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Diaminodiphenyl sulfone–induced parkin ameliorates age-dependent dopaminergic neuronal loss

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ABSTRACT

During normal aging, the number of dopaminergic (DA) neurons in the substantia nigra progressively diminishes, although massive DA neuronal loss is a hallmark sign of Parkinson's disease. Unfortunately, there is little known about the molecular events involved in age-related DA neuronal loss. In this study, we found that (1) the level of parkin was decreased in the cerebellum, brain stem, substantia nigra, and striatum of aged mice, (2) diaminodiphenyl sulfone (DDS) restored the level of parkin, (3) DDS prevented age-dependent DA neuronal loss, and (4) DDS protected SH-SY5Y cells from 1-methyl-4-phenylpyridinium and hydrogen peroxide. Furthermore, pretreatment and/or post-treatment of DDS in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–induced Parkinson's disease model attenuated DA neuronal loss and restored motor behavior. DDS transcriptionally activated parkin via protein kinase RNA-like endoplasmic reticulum kinase–activating transcription factor 4 signaling and DDS not only failed to induce parkin expression but also failed to rescue SH-SY5Y cells from 1-methyl-4-phenylpyridinium in the absence of ATF4. Herein, we demonstrated for the first time that DDS increased parkin level and served as a neuroprotective agent for age-dependent DA neuronal loss. Thus, DDS may be a potential therapeutic agent for age-related neurodegeneration.

1. Introduction

Aging beyond young adulthood is associated with progressive decline of cognition and motor control as well as an increased susceptibility to neurodegenerative disorders. Although many studies explain the onset and progression of aging, it is generally considered that aging is a complex phenomenon that may be accelerated by diverse causes. For example, aging is the greatest risk factor for the development of Parkinson's disease (PD), which is characterized by massive loss of dopaminergic (DA) neurons in the substantia nigra (SN). Healthy human populations also show a progressive loss of DA neurons during normal aging (7%–10% reduction per decade; Cabello et al., 2002; Gibb and Lees, 1991; McGeer et al., 1988; Rodriguez et al., 2015), contributing to age-related abnormalities in terms of cognition, reward learning, and voluntary movement. Recent electrophysiological evidence has shown that DA neurons of SN from aged mice (25–30 months of age) exhibit slower firing rates, narrower spike widths, and more variable interspike intervals compared to DA neurons from young mice (2–7 months of age). The DA neurons from aged mice also harbor smaller L-type calcium channel currents, explaining the decrease in voluntary movement and other dopamine-mediated behaviors observed in aged populations (Branch et al., 2014). However, how aging accelerates DA neuronal dysfunction and the molecular mechanisms involved in age-related DA neuronal loss are not fully understood.

Diaminodiphenyl sulfone (DDS) has been commonly used as both an antibiotic and an anti-inflammatory agent for the treatment of leprosy as well as for the prevention of various skin diseases. DDS

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is able to protect from kainic acid–induced neurotoxic damage and to improve recovery after spinal cord injury (Diaz-Ruiz et al., 2011, 2013). Furthermore, DDS has been demonstrated to act as an antioxidant and/or antiapoptotic agent to prevent secondary mechanisms of ischemic injury (Diaz-Ruiz et al., 2008; Ríos et al., 2004). Our previous study revealed that DDS exerts a protective effect against paraquat-induced lung injury (Cho et al., 2011). Interestingly, another antileprosy drug, rifampicin, lowers the probability of senile dementia and prevents 1-methyl-4-phenylpyridinium (MPP^+)-induced apoptosis through inhibition of α-synuclein fibrillation (Li et al., 2004). Moreover, Japanese leprosy patients administered with DDS have a significantly lower prevalence of senile dementia (McGeer et al., 1992), suggesting that DDS may be of therapeutic value in treating neuronal cell damages.

Because little evidence has been presented regarding the molecular events that take place during DA neuronal loss in the aging process, we monitored levels of parkin and DJ-1, which are key molecules for DA neuronal survival in PD pathogenesis. Parkin expression declined in the SN of aged mice, and the intervention of DDS in aged mice restored the level of parkin via mild endoplasmic reticulum (ER) stress-mediated protein kinase RNA-like ER kinase (PERK)-activating transcription factor 4 (ATF4) signaling pathway. In addition, DDS intervention significantly rescued DA neuronal toxicity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication rodent model. Our findings suggest that the induction of parkin by DDS may be a potential therapeutic strategy for age-related neurodegenerative diseases such as PD.

2. Materials and methods

2.1. Reagents

The following reagents were used: 4-aminophenyl sulfone (Sigma-Aldrich, St. Louis, USA, A74807), thapsigargin (Sigma-Aldrich, T9033), 4-phenylbutyric acid (Sigma-Aldrich, P21005), MPP^+ iodide (Sigma-Aldrich, D048), and hydrogen peroxide (H_2O_2) solution (Sigma-Aldrich, H1009).

2.2. Cell culture and transfections

SH-SY5Y neuroblastoma cells were maintained under 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, USA) containing 10% fetal bovine serum (Gibco) and 1% streptomycin and penicillin (Gibco). SH-SY5Y cells were transfected with Lipofectamine Plus (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. For RNA interference, SH-SY5Y cells were transfected using Lipofectamine RNAiMAX (Invitrogen). Human ATF4/CREB-2 small interfering (siRNA; sc-35112) and human Parkin siRNA (sc-42158) were purchased from Santa Cruz biotechnology (Dallas, USA).

2.3. Immunoblot analysis

Protein samples (up to 40 μg) were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5%–15%) (Mini Protean II, Bio Rad, Hercules, CA, USA) using the Laemmli sample buffer system (Santa Cruz Biotechnology), and proteins were transferred to nitrocellulose membranes (Hybond ECL, Amersham, Amersham, UK). Membranes were blocked with 5% (w/v) nonfat dried milk in Tris-buffered saline, 0.1% Tween 20 for 3 hours at room temperature and probed with primary antibodies overnight at 4 °C followed by the appropriate horseradish peroxidase–conjugated secondary antibody for 1 hour (Santa Cruz Biotechnology). The following antibodies were used: anti-parkin mAb (Cell signaling, Danvers, USA, #4211), anti-DJ-1 pAb (Abcam, Cambridge, USA, ab18257), anti-ATF4/CREB2 pAb (Santa Cruz Biotechnology, sc-200), anti-pPERK mAb (Cell Signaling, #3179), anti-pPERK pAb (Santa Cruz, sc-32577), anti-PERK mAb, anti-IRE1α mAb, anti-Bip mAb (Cell Signaling, #9956).

2.4. Cell viability analysis

SH-SY5Y cells were plated in a 12-well plate at a seeding density of 0.1×10^6 cells/well. Before toxin treatment, cells were preincubated with DDS at the indicated concentration for 24 hours. Subsequently, MPP^+ (150 μM) or H_2O_2 (100 μM) was coincubated with DDS for 48 hours. For RNA interference, siRNA-ATF4 or siRNA-Parkin was transfected into SH-SY5Y before DDS treatment. The cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum for 3 days and were trypsinized for single-cell suspensions. Cells were washed twice with PBS and resuspended with phosphate-buffered saline (PBS). Resuspended cells were mixed with an equal volume of 0.4% trypan blue and incubated for 2 minutes at room temperature. Live and dead cells were counted (Eve, nanoENTEK, Seoul, Korea).

2.5. Luciferase assays

SH-SY5Y cells were transiently transfected with pGL3-Basic, wild-type (WT) parkin-Luc, ATF4 responsive element (ATF4RE) deletion parkin-Luc, 3X ATF4RE-Luc (generously provided by K. F. Winklhofer, Ludwig Maximilians University, Munich, Germany), and 10 pRL-TK vector (Promega, Fitchburg, USA) was used for the Renilla luciferase control. At 8 hours after transfection, cells were treated with DDS (50 μM) or dimethyl sulfoxide (DMSO; 0.1%). After 56 hours, cells were harvested and assayed sequentially for firefly and Renilla luciferases using the Dual-Luciferase Reporter Assay System (Promega) with a Glomax 20/20 luminometer (Promega) according to the manufacturer's instructions. Firefly luciferase readings were normalized to Renilla readings.

2.6. Real-time qRT-PCR

Total RNA was extracted with the MiniBEST RNA extraction kit (Takara Bio Inc, Otsu, Japan). To eliminate DNA, dissolved RNA was treated with DNase I (RNase free, Stratagene, Agilent Technologies, Waldbronn, Germany) for 15 minutes at 37 °C and purified using a RNeasy kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized from total RNA (1.5 μg) using a first-strand cDNA synthesis kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). Real-time qRT-PCR was performed using a RotorgeneQ (Qiagen), and Rotorgene SYBR green PCR kit (Qiagen). The primers used for RT-PCR were as follows: parkin, 5′-CCT GCT GTT CTC TCG CGC-3′ (forward) and 5′-GGT CAG AGA CCC GGA CCC-3′ (reverse); DJ-1, 5′-GGA GAC GGT CAT CCC TGT AG-3′ (forward) and 5′-TTC ACA GCA GCA GAC TCA GA-3′(reverse); actin, 5′-TGT TAC CAA CTG GGA CGA CA-3′ (forward) and 5′-GGG GTG TTG AAG GTC TCA AA-3′ (reverse).

2.7. Animals

All experiments involving animals were approved and confirmed by the Sungkyunkwan University Ethical Committee in accordance with international guidelines. Male and female C57BL/6J background mice (3 months and 21 months of age) were obtained from Korea Basic Science Institute and maintained at 12-hour dark/light cycles in air-controlled rooms, with free access to food and water. The animals were group-housed (4 mice/cage) and all efforts were made to minimize animal suffering from pain and discomfort and to reduce the number of animals used.