observed in this study may occur via mechanisms similar to those that mediate the palliative effects of GPS on affective behaviors and L-

DOPA-induced dyskinesia in PD models.[7,25] Therefore, GPS may be helpful in preventing the neurotoxic effects of L-DOPA in patients with PD and in slowing the progression of PD symptoms.

In conclusion, GPS showed protective effects against dopaminergic neuronal cell death in the 6-OHDA rat model of PD, both with and without L-DOPA treatment. These results are similar to previously observed protective effects of GPS in the MPTP mouse model of PD. Clinical applications of GPS and GP-EX will require further study.

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References

(1) Fearnley, J. M.; Lees, A. J. *Brain* **1991**, *114*, 2283-2301.
(2) Fahn, S. *Ann. N. Y. Acad. Sci.* **2003**, *991*, 1-14.
(3) Marsden, C. D. *J. Neurol. Neurosurg. Psychiatry* **1994**, *57*, 672-681.
(4) Jankovic, J. *Mov. Disord.* **2005**, *20*, S11-S16.
(5) Cheng, N.; Maeda, T.; Kume, T.; Kaneko, S.; Kochiyama, H.; Akaike, A.; Goshima, Y.; Misu, Y. *Brain Res*. **1996**, *16*, 278-283.
(6) Walkinshaw, G.; Waters, C. M. *J. Clin. Invest*. **1995**, *95*, 2458-2464.
(7) Shin, K. S.; Zhao, T. T.; Park, K. H.; Park, H. J.; Hwang, B. Y.; Lee, C. K.; Lee, M. K. *BMC Neurosci*. **2015**, *16*, 23.
(8) Bové, J.; Perier, C. *Neuroscience* **2012**, *211*, 51-76.
(9) Jackson-Lewis, V.; Blesa, J.; Przedborski, S. *Parkinsonism Relat. Disord.* **2012**, *18*, S183-S185.
(10) Seidl, S. E.; Potashkin, J. A. *Front. Neurol.* **2011**, *2*, 68.
(11) Razmovski-Naumovski, V.; Huang, T. H. W.; Tran, V. H.; Li, G. Q.; Duke, C. C.; Roufogalis, B. D. *Phytochem. Rev.* **2005**, *4*, 197-219.
(12) Im, S. A.; Choi, H. S.; Hwang, B. Y.; Lee, M. K.; Lee C.K. *Kor. J. Pharmacogn.* **2009,** *40*, 35-40.
(13) Choi, H. S.; Zhao, T. T.; Shin, K. S.; Kim, S. H.; Hwang, B. Y.; Lee, C. K.; Lee, M. K. *Molecules* **2013**, *18*, 4342-4356.
(14) Choi, H. S.; Park, M. S.; Kim, S. H.; Hwang, B. Y.; Lee, C. K.; Lee, M. K. *Molecules* **2010**, *15*, 2814-2824.
(15) Shang, L. S.; Liu, J. C.; Zhu, Q. J.; Zhao, L.; Feng, Y.; Wang, X.; Cao, W.; Xin, H. *Brain Res.* **2006**, *1102*, 163-174.
(16) Wang, P.; Niu, L.; Guo, X. D.; Gao, L.; Li, W. X.; Jia, D.; Wang, X. L; Ma, L. T.; Gao, G. D. *Brain Res. Bull.* **2010**, *83*, 266-271.
(17) Zhang, G.; Zhao, Z.; Gao, L.; Deng, J.; Wang, B.; Xu, D.; Liu, B.; Qu, Y.; Yu, J.; Li, J.; Gao, G. *Pharmacol. Biochem. Behav.* **2011**, *99*, 42-51.
(18) Wang, P.; Niu, L.; Gao, L.; Li, W. X.; Jia, D.; Wang, X. L.; Gao G. D. *J. Int. Med. Res.* **2010**, *38*, 1084-1092.
(19) Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates 2nd ed: Academic Press; Australia, **1986**.
(20) Schwarting, R. K. W.; Huston, J. P. *Prog. Neurobiol.* **1996**, *50*, 275-331.
(21) Mo, J.; Zhang, H.; Yu, L. P.; Sun, P. H.; Jin, G. Z.; Zhen, X. *Neurobiol. Aging* **2010**, *31*, 926-936
(22) Izurieta-Sánchez, P.; Sarre, S.; Ebinger, G.; Michotte, Y. *Eur. J. Pharmacol.* **1998**, *353*, 33-42.
(23) Ljungberg, T.; Ungerstedt, U. *Eur. J. Pharmacol.* **1977**, *46*, 147-151.
(24) Blesa, J.; Phani, S.; Jackson-Lewis, V.; Przedborski, S. *J. BioMed. Biotechnol.* **2012**, *2012*, 845618.
(25) Shin, K. S.; Zhao, T. T.; Choi, H. S.; Hwang, B. Y.; Lee, C. K.; Lee, M. K. *Brain Res.* **2014**, *1567*, 57-65.
(26) Cenci, M. A. *Parkinsonism Relat. Disord.* **2007**, *13*, S263- S267.
(27) Cohen, G. *Neurotoxicology* **1984**, *5*, 77-82.
(28) Soto-Otero, R.; Méndez-Álvarez, E.; Hermida-Ameijeiras, Á.; Muñoz-Patiño, A. M.; Labandeira-Garcia, J. L. *J. Neurochem.* **2000**, *74*, 1605-1612.
(29) Cadet, J. L.; Brannock, C. *Neurochem. Int*. **1998**, *32*, 117-131.
(30) Fahn, S.; Cohen, G. *Ann. Neurol.* **1992**, *32*, 804-812.
(31) Schober, A. *Cell Tissue Res.* **2004**, *318*, 215-224.
(32) Blandini, F.; Armentero, M. T.; Martignoni, E. *Parkinsonism Relat. Disord.* **2008**, *14*, S124-S129.
(33) Severson, J. A.; Marcusson, J.; Winblad, B.; Finch, C. E. *J. Neurochem.* **1982**, *39*, 1623-1631.
(34) Linert, W.; Herlinger, E.; Jameson, R. F.; Kienzl, E.; Jellinger, K.; Youdim, M. B. *Biochim. Biophys. Acta* **1996**, *1316*, 160-168.
(35) Maharaj, H.; Sukhdev Maharaj, D.; Scheepers, M.; Mokokong, R.; Daya, S. *Brain Res.* **2005**, *1063*, 180-186.
(36) Basma, A. N.; Morris, E. J.; Nicklas, W. J.; Geller, H. M. *J. Neurochem.* **1995**, *64*, 825-832.
(37) Migheli, R.; Godani, C.; Sciola, L.; Delogu, M. R.; Serra, P. A.; Zangani, D.; De Natale, G.; Miele, E.; Desole, M. S. *J. Neurochem.* **1999**, *73*, 1155-1163.
(38) Zhang, L.; Dawson, V. L.; Dawson, T. M. *Pharmacol. Ther.* **2006**, *109*, 33-41.
(39) Padovan-Neto, F. E.; Echeverry, M. B.; Tumas, V.; Del-Bel, E. A. *Neuroscience* **2009**, *159*, 927-935.
(40) Nicklas, W. J.; Vyas, I.; Heikkila, R. E. *Life Sci.* **1985**, *36*, 2503-2508.
(41) Yacoubian, T. A.; Standaert, D. G. *Biochim. Biophys. Acta* **2009**, *1792*, 676-687.
(42) Cai, T. S.; Zhang, S. F.; Wang M. C. *Chin. J. Clin. Rehabil.* **2005**, *9*, 106-107.
(43) Tanner, M. A.; Bu, X.; Steimle, J. A.; Myers, P. R. *Nitric Oxide* **1999**, *3*, 359-365.

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