Protective Effect of Sulforaphane against Dopaminergic Cell Death

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ABSTRACT

Parkinson’s disease (PD) is a progressive neurodegenerative disorder associated with a selective loss of dopaminergic neurons in the substantia nigra. Evidence suggests oxidation of dopamine (DA) to DA quinone and consequent oxidative stress as a major factor contributing to this vulnerability. We have previously observed that exposure to or induction of NAD(P)H:quinone reductase (QR1), the enzyme that catalyzes the reduction of quinone, effectively protects DA cells. Sulforaphane (SF) is a drug identified as a potent inducer of QR1 in various non-neuronal cells. In the present study, we show that SF protects against compounds known to induce DA quinone production (6-hydroxydopamine and tetrahydrobiopterin) in DAergic cell lines CATH.a and SK-N-BE(2)C as well as in mesencephalic DAergic neurons. SF leads to attenuation of the increase in protein-bound quinone in tetrahydrobiopterin-treated cells, but this does not occur in cells that have been depleted of DA, suggesting involvement of DA quinone. SF pretreatment prevents membrane damage, DNA fragmentation, and accumulation of reactive oxygen species. SF causes increases in mRNA levels and enzymatic activity of QR1 in a dose-dependent manner. Taken together, these results indicate that SF causes induction of QR1 gene expression, removal of intracellular DA quinone, and protection against toxicity in DAergic cells. Thus, this major isothiocyanate found in cruciferous vegetables may serve as a potential candidate for development of treatment and/or prevention of PD.
supplemented with B-27, 2 mM glutamine, 100 IU/l penicillin, and Vector Laboratories (Burlingame, CA). Laminin, TRIzol reagent, and biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA) were used. The ABC Vecstatin kit was from Sigma Chemical and nitroblue tetrazolium (NBT) were purchased from University of Ulsan Asan Institute for Life Sciences. Pregnant cephalic neurons, all procedures were performed in compliance with the guidelines set forth by the Laboratory Animal Manual of the University of Ulsan Asan Institute for Life Sciences. Pregnant Sprague-Dawley rats were obtained from Orient Charles River Technology (Seoul, Korea). The ventral mesencephalon was removed from 14-day gestation embryo and incubated with 0.01% trypsin in Hank's balanced salt solution for 15 min at 37°C. After trituration, 3 × 10^5 cells were plated on each polystyrene cover slide that had been treated with 10% FBS. Cells were grown as monolayers in the presence of 100 IU penicillin and 10 µg/ml streptomycin at 37°C in 95% air and 5% CO2 in a humidified atmosphere. For primary culture of mesencephalic neurons, all procedures were performed in compliance with the guidelines set forth by the Laboratory Animal Manual of the University of Ulsan Asan Institute for Life Sciences. Pregnant Sprague-Dawley rats were obtained from Orient Charles River Technology (Seoul, Korea). The ventral mesencephalon was removed from 14-day gestation embryo and incubated with 0.01% trypsin in Hank's balanced salt solution for 15 min at 37°C. After trituration, 3 × 10^5 cells were plated on each polystyrene cover slide that had been treated with 100 µg/ml penicillin and 4 µg/ml laminin and placed in a 24-well culture plate. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2 in neurobasal medium supplemented with B-27, 2 mM L-glutamine, 100 IU penicillin, and 10 µg/ml streptomycin. At 5 or 6 days in vitro, the neurons were fed with fresh medium and treated. BH4, 6-OHDA, MPP+, AMPT, and SF were dissolved in distilled H2O immediately before use and added to the culture at appropriate time points.

**Immunocytochemistry.** Primary mesencephalic neurons were fixed in cold 4% paraformaldehyde in 0.1 M PBS, pH 7.4, for 30 min at room temperature. After washing twice in PBS, the cells were incubated for 1 h in blocking solution (5% FBS and 0.3% Triton X-100 in 0.1 M PBS). The cells were then incubated overnight with TH antibody (1:5000) in 0.1 M PBS containing 5% FBS and 0.2% Triton X-100 at 4°C and washed twice in PBS. The samples were incubated with secondary antibody (1:200) and subjected to staining according to the method described for the ABC Vecstatin kit.

**Measurement of TH Immunoreactive Processes.** To measure the process length of DAergic neurons, the mesencephalic neurons immunostained against TH were measured as reported previously (Lee et al., 2007). The length of the longest process of each TH-positive neuron was measured both manually and by the computer program (Mousotron 3.8.3; Blacksun Software, Turnhout, Belgium). On average, 20 measurements were made for each condition.

**Lactate Dehydrogenase Assay.** Degrees of cell death were assessed by activity of LDH released into the culture medium using the method described previously (Hwang et al., 2001). SK-N-BE(2)C and CATH.a cells cultured at densities of 1 × 10^5 and 2 × 10^5 cells per 24-well culture plate, respectively, were used. Aliquots (50 µl) of cell culture medium were incubated with 0.26 mM NADH, 2.87 mM sodium pyruvate, and 100 mM potassium phosphate buffer (pH 7.4) in a total volume of 200 µl. The rate of NAD+ formation was monitored for 5 min at 2-s intervals at 340 nm using a microplate spectrophotometer (SPECTRA MAX 340 pc; Molecular Devices, Menlo Park, CA).

**Reactive Oxygen Species Assay.** The level of ROS was determined by flow cytometry analysis and confocal microscopy. For flow cytometry analysis, CATH.a cells cultured on 60-mm dishes were incubated in medium containing 10 µM DCFH-DA for 15 min at room temperature in the dark. After washing in PBS, the cells were harvested, resuspended in 200 µl of PBS, and subjected to flow cytometry analysis using FACS Calibur (BD Biosciences, San Jose, CA). Dichlorofluorescein was determined by excitation at 488 nm using an argon laser and emission using a 520/20 nm filter; 1 × 10^4 cells were analyzed, and the average was calculated using CELL Quest software. For confocal microscopy, 3 × 10^5 cells cultured in 6-well plates were incubated in 1 ml of KR buffer (150 mM NaCl, 6 mM KCl, 10 mM HEPES, 1 mM MgCl2, 1.5 mM CaCl2, and 5.5 mM glucose) containing 2 µM DCFH-DA at room temperature for 15 min. The cells were analyzed by a Leica DM IRE2 inverted microscope 20 × HCX Fluota lens/TC5-SP2 confocal system using the same excitation and emission wavelengths used above.

**Analysis of Chromosomal Condensation.** CATH.a cells (3 × 10^5) cultured on a 22-mm coverslip in a 6-well plate were washed, permeabilized in 0.1% Triton X-100, and exposed to 10 µM propidium iodide for 30 min in the dark. After washing, the coverslips were mounted on glass slides and analyzed by confocal microscopy (with a 100 × HCX Fluota oil NA 1.3 lens) using excitation at 488 nm and excitation at 560 to 620 nm.

**Analysis of DNA Fragmentation.** CATH.a cells were harvested, washed, and fixed in 70% cold ethanol for 30 min at 4°C. After centrifugation and washing in PBS, the cells were incubated in PBS containing 0.1% Triton X-100, 10 µg/ml RNase A, and 10 µg/ml propidium iodide for 30 min in the dark. The cells were filtered through a 50-µm Falcon strainer and transferred to Falcon 2052 tubes. Flow cytometric analysis was carried out using FACS Calibur with excitation at 488 nm (argon laser) and emission at 585/42 nm (filter 2; red). 1 × 10^6 cells were acquired using CELL Quest software, and cell cycle calculations were performed using ModFit 2.0.

**Measurement of Protein Modification.** For detection and quantitation of protein-bound quinones, a NBT/glycinate assay was performed as described previously (Choi et al., 2003a). CATH.a cells were harvested and briefly washed in 50 mM phosphate buffer (pH 7.4), and proteins were precipitated in the presence of trichloroacetic acid.
acid. The precipitate was washed twice with ethanol, further treated with chloroform-methanol (2:1, v/v), and pelleted. To the delipidated protein precipitate was added 1 ml of NBT reagent (0.24 mM NBT in 2 M potassium glycinate, pH 10), after which the reaction mixture was incubated in the dark for 1 h on a shaker. The blue-purple color developed in the reaction mixture was suitably diluted, and absorbance was taken at 530 nm.

Observation of Morphology and Membrane Damage. Changes in cell morphology were observed using differential interference contrast. The cells were also subjected to propidium iodide staining (10 μM) for 5 min at room temperature without the permeabilization step. After washing, the cells were analyzed under the Leica DM IRE2 inverted microscope using the red filter (583/30 nm).

QR1 Activity Assay. QR1 activity was determined as described previously (Choi et al., 2003a). Crude CATH.a cell lysate was resuspended in 25 mM Tris-HCl buffer (pH 7.4) containing 1 mM NADH, 230 μg/ml bovine serum albumin, and 1 mM FAD. The enzyme reaction was initiated by the addition of DCPIP to the final concentration of 10 μM, and reduction of DCPIP was monitored spectrophotometrically as a decrease in absorbance at 600 nm. Specific enzyme activity was defined as the difference between the slope as measured above and that obtained in the presence of the QR1 inhibitor dicoumarol (10 μM).

RT-PCR. Total cellular RNA was isolated using TRIzol reagent, 5 μg of which was used for reverse transcription using a Superscript III first strand synthesis kit with random decamers. Synthetic oligonucleotides were designed on the basis of mouse and human QR1 sequences in the GenBank Sequence Database at http://www.ncbi.nlm.nih.gov/BLAST/ and used for the PCR: mouse QR1 sense, 5'-CTCGAGCTTTAGGTCTGCTTg-3'; mouse QR1 antisense, 5'-AAGC-TCTGCACCTTGGCCT-3'; human QR1 sense, 5'-CCATCATTTCCAGAAAGGACATC-3'; and human QR1 antisense, 5'-TGAGACCCACTGTATTTTGCTCC-3'. The amplifications were performed basically with the following protocol: 25 cycles of 94°C for 30 s, 57°C for 40 s, and 72°C for 40 s. The resulting PCR fragments were subjected to electrophoresis through a 1.5% agarose gel and visualized by ethidium bromide staining and UV irradiation. As a loading control, RT-PCR for mouse 2-macroglobulin and human glyceraldehyde-3-phosphate dehydrogenase was performed on CATH.a and SK-N-BE(2)C samples, respectively.

Statistical Analyses. All statistical analyses were performed using the SPSS package, and results are expressed as means ± S.E.M. Data were analyzed by the Student’s paired t test or one-way ANOVA followed by a comparison of means by Dunnett’s test. All experiments were performed on four to five samples for each condition and were repeated on at least three different culture preparations.

Results

SF Protects against 6-OHDA-Induced DAergic Cell Death. We first asked whether SF might protect DAergic cells from cytotoxicity using DAergic toxins that are known to lead to production of DA quinone: 6-OHDA and BH4. This theory was tested in two different DAergic cell lines, SK-N-BE(2)C and CATH.a, and the degree of cell death was assessed by LDH activity released into the medium.

As shown in Fig. 1A, treatment with 50 μM 6-OHDA for 24 h led to cell death of SK-N-BE(2)C cells (197 ± 2% of the
untreated control), and this was blocked by a 24-h pretreatment with SF in a concentration-dependent manner between 1 and 5 μM. At 5 μM SF, the 6-OHDA-induced cell death was only 120 ± 5% of the untreated control. SF alone had no cytotoxic effect in the concentrations tested (not shown). CATH.a cells treated with 50 μM 6-OHDA were also damaged, with the released LDH activity increased to 244 ± 31% of the untreated control (Fig. 1B). Pretreatment with SF at 1 μM resulted in a significant decrease in the LDH value to 156 ± 14% of the untreated control, and higher concentrations did not significantly further increase the protective effect. Therefore, SF seemed to effectively protect against 6-OHDA-induced cell death in the range of 1 to 5 μM in both SK-N-BE(2)C and CATH.a cells.

SF Protects against BH4-Induced DAergic Cell Death. As shown in Fig. 2A, 200 μM BH4 caused an increase in LDH activity to 169 ± 12% of the untreated control in SK-N-BE(2)C cells. This activity was suppressed by the pretreatment with SF in a dose-dependent manner. At 2.5 and 5 μM SF, the LDH activity was decreased to 106 ± 12 and 100 ± 7%, respectively. Likewise, BH4 treatment resulted in the death of CATH.a cells to 155 ± 4% of the untreated control cells, which was blocked by the pretreatment with 0.5, 1, and 2.5 μM SF (118 ± 5, 98 ± 2, and 121 ± 7%, respectively) (Fig. 2B). Therefore, SF protected both SK-N-BE(2)C and CATH.a cells from BH4-induced cytotoxicity.

SF Protects Primary Cultured Mesencephalic DAergic Neurons. To confirm the protective effect of SF in a more physiologically relevant system, we used primary cultured DAergic neurons isolated from rat mesencephalon. As shown in Fig. 3A, the TH-immunopositive DAergic neurons were preferentially vulnerable to BH4, with shortened neurites, whereas MAP-2 positive neurons seemed mostly unaffected. Pretreatment with SF prevented the BH4-induced DAergic neurite loss in a dose-dependent manner. Quantitative analysis (Fig. 3B) revealed that the neurites were decreased in length to 20.4% of the untreated control by the BH4 treatment. Significant protection was observed by SF pretreatment at a concentration as low as 0.5 μM, with the neurite length being 2.0-fold of the BH4-alone treated (41.0% of the untreated control). Full protection was achieved at 2.5 and 5 μM SF, as the neurite lengths were not significantly different from untreated controls (p > 0.05).

SF Does Not Protect against MPP⁺-Induced DAergic Cell Death. If the protective action of SF is achieved indeed via removal of DA quinone in these cells, the SF protection would be effective principally under a damaging condition that accompanies DA quinone generation. To test this notion, we used another DAergic toxin, MPP⁺, whose principal mechanism of action does not involve DA quinone. As shown in Fig. 4, 2 mM MPP⁺ caused increases in LDH activity to 198 ± 15 and 140 ± 1% of untreated controls in SK-N-BE(2)C and CATH.a cells, respectively. Pretreatment with SF did not attenuate the toxic effect at any of the concentrations tested (p > 0.05 versus MPP⁺-treated). Therefore, SF seemed to exert protection particularly against the mechanism that involves quinone generation in DA cells.

SF Attenuates Formation of Quinone-Modified Proteins. DA quinone can attack and modify cellular nucleophiles, including sulphydryl groups in proteins. We tested whether pretreatment with SF might prevent accumulation of intracellular quinone proteins. As shown in Fig. 5A, a significant increase (161 ± 4%) in the protein-bound quinone, as determined by a NBT/glycinate assay, was observed after BH4 treatment. This increase was blocked in the cells pre-
treated with SF; at 2.5 μM SF, the quinone protein level remained at the untreated control level (102 ± 2%). Thus, SF effectively prevented the formation of protein-bound quinone products in DAergic cells.

To confirm that the protein-bound quinone is derived from DA, we also tested the effect of lowering endogenous DA content. We have previously shown that treatment with the TH inhibitor AMPT (100 μM) lowers intracellular dopamine content by 78% (Choi et al., 2003a). As shown in Fig. 5B, with the AMPT pretreatment, BH4 did not cause the increase in protein-bound quinone (110 ± 17% of the untreated control; p > 0.05). Under this condition, cotreatment with SF did not result in statistically significant lowering of the protein-bound quinone level (p > 0.05 versus BH4/AMPT for both 1 and 2.5 μM SF).

**SF Attenuates Accumulation of ROS.** Because the formation of quinones can cause generation of ROS, it was possible that the SF treatment might lead to reduction of cellular ROS. To test this theory, cells pretreated with SF were exposed to BH4, and ROS was assessed by flow cytometric analysis after loading the cells with the ROS-sensitive dye DCFH-DA. As shown in Fig. 6A, an increase in DCF was observed in the BH4-treated cells, with the mean fluorescence intensity increased by 6.2-fold. This phenomenon was attenuated by SF pretreatment: 2.5 μM SF caused the mean fluorescence intensity to decrease by 50% of BH4-treated control. For confirmation, the cells treated under the same conditions were also subjected to confocal microscopy. As shown in Fig. 6B, a dramatic increase in fluorescence was observed in the treated cells, which was reversed by pretreatment with SF.

**SF Protects against Loss of Membrane Integrity.** Exposure to BH4 leads to lipid peroxidation (Choi et al., 2003a), causing membrane modification and permeabilization. We asked whether such permeabilization of membrane might occur after the exposure to BH4 and whether SF might attenuate this. Cells were treated with the nucleus staining dye propidium iodide. As propidium iodide does not normally penetrate the cell membrane, any increase in the intracellular staining would suggest breakages in the membrane. As shown in typical micrographs (Fig. 7), although untreated cells had no cells that were stained positive (0.2%), many of the cells treated with BH4 alone showed intracellular propidium iodide staining (29.7%; p < 0.01 versus the untreated control). On the other hand, the cells pretreated with SF before the BH4 exposure showed dramatically lower staining (2.9%; p < 0.01 versus BH4 alone). Therefore, SF seemed to protect the cell membrane from BH4-induced breakages in the membrane.

**SF Inhibits Chromosomal Condensation and DNA Fragmentation.** Apoptotic cells undergo nuclear fragmentation, and BH4 has been observed to lead to chromatin condensation (Choi et al., 2003a) and DNA fragmentation.
We tested whether SF might attenuate this phenomenon. For this, CATH.a cells were treated with BH4, membrane-permeabilized, and stained with propidium iodide. As shown in Fig. 8A, the cells treated with BH4 revealed punctate nuclear staining, indicative of chromosomal condensation, in almost all cells. In comparison, cells pretreated with SF exhibited a nuclear staining pattern that was more similar to that for the untreated control.

We also quantitated the degrees of DNA fragmentation by fluorescence-activated cell sorting analysis after the propidium iodide staining. As shown in Fig. 8B, BH4 exposure caused an increase in the level of debris from DNA fragmentation at the sub-G0–1 phase from 4.5% (untreated control) to 40.4% (BH4-treated; p < 0.01 versus the untreated control). SF pretreatment resulted in attenuation of the effect, with the value reaching only 21.1% (p < 0.01 versus BH4 alone). There was no significant difference in cell cycle (data not shown). Therefore, SF seemed to effectively attenuate the BH4-induced DNA fragmentation in DAergic cells.

**SF Induces QR1 Gene Expression.** Because SF seemed to remove quinone species in DAergic cells, whether this might involve induction of QR1 was tested. SK-N-BE(2)C and CATH.a cells were treated with various concentrations of SF, and the levels of QR1 mRNA were assessed by RT-PCR. As shown in Fig. 9A, SK-N-BE(2)C cells showed increased QR1 mRNA level in a manner dependent on SF concentration. Densitometric analysis revealed that 3 μM SF resulted in a maximal increase in QR1 mRNA, reaching 3.1 ± 0.3-fold of the untreated control. As shown in Fig. 9B, CATH.a also showed similar induction of QR1: at 2 and 3 μM SF, the QR1 mRNA level was increased by 2.6 ± 0.3- and 2.5 ± 0.3-fold, respectively.

Whether QR1 protein activity might be enhanced was tested. Lysate of cells treated with various concentrations of SF for 30 h was subjected to QR1 activity assay. As shown in Fig. 9C, specific activity of QR1 was indeed augmented after the SF treatment in a dose-dependent manner. Cells exposed to 1 and 3 μM SF showed increases to 115 ± 1 and 143 ± 3%, respectively, compared with the untreated control. Therefore, the SF treatment caused increases in enzyme activity as well as gene expression of QR1.

Fig. 6. SF prevents formation of ROS. CATH.a cells were pretreated with 2.5 μM SF for 24 h followed by 200 μM BH4 for an additional 24 h. Typical flow cytometric analysis (A) and photographs of confocal microscopy (B) of DCFH-DA-stained CATH.a cells are shown. Scale bar, 200 μm. Values of mean fluorescence intensity for each group were as follows: untreated control, 2.4 ± 0.2; BH4-treated, 15.1 ± 0.7; and BH4/SF-treated, 7.8 ± 0.2 (p < 0.001 versus BH4-treated cells). Data are expressed as means ± S.E.M. DCF, dichlorofluorescein; DIC, differential interference contrast.

Fig. 7. SF reduces BH4-induced membrane breakages in DAergic cells. CATH.a cells were pretreated with 2.5 μM SF for 24 h and subsequently exposed to 200 μM BH4 for 24 h. Morphological changes were examined by differential interference contrast (DIC) inverted microscopy and membrane integrity was assessed by propidium iodide (PI) staining. Scale bar, 200 μm. CTL, control.

Fig. 8. SF attenuates DNA fragmentation in DAergic cells. CATH.a cells were pretreated with 2.5 μM SF for 24 h and subsequently exposed to 200 μM BH4 for 24 h. After membrane permeabilization and propidium iodide staining, the cells were subjected to confocal microscopic observation of chromosomal condensation (A) and flow-cytometric analysis of DNA fragmentation (B). Scale bar, 15 μm.
Discussion

In the present study we demonstrate that SF can protect DAergic cells from the cytotoxicity of 6-OHDA and BH4, the compounds known to generate DA quinone products and oxidative stress and cause selective death of DAergic cells. This protective effect seems to be mediated by removal of quinone products, because QR1 enzyme activity and mRNA level are increased and quinone-modified proteins are decreased by the SF treatment. SF pretreatment also leads to attenuation of the BH4-induced ROS production, DNA fragmentation, and membrane breakage. This is the first report to our knowledge that SF protects DA cells.

Accumulating evidence suggests that the particular vulnerability of DAergic neurons in the substantia nigra is due to oxidative metabolism of DA and consequent oxidative stress. Both enzymatic oxidation of DA by monoamine oxidase and autoxidation of DA lead to production of DA quinones (Mak et al., 1981; Hastings and Zigmond, 1997). The quinone can readily bind to cellular nucleophiles including the reduced form of glutathione and also cause cellular damage by covalently binding and inactivating essential cellular macromolecules such as DNA and proteins (Graham, 1978; Asanuma et al., 2003). Interestingly, DA quinone was recently found to bind α-synuclein and promote its conversion to protofibrils, which have been shown to be cytotoxic and thought to be involved in the pathogenesis of PD (Conway et al., 2001). In addition, the quinone can result in further generation of ROS by cyclizing to hydroquinone, from accumulation of the quinone products. One such mechanism is induction of QR1 activity. By catalyzing reduction of quinone to hydroquinone (Joseph et al., 2000; Cavelier and Amzel, 2001), QR1 can prevent toxic accumulation of DA quinone products in DA cells. In addition, QR1 is known to maintain both α-tocopherol and coenzyme Q10 in their reduced, antioxidant state (Beyer et al., 1997; Siegel et al., 1997). Therefore, a means to induce QR1 activity in DAergic cells would prove to be beneficial for protection of DAergic cells. In the present study, we show that SF can induce QR1 expression and effectively protect DAergic cells from cytotoxic compounds. This result is further corroborated by the findings that the BH4-induced increases in quinone-bound proteins and ROS are attenuated by the SF treatment. The protective role of QR1 has indirectly been suggested by the epidemiological data showing that its polymorphism (C609T), which results in a decrease or total loss of its expression (Siegel et al., 2001), is associated with PD (Harada et al., 2001).

Although QR1 is abundant in the liver where it participates in phase II detoxification, the enzyme is also expressed in the brain (Stringer et al., 2004). In addition to its predominant expression in astrocytes and endothelial cells (van Muiswinkel et al., 2000; Flier et al., 2002), QR1 is reported to be also expressed, albeit to a lesser degree, in DAergic neurons in the substantia nigra (van Muiswinkel et al., 2004). Moreover, a marked increase in the neuronal expression of QR1 was consistently observed in the Parkinsonian substantia nigra (van Muiswinkel et al., 2004). Therefore, it is possible to speculate that QR1 gene expression might be induced in response to conditions eliciting oxidative stress, such as accumulation of DA quinones, in the nigral DAergic neurons.

SF has been shown to increase the expression of a number of genes, via activating the transcription factor Nrf2 (Lee and Johnson, 2004; McWalter et al., 2004), including those that belong to phase II detoxification pathway (glutathione S-transferase, epoxide hydrolase, and glucuronosyltransferase) (Zhang et al., 1992) as well as thioredoxin (Tanito et al., 2005) and thioredoxin reductase (Wang et al., 2005). Therefore, one cannot eliminate the possibility that induction of the other antioxidant enzymes also contribute to the protection. It is possible that the concurrent expression of these enzymes along with QR1 can facilitate the removal of ROS...
that is accumulated as a result of DA quinone generation, indirectly assisting the protective action of QR1.

We have previously reported that the QR1 inducers dimethyl fumarate and butylated hydroxyanisole protect CATH.a cells from BH4-induced death and that their effective concentrations are 10 and 50 µM, respectively (Choi et al., 2003a). The fact that SF exhibits similar protective capacity at lower concentration range (1–2.5 µM) suggests that SF is a more effective drug. Because SF is a component of the human diet, found in cruciferous vegetables such as broccoli, cabbage, cauliflower, and radish, and is derived from its naturally occurring glucosinolate precursor glucoraphanin, it is most probably safe for administration and can be orally administered. Questions such as whether SF crosses the blood-brain barrier and how much dietary intake of SF-containing vegetables should be made to reach an effective level in vivo should be addressed in future studies. Taken together, the results of the present study suggest a potential use of this drug toward development of treatment and/or prevention of PD.

References


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