A dibenzoylmethane derivative protects dopaminergic neurons against both oxidative stress and endoplasmic reticulum stress

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Takano K, Kitao Y, Tabata Y, Miura H, Sato K, Takuma K, Yamada K, Hibino S, Choshi T, Iinuma M, Suzuki H, Murakami R, Yamada M, Ogawa S, Hori O. A dibenzoylmethane derivative protects dopaminergic neurons against both oxidative stress and endoplasmic reticulum stress. Am J Physiol Cell Physiol 293: C1884–C1894, 2007.—The enhancement of intracellular stresses such as oxidative stress and endoplasmic reticulum (ER) stress has been implicated in several neurodegenerative disorders including Parkinson’s disease (PD). During a search for compounds that regulate ER stress, a dibenzoylmethane (DBM) derivative 14-26 (2,2'-dimethoxydibenzoylmethane) was identified as a novel neuroprotective agent. Analysis in SH-SY5Y cells and in PC12 cells revealed that the regulation of ER stress by 14-26 was associated with its anti-oxidative property. 14-26 prevented the production of reactive oxygen species (ROS) when the cells were exposed to oxidants such as hydrogen peroxide and 6-hydroxydopamine (6-OHDA) or an ER stressor brefeldin A (BFA). 14-26 also prevented ROS-induced damage in both the ER and the mitochondria, including the protein carbonylation in the microsome and the reduction of the mitochondrial membrane potential. Further examination disclosed the presence of the iron-chelating activity in 14-26. In vivo, 14-26 suppressed both oxidative stress and ER stress and prevented neuronal death in the substantia nigra pars compacta (SNpc) after exposure to oxidants such as hydrogen peroxide and 6-hydroxydopamine (6-OHDA) or an ER stressor brefeldin A (BFA). 14-26 also prevented ROS-induced damage in both the ER and the mitochondria, including the protein carbonylation in the microsome and the reduction of the mitochondrial membrane potential. Further examination disclosed the presence of the iron-chelating activity in 14-26. In vivo, 14-26 suppressed both oxidative stress and ER stress and prevented neuronal death in the substantia nigra pars compacta (SNpc) after injection of 6-OHDA in mice. These results suggest that 14-26 is an antioxidant that protects dopaminergic neurons against both oxidative stress and ER stress and could be a therapeutic candidate for the treatment of PD.

neuronal cell death; stress response; Parkinson’s disease

NEURODEGENERATION is a multifaceted process that leads to progressive neuronal death, and one of the key factors underlying it is intracellular stress. Increased oxidative stress and dysfunction of the mitochondria are implicated in many neurodegenerative diseases (3). Several neurotoxins, including 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), have been used to model Parkinson’s disease (PD); most of these agents are associated with mitochondrial damage and enhanced oxidative stress.

Recent studies, however, have raised the possibility that the endoplasmic reticulum (ER) also plays an important role in maintaining neurons in the neuropathological situations. The ER is a target for two types of intracellular stresses: ER stress and oxidative stress. ER stress is characterized by the accumulation of unfolded proteins in the ER, which occurs in conditions such as glucose starvation, oxygen deprivation, inhibition of protein modification, and disturbance of Ca2+ homeostasis. Eukaryotic cells, including neurons, respond to ER stress by activating a set of pathways known as the unfolded protein response (UPR) (36). The UPR is transmitted through the activation of ER resident proteins, such as inositol-required enzyme 1 (IRE1α/β), protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6), and the UPR-targeted genes include molecular chaperones, folding catalysts, subunits of translocation machinery (Sec61 complex) in the ER, ER-associated degradation (ERAD) molecules, and antioxidant genes (16, 36, 43). However, if the protein load in the ER exceeds its folding capacity, cells tend to die, typically, with apoptotic features (ER stress-induced cell death). Important roles for ER stress and ER stress-induced cell death have been demonstrated in various pathological situations, including brain ischemia and neurodegeneration (12, 20, 30, 34, 40).

The ER is also a place where oxidative stress is generated. In the lumen of the ER, the redox status of glutathione (GSH) is shifted to the oxidized form (GSSG), and disulfide bonds of proteins are effectively formed through the relay of electrons by the ER-resident proteins; protein disulfide isomerase (PDI) and a novel flavoprotein Ero1(5, 29). As a result, reactive oxygen species (ROS), which are believed to be eliminated by GSH, are generated in the ER (6). It has been reported that branches of the UPR such as the PERK-eIF2α-ATF4 pathway are critical for the regulation of oxidative stress derived from the ER or other sources (36). Cells lacking PERK or ATF4 accumulate ROS (11) and are more susceptible to PD-related neurotoxins such as 6-OHDA (34).

Herp is a UPR-dependent ubiquitin-like protein that is located in the ER in a wide range of cells including neurons (16, 24, 35). We recently reported that targeted disruption of the Herp gene rendered F9 embryonic carcinoma cells vulnerable to ER stress (16). Using this cell line and other neuronal cell lines including SH-SY5Y cells and PC12 cells, we evaluated the protective effect of 300 compounds against ER stress-induced cell death. We report here that a dibenzoylmethane

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(DBM) derivative, 14-26 (2',2'-dimethoxydibenzoylmethane) protects dopaminergic neurons against both ER stress and oxidative stress. 14-26 prevents ROS production under those conditions and inhibits ROS-induced damage in both the ER and mitochondria. In vivo, 14-26 prevented neuronal death in the substantia nigra pars compacta (SNpc) following injection of 6-OHDA in mice.

EXPERIMENTAL PROCEDURES

Cell cultures and stress conditions. F9 Herp null cells were developed as previously described (16) and were maintained in DMEM containing 20% fetal bovine serum. Human catecholaminergic neuronal cell line F9 (Kanazawa University) was used for Western blot analysis. For the administration of 14-26 or vehicle, mice were intraperitoneally injected with 14-26 (10 mg/kg) or 6-OHDA (30 or 75 μM) after pretreatment of the cells with 200 synthetic compounds, including DBM derivatives, Carbazole derivatives, and pyrimidine plant-derived compounds (38, 39) and 200 synthetic compounds, which were subjected to Northern blot analysis using cDNA fragments specific for glucose-regulated protein 78 (GRP78), a molecular chaperone in the ER, C/EBP-homologous protein (CHOP), a mediator of ER stress-induced cell death (36), or β-actin as described previously (16).

Western blot analysis. SH-SY5Y cells or PC12 cells were treated with each compound or cultured in the medium alone for 16 h, after which the medium was replaced with fresh medium containing 6-OHDA. The cells were subjected to Northern blot analysis using cDNA fragments specific for glucose-regulated protein 78 (GRP78), a molecular chaperone in the ER, C/EBP-homologous protein (CHOP), a mediator of ER stress-induced cell death (36), or β-actin as described previously (16).
antibodies were used for the visualization of immunolabeling. TH (+) neuronal cells in the SNpc were counted in two representative sections (Bregma-3.16 and -3.64 mm) as described before (25) after digital images were acquired using a CCD camera (Hamamatsu Photonics, Shizuoka, Japan).

Laser densitometric analysis and statistical analysis. Laser densitometric analysis was performed to standardize the results of Western and Northern blot analyses as described previously (16). For statistical evaluation, Bonferroni/Dunnett test following one-way ANOVA or Student’s t-test was employed.

Fig. 1. Effect of dibenzoylmethane (DBM) derivatives on endoplasmic reticulum (ER) stress and oxidative stress in neuronal cell lines. A: structure of DBM derivatives. B and C: protection of SH-SYSY cells against ER stress-induced cell death. SH-SYSY cells (5 × 10⁵ cells/condition) were treated with tunicamycin (Tm, 1.5 μg/ml; B) or brefeldin A (BFA, 1 μg/ml; C) in the absence or presence of compounds for 48 h. Cell viability was evaluated using the 3-(4,5-dimethyl-2-thiazolyl)2,5-diphenyltetrazolium bromide (MTT) assay. NAC, N-acetyl-cysteine (NAC). Values shown are means (SD) of 4 experiments. *P < 0.01, **P < 0.001. D and E: protection of SH-SYSY cells against oxidative stress-induced cell death. SH-SYSY cells (5 × 10⁵ cells/condition) were pretreated with medium alone or with the indicated compounds for 24 h and incubated with H₂O₂ (44 μM; D) or with 6-hydroxydopamine (6-OHDA, 30 μM; E) for a further 24 h in the presence or absence of compounds. Cell viability was evaluated using the MTT assay as above. Values shown are means (SD) of 4 experiments. *P < 0.01, **P < 0.001. F: enhancement of the cell protection against oxidative stress by pre-treatment with 2,2′-dimethoxydibenzoylmethane (14-26). SH-SYSY cells (5 × 10⁵ cells/condition) were pretreated with 14-26 for the indicated times and incubated with H₂O₂ (44 μM) for a further 24 h in the presence or absence of 14-26. Cell viability was evaluated using the MTT assay as above. Values shown are means (SD) of 4 experiments. *P < 0.01, **P < 0.001.
RESULTS

Prevention of ER stress- and oxidative stress-induced cell death by DBM derivatives. We previously reported that Dantrolene, an antagonist of the ryanodine receptors in the ER, and some antioxidants such as α-tocopherol, partly prevented Tm-induced cell death in F9 Herp null cells (39). With the use of these agents as positive controls, 103 plant-derived compounds and 200 synthetic compounds were screened in F9 Herp null cells treated with Tm. The DBM derivative 14-26 (2,3′-dimethoxydibenzoylmethane) was identified as a protective agent against ER stress (supplemental figure 1, A and B). 14-26 (20–40 μM) improved cell viability after Tm treatment at a level similar to that achieved by Dantrolene (30 μM) in F9 Herp null cells (supplemental figure 1). To further investigate the cell protection by DBM derivatives under stress, two neuronal cell lines, SH-SY5Y cells and PC12 cells, were exposed to ER stress or oxidative stress, and the protective property of DBM derivatives were compared (Fig. 1A). When SH-SY5Y cells (Fig. 1B) and PC12 cells (data not shown) were treated with ER stressors such as Tm (Fig. 1B) and BFA (Fig. 1C) for 48 h, cell viability dropped to 38 (SD 5) % and 28 (SD 6) %, respectively. Among the DBM derivatives, 14-26 restored cell viability to 65 (SD 8) % and 62 (SD 7) % in each condition (Fig. 1, B and C). 14-26 also protected SH-SY5Y cells against ER stress to a slightly lesser degree. Similar results were obtained when PC12 cells were treated with ER stressors (data not shown). The strong cell protection by 14-26 and 14-28 against oxidative stress was also observed when exposing SH-SY5Y cells (Fig. 1, D and E) or PC12 cells (data not shown) to oxidants such as H2O2 (Fig. 1D), 6-OHDA (Fig. 1E), and BSO (data not shown) for 24 h, after pretreatment of the cells with each compound for 24 h (Fig. 1, D and E). The protective activity of 14-26 that is equivalent to NAC (1,000 μM), the precursor of glutathione, was obtained at the range of 40–60 μM (Fig. 1, D and E). Similar levels of cell protection against H2O2 were obtained when SH-SY5Y cells were pretreated with 14-26 for shorter periods (4–16 h), but, in the condition without pretreatment, 14-26 was less effective (Fig. 1F). When SH-SY5Y cells were exposed to higher concentration of H2O2 (440 μM) for a shorter period (6 h), 14-26 also protected cells, but to a lesser degree (supplemental Figure 1C).

Effect of 14-26 on the expression of the UPR-targeted genes. To analyze the protective property of 14-26 against ER stress, SH-SY5Y cells (Fig. 2, A and D) and PC12 cells (Fig. 2, B and C) were treated with ER stressors such as Tm (2 μg/ml) or BFA (1 μg/ml) in the presence or absence of the indicated compounds for 6 h after pretreatment of the cells with each compound or vehicle (culture medium) for 16 h. Total RNA (10 μg/condition) was isolated and subjected to Northern blot analysis with specific probes against GRP78, CHOP, and β-actin. The intensities of GRP78 mRNA and CHOP mRNA were quantified and standardized with those of β-actin (Fig. 2A).

Fig. 2. Effect of 14-26 on the unfolded protein response (UPR). A–C: expression of glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) mRNA in normal and ER stress conditions. SH-SY5Y cells (A) or PC12 cells (B and C) were treated with Tm (2 μg/ml) or BFA (1 μg/ml) in the presence or absence of the indicated compounds for 6 h after pretreatment of the cells with each compound or vehicle (culture medium) for 16 h. Total RNA (10 μg/condition) was isolated and subjected to Northern blot analysis with specific probes against GRP78, CHOP, and β-actin. The intensities of GRP78 mRNA and CHOP mRNA were quantified and standardized with those of β-actin (A). D: expression of GRP78 and GRP94 protein in normal and ER stress conditions. SH-SY5Y cells were treated with Tm (2 μg/ml) or BFA (1 μg/ml) in the presence or absence of the 14-26 for 12 h after pretreatment of the cells with 14-26 or vehicle (culture medium) for 16 h. Cell lysates were subjected to Western blot analysis with anti-KDEL antibody and anti-β-actin antibody. The intensities of GRP78 protein and GRP94 protein were quantified and standardized with those of β-actin.
suppressed at the mRNA level by treatment of the cells with 14-26 (Fig. 2, A and B), but not with IN21 (Fig. 2C). Similarly, the enhanced levels of expressions of GRP78 and GRP94, the latter another UPR-targeted gene, under ER stress were suppressed at protein level by treating cells with 14-26. These results suggest that the cell protection by 14-26 against ER stress was not related to the activation of the UPR, which is the case for methoxyflavones (39). Instead, these observations suggest that 14-26 improves the ER luminal environment that reduces the level of ER stress after treating the cells with ER stressors.

**Effect of 14-26 on ROS production in response to oxidative stress and ER stress.** We hypothesized that the protective effect of 14-26 and 14-28 against ER stress may be related to their anti-oxidative properties. To analyze the effect of 14-26 on ROS production in response to both oxidative stress and ER stress, SH-SY5Y cells were treated with oxidants such as H2O2 (44 μM) and 6-OHDA (75 μM) for 6 h or an ER stressor BFA (1 μg/ml) for 16 h, and the intracellular production of ROS was estimated using a fluorescent probe DCF. Enhanced levels of intracellular ROS were observed in all stress conditions (Fig. 3, A,II; B,II; C,II). Treatment of the cells with Tm also generated ROS to a lesser degree (data not shown). Treatment of the cells with 14-26 or NAC after pretreatment for 16 h prevented both oxidative stress- and ER stress-derived ROS (Fig. 3, A,III; A,IV; B,III; B,I; C,III; C,IV). Similar tendency was observed when DCF-derived fluorescence was measured by a fluorometer (Fig. 3, D–F).

**Effect of 14-26 on ROS-induced damage in the ER and mitochondria.** As the oxidative modification of proteins is an important factor in ROS-induced damage to organelles, especially in the ER (9, 33), the protein carbonylation levels were estimated in both whole cell lysates (Fig. 4A) and crude cell fractions (Fig. 4B) from SH-SY5Y cells after treatment of the cells with 6-OHDA for 90 min as described previously (14). Carbonylated proteins were observed in whole cell lysates after 6-OHDA treatment in a dose-dependent manner (Fig. 4A), and they were predominantly located in the microsome fraction. Treatment of cells with 14-26 diminished carbonylated proteins in both whole cell lysates and in the microsome fraction.
(Fig. 4, A and B). A similar tendency was observed when the cells were treated with H\textsubscript{2}O\textsubscript{2} (data not shown). As the mitochondria is also a target organelle of the ROS-induced injury, the levels of the mitochondrial membrane potential was assessed after treatment of SH-SY5Y cells with H\textsubscript{2}O\textsubscript{2} (44 \textmu M; Fig. 4C,II), 6-OHDA (75 \textmu M; Fig. 4D,II) or BFA (1 \textmu g/ml; Fig. 4E,II). The reduction of the mitochondrial membrane potential was observed in all stress conditions, although the effect of BFA may be smaller than those of H\textsubscript{2}O\textsubscript{2} or 6-OHDA (Fig. 4, C,II; D,II; E,II). Treatment of the cells with 14-26 restored the membrane potential in all cases (Fig. 4, C,III; D,III; E,III).

**Iron chelation by DBM derivatives.** To dissect in more detail the anti-oxidative properties of 14-26, experiments were performed in a cell-free system. As it has been reported that DBM has the iron-chelating activity and can be used for the analysis of serum iron (44), the binding properties of 14-26 to iron, copper, and zinc were measured using the fluorescence indicator calcein. When calcein (100 nM) was incubated with Fe(II), Cu(II), or Zn(II), the fluorescence intensity went down to 39 (SD 4) % (Fig. 5A,I), 50 (SD 6) % (Fig. 5A,II), and 58 (SD 7) % (data not shown), respectively, of the intensity seen when calcein was incubated with the control solution without metals. The addition of 14-26 restored the fluorescence inten-
sity of calcein in the calcein-iron solution in a dose-dependent manner (Fig. 5A,I), although this effect was milder than that of a common iron chelator deferoxamine (DFO). NAC did not affect the binding of iron to calcein (Fig. 5A,I). 14-26 also showed chelating activity, to a much lesser degree, for Cu(II) (Fig. 5A,II) but not for Zn(II) (data not shown). Further analysis with different DBM derivatives revealed that 14-26 and 14-28, but not other DBM derivatives, chelated iron effectively (Fig. 5B). These observations suggest that the iron-chelating property of 14-26 may play a role in its protective property.

Neuroprotective effect of 14-26 in a mouse model of PD. It has been reported that PD-related neurotoxins such as 6-OHDA and MPTP induce both oxidative stress- and ER stress-signaling pathways (13, 34, 37). As 14-26 prevented 6-OHDA-induced cell death in SH-SY5Y cells (Fig. 2D), the neuroprotective properties of 14-26 were assessed in a unilateral 6-OHDA lesion model of PD in mice (19). When mice were injected with 6-OHDA (5.25 μg/1.5 μl) into the left MFB, the number of TH-positive cells in the left SNpc decreased to 42 (SD 10) % of the number observed in the right SNpc (noninjected side) 1 wk after lesioning (Fig. 6A, a and b). By 3 wk after lesioning, this number decreased to 21 (SD 8) % of the control figure (Fig. 6A, a, b, c, and Fig. 6B,II). Nissl staining confirmed neuronal loss in the left SNpc at 3 wk after 6-OHDA lesioning (Fig. 6C, a and b). Consistently, loss of TH immunoreactivity was observed in the left striatum, both 1 wk (data not shown) and 3 wk (Fig. 6D,a) after 6-OHDA lesioning. By contrast, when mice were intraperitoneally injected with 14-26 (10 mg/kg) for 3 consecutive days, beginning the day before 6-OHDA injection, the decrease in the number of TH (+) neurons (Fig. 6A, d, e, f, and Fig. 6B) and neuronal death (Fig. 6C,b) were prevented. The number of TH (+) cells in the left SNpc was recovered to 71 (SD 9) % of the number observed in the right SNpc at 1 wk after lesioning (Fig. 6B,II). The loss of TH immunoreactivity in the left striatum was also improved by 14-26 administration (Fig. 6D).

To assess whether the neuroprotective effect of 14-26 in vivo correlates with the regulation of oxidative stress and ER stress, the levels of immunoreactivities of 4-HNE-modified proteins (oxidative stress markers) and GRP78 and/or GRP94 proteins (ER stress markers) were evaluated 1 wk after 6-OHDA lesioning in mice. Enhanced immunoreactivities for both stress markers were observed in vehicle-treated mice (Fig. 7, A,a and B,a), but not in 14-26-treated mice (Fig. 7 A,c and B,c).
Further behavioral examination revealed that administration of 14-26 significantly reduced amphetamine (2.5 mg/kg)-induced rotation, which is caused by the asymmetrical loss of TH (H11001) neurons in SNpc (19) at 3 wk after 6-OHDA lesions (Fig. 8).

**DISCUSSION**

DBM and its derivatives are small β-diketone compounds and are minor constituents of licorice (8). Several beneficial bioactivities of DBM, such as anti-tumor activity (21, 27, 31), anti-inflammatory activity (18), anti-mutagenesis (7), and iron chelation (44), have previously been reported. Some DBM derivatives have also been used as sunscreens because of their UVA-absorbing activities, but an inability of these derivatives to protect keratinocytes against UVA-induced cytotoxicity has also been reported (1).

In this report, we demonstrated that 14-26 (2,2'-dimethoxydibenzyolmethane), a DBM derivative, possesses neuroprotective properties against both ER stress and oxidative stress. Recent studies have revealed that the protection of cells against ER stress/ER stress-induced cell death can be obtained in several ways: enhancement of the chaperone activity in the ER (40), suppression of general protein synthesis (16), maintenance of the Ca2+ homeostasis in the ER (26), and activation of the UPR branches (4, 39). However, the regulation of ER stress by 14-26 was associated with its antioxidative property.
Comparative analysis between DBM derivatives revealed that the presence of methoxy groups in the structure may play an important role in the neuroprotective properties of 14-26 (Fig. 1, B and D).

Whereas the ER is where ROS are produced through the formation of disulfide bonds in proteins, it has weaker defense systems against oxidative stress when compared with either the cytosol or mitochondria. As a result, ER proteins become major targets for oxidative modifications such as carbonylation as described previously (9, 33). Protein carbonyls are generated by a direct metal-catalyzed oxidative reaction or by secondary reactions with reactive carbonyl compounds on carbohydrates, lipids, and advanced glycation/lipoxydation end products (32). 14-26 prevented ROS production in response to both oxidative stress and ER stress (Fig. 3) and reduced the levels of ROS-induced protein carbonyls in the microsome (Fig. 4, A and B). 14-26 also prevented the reduction of the mitochondrial membrane potential after exposure of the cells to both oxidative stress and ER stress (Fig. 4, C–E). These results suggest that 14-26 protects cells against stresses by suppressing ROS generation and ROS-derived damage in both the ER and mitochondria. Although dopaminergic neurons are highly susceptible to oxidative stress because of the presence of dopamine and its metabolites, the protective effects of 14-26 may be more general. Our preliminary results revealed that 14-26 protected mouse insulinoma MIN6 cells against both oxidative stress and ER stress, in a similar manner to SH-SYSY and PC 12 cells.

Interestingly, NAC also prevented the production of ROS in response to both oxidative stress and ER stress (Fig. 3), but, unlike 14-26, failed to protect against Tm-induced cell death in our experiments (Fig. 1B). A possible explanation for this discrepancy is the mechanism underlying the antioxidative property of each compound. 14-24 possessed the iron-chelating activity (Fig. 5A) that may play a role in its cytoprotective property (Fig. 5B). By contrast, NAC plays an important role in prevention of ROS, but it can shift the redox status of proteins to the reduced forms (6). As a result, secretory proteins in the reduced forms accumulate in the ER and cause ER stress. Another possibility is that, besides the antioxidative property, other mechanisms are involved in the prevention of ER stress-induced cell death by 14-26. In this context, our preliminary results indicated that 14-26 slightly suppresses general protein synthesis (15–20% decrease after 24h treatment), which is an alternative mechanism of regulating ER stress. However, it is not clear at this moment to what extent this slight change of the levels of general protein synthesis contributes to the cell protection by 14-26.
In vivo, 14-26 suppressed neuronal death in the SNpc and improved amphetamine-induced rotational behavior after injection of 6-OHDA into mice. The neuroprotection by 14-26 in vivo correlated with the regulation of both oxidative stress and ER stress (Fig. 7).

Total iron levels in the SNpc are increased in both PD patients and animals after administration of PD-related neurotoxins such as 6-OHDA (2) and MPTP (41). As iron can induce ROS, oxidative stress, and aggregation of α-synuclein, iron chelation is believed to have the potential as a neuroprotective strategy for PD, which requires long-term treatment (45). Unfortunately, however, some iron chelators, such as DFO, have poor penetration across the blood-brain barrier due to their hydrophilic nature; others, such as clioquinol (CQ), have high toxicity (45). Therefore, it is important to find small, hydrophobic, and nontoxic compounds with iron-chelating activity, which can be used in a long-term treatment. The nature of 14-26 and other DBM derivatives described here makes these good candidates for use in PD. 14-26 is a small hydrophobic compound, and our results suggest that 14-26 crosses the blood-brain barrier. Although it is not clear whether it counteracts 6-OHDA in the cells or predominantly in the extracellular spaces in vivo, our results in cultured cells support its ability to penetrate the cells.

It is noteworthy that curcumin ( diferuloylmethane), another β-diketone molecule and a component of turmeric, also has extracellular spaces in vivo, our results in cultured cells suggest that 14-26 crosses the blood-brain barrier. Although it is not clear whether it counteracts 6-OHDA in the cells or predominantly in the extracellular spaces in vivo, our results in cultured cells support its ability to penetrate the cells.

In summary, a DBM derivative, 14-26, was identified as a novel neuroprotective agent against both ER stress and oxidative stresses. The regulation of ER stress by 14-26 was associated with its antioxidative property. Dopaminergic neuronal death in the SNpc was reduced by 14-26 administration following injection of 6-OHDA in mice. These results suggest that 14-26 could be a therapeutic candidate for the treatment of PD.

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