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A possible novel pharmacological therapy for Parkinson's disease using the pleiotropic property of Selegiline

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ABSTRACT

Monoamine oxidase type B (MAO-B) inhibitors have neuroprotective properties in addition to their primary pharmacologic function. We examined the effects of selegiline, an irreversible MAO-B inhibitor, on the expression levels of *survivin* mRNA and lipid peroxidation in the corpus striata of 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonian mice. Mice were treated with normal saline (vehicle as control), MPTP alone (30 mg/kg of body weight), MPTP with low-dose of selegiline (0.1 mg/kg of body weight), or MPTP with high-dose of selegiline (1.0 mg/kg of body weight). A consecutive 7-day oral treatment of MPTP alone did not influence *survivin* mRNA expression. Selegiline treatment together with MPTP significantly upregulated survivin expression at both low- and high-doses. Interestingly, lipid peroxidation was significantly suppressed by a low-dose of selegiline but not by a high-dose. These results suggest that selegiline has pleiotropic effects; the upregulation effects on *survivin* mRNA expression are likely to be independent from its primary pharmacological properties (MAO-B inhibition), whereas the inhibitory effects on lipid peroxidation seem to be associated with its primary pharmacological properties. In addition to conventional dopamine supplementation therapy, selegiline might serve as a novel pharmacological therapeutic strategy for Parkinson's disease through its antiapoptotic properties via survivin induction as well as its antioxidative properties.

KEYWORDS: survivin, neuroprotection, selegiline, Parkinson's disease, lipid peroxidation

INTRODUCTION

Parkinson's disease (PD) is a major neuro-degenerative disorder characterized by akinesia, muscular rigidity, rest tremor, and postural instability [1]. The loss of striatonigral dopaminergic (DAergic) neurons, which originate in the substantia nigra (SN) and terminate in the corpus striatum (CS), plays a major pathological role in the motor symptoms of this disorder [1]. Although the fundamental mechanism of neuronal degeneration remains unresolved, oxidative stress, mitochondrial dysfunction, and/or apoptotic processes are assumed to be involved [1-4]. Iron ions in the SN have been attributed to provoke a form of oxidative stress known as Fenton-like reactions [1-4]. The supplementary administration of dopamine (DA) and/or DA agonists has been a reasonable pharmacological therapy in view of the pathology of this disease [1]. Recent advances have revealed that monoamine oxidase type B (MAO-B) inhibitors as well as DA agonists have neuroprotective functions in addition to their

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primary pharmacological properties [5, 6]. This pleiotropic property highlights the value of MAO-B inhibitors and might influence treatment strategies for PD.

The mechanism of neuroprotection falls under the following three categories: antioxidative properties, antiapoptotic properties, and neurotrophic properties. The neuroprotective function of MAO-B inhibitors is ascribed to these properties [7, 8]. Survivin is a protein that is present in the cytoplasm, nucleus, and mitochondria, modulating the cell cycle at the G_2/M phase [9-14]. Survivin is a member of the inhibitor of the apoptosis protein (IAP) family and suppresses caspase activities, which serve as the executioners of the final apoptotic pathway, and result in cell survival and/or cell proliferation [9-14]. The expression of survivin is higher in immature cells and tumor cells than in mature cells [9-14]. Survivin is critically required for the survival of developing CNS neurons [15, 16]. Therefore, we examined the effects of selegiline, an irreversible MAO-B inhibitor approved for clinical use, on *survivin* mRNA expression in the CS in a mouse model for Parkinson's disease produced using 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) treatment.

MATERIALS AND METHODS

Animals and preparation of rodent model for PD using MPTP treatment

Twelve 8-week-old C57/6J mice were purchased from Sankyo Laboratory Service (Tokyo, Japan) for use in the present study. The mice were kept under standard conditions: a 12-h on/off light cycle, a room temperature between 22 ºC and 25 ºC, and food and water *ad libitum*. The mice were randomly assigned to the following 4 groups (3 each): 1) a control (vehicle) group, 2) an MPTP (30 mg/kg of body weight) alone group, 3) an MPTP (30 mg/kg of body weight) with high-dose (1.0 mg/kg of body weight) selegiline group, and 4) an MPTP (30 mg/kg of body weight) with low-dose (0.1 mg/kg of body weight) selegiline group. The drugs were dissolved in normal saline as a vehicle. Each group of mice received a daily administration of a single dose of normal saline (control group) or one of the above-mentioned drug solutions via a gastric tube for 7 consecutive days. Seven days were sufficient to enable the development of pathological changes in DAergic neurons in the CS, as shown in previous studies [17-23]. Thereafter, all the mice were killed by decapitation under intraperitoneal pentobarbital sodium (40-50 mg/kg of body weight) anesthesia 7 days after the last administration of normal saline (control group) or drug(s) (MPTP alone group, MPTP with low-dose selegiline group, and MPTP with high-dose selegiline group) [17-23]. The whole brains were immediately removed, weighed, and placed on a glass plate over crushed ice; the corpus striata were then dissected. The ipsilateral sides were used for LPO (lipid peroxide) determination, and the other sides were frozen at -80 ºC for subsequent use in the *survivin* mRNA measurements.

The present study was approved by the Ethical Committee for Laboratory Animal Care and Use at the School of Pharmacy, Nihon University.

Confirmation of PD-like disease in MPTP-treated mice

We confirmed the development of a PD-like disease using kinesiological parameters as surrogate markers for a histological study (neuronal degeneration at SN) or a biochemical study (DA depletion at CS), as described elsewhere [23]. The horizontal and vertical motor activities of the mice were measured using an infrared beam test using Digiscan (Omnitech Electronics Inc., Columbus, OH) before and after drug administration, as described previously [23-25].

Chemicals

Selegiline was kindly donated by Fujimoto Pharmacy (Tokyo, Japan). Ethidium bromide, diethylpyrocarbonate (DEPC), and MPTP were obtained from Sigma (St. Louis, MO). The total RNA isolation reagent (TRIzol), Moloney murine leukemia virus reverse transcriptase, *Thermus aquaticus* DNA polymerase, oligo (dT) primer, dNTP (2'-deoxynucleotide 5'-triphosphate), DTT (dithiothreitol), 10 × PCR buffer (200 mM Tris-HCl (pH 8.0), and 500 mM KCl), and electrophoretic -grade agarose were purchased from Invitrogen Life Technologies (Carlsbad, CA).

Survivin measurement

The expression of *survivin* mRNA was measured using a semi-quantitative reverse-transcription

polymerase reaction (RT-PCR). Total RNA was isolated from the brain samples and the following RT reactions were performed, as described previously [23, 25, 26]. The primers for mouse *survivin* and *glyceroaldehyde-3-phosphate* (GAPDH) were obtained from TaKaRa Bio (Gunma, Japan). The sequences of the primers were as follows: *survivin*: 5'-ATC GCC ACC TTC AAG AAC TG-3' (forward) and 5'-TGA CTG ACG GGT AGT CTT TGC-3' (reverse) [13] and GAPDH: 5'-TAT TGG GCG CTT GGT CAC CA-3' (forward) and 5'-CCA CCT TCT TGA TGT CAT CA-3' (reverse). The predicted sizes of the amplified products (cDNA) were 348 bp for *survivin* and 752 bp for GAPDH [23, 25, 26], respectively.

PCR was performed with denaturation at 94 ºC for 45 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 1.5 min. The last extension was 10 min. Within a logarithmic linear range, 30 cycles were used to determine the *survivin*/GAPDH ratio [13]. The cDNAs were electrophoresed on an agarose gel and were visualized using ethidium bromide staining. The density of the cDNA bands was determined using Scion Image Software (Frederick, MD). The relative amount of *survivin* mRNA standardized against the amount of GAPDH mRNA was calculated. The values of the MPTP alone group, the MPTP with high-dose selegiline group, and the MPTP with low-dose selegiline group were standardized against the value of the control group.

LPO measurement

The LPO concentration was determined using the thiobarbituric acid (TBA) method, as described elsewhere [23, 25-27]. Briefly, the brain samples were homogenized in ice-cold PBS. All the subsequent steps were performed using amber-colored tubes. The homogenized samples were added to 40 mM sulfuric acid and 10% phosphotungstic acid and then centrifuged at 1,500 x g for 10 min. A TBA agent containing 8.8 M acetic acid and 20 mM TBA was then added. The samples were incubated in a boiling bath for 1 h. After cooling with tap water, 1-butanol was added to extract malondialdehyde, and the mixture was shaken vigorously by hand. After centrifugation at 1,500 x g for 10 min, the 1-butanol phase was saved. The absorbance of the supernatant (1-butanol-phase) was measured using a spectrofluorometer (FP-6200; Nihon Bunko, Tokyo, Japan) at an excitation wavelength of 515 nm and an emission wavelength of 553 nm. The LPO level was estimated using 1,3,3-tetraetoxypropane as a reference standard. The results were expressed as nanomoles of malondialdehyde reactive substances per gram of wet tissue weight.

Statistical analysis

All the values were expressed as the mean ± S.E.M. The motor activities among the 4 groups before or after drug treatment were assessed using a one-way ANOVA (analysis of variance), while the motor activities of each group of mice before and after drug treatment were analyzed using a paired t-test. The body-weight change before and after drug treatment was also assessed using a paired t-test. The brain weight, *survivin* mRNA expression, and LPO levels were analyzed using a one-way ANOVA. A Tukey-Kramer post-hoc test was used if the ANOVA table produced a P value < 0.05.

RESULTS

Motor activity changes

A summary of the horizontal motor activities of the mice is shown in Figure 1. The mice activities in all 4 groups showed a tendency to decrease after normal saline or drug(s) treatment. However, only the MPTP treatment (MPTP alone group, MPTP with low-dose selegiline group, and MPTP with high-dose selegiline group) significantly reduced the activities. The vertical motor activities showed the same trend as the horizontal activities (data not shown).

Body weight change and brain weight

No significant changes in body weight were observed after normal saline or drug(s) treatment among the 4 groups (Figure 2). Furthermore, no significant changes in brain weight were observed after normal saline or drug(s) treatment (Figure 3).

Expression of *survivin* mRNA in the corpus striatum

The expression of *survivin* mRNA is shown in Figure 4. Interestingly, treatment with MPTP

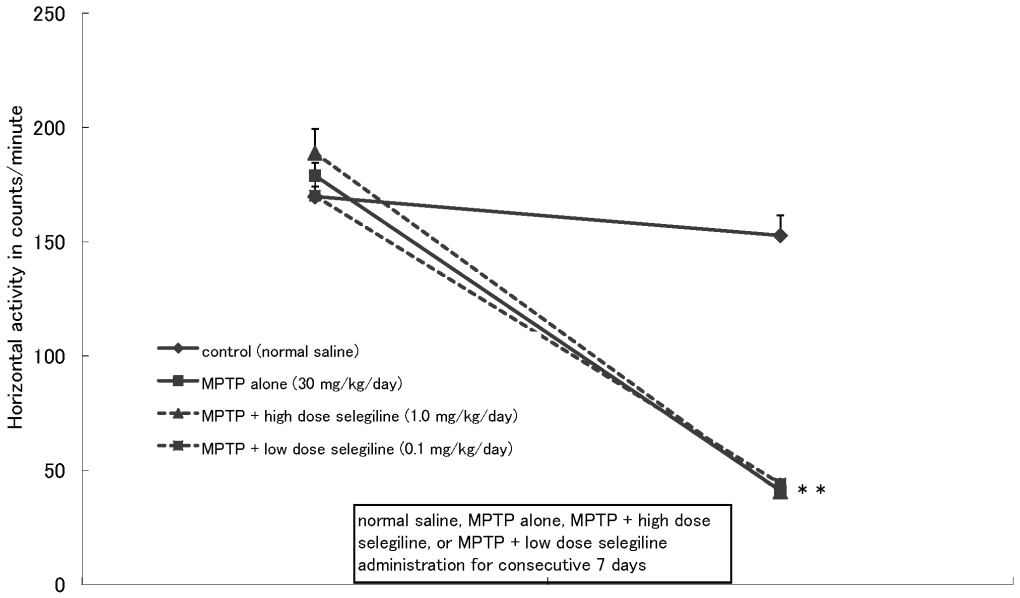

Figure 1. Horizontal motor activities of mice. No significant differences in motor activities were observed among the 4 groups at the time of group allocation (P = 0.629, using a one-way ANOVA). No significant decrease in activity was detected in the control group (P = 0.321, using a paired t-test). The activities were significantly decreased after the administration of MPTP for 7 consecutive days in all the groups (treated with MPTP alone group, MPTP with high-dose selegiline group, and low-dose selegiline group) according to a paired t-test (**P < 0.01). MPTP: 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine.

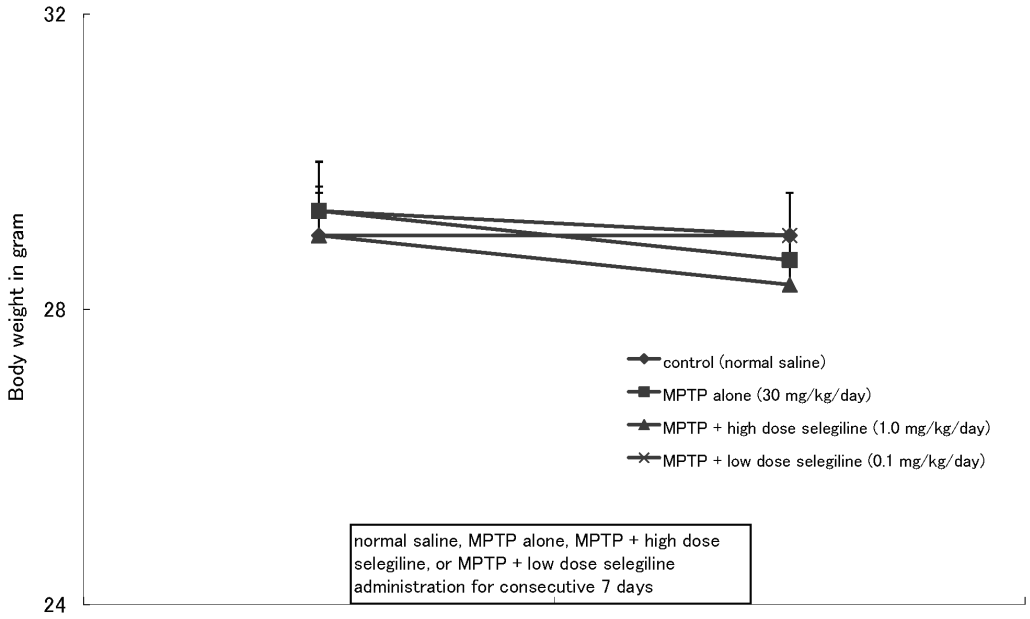

Figure 2. Body weight change. At allocation, no significant differences in body weight were observed among the 4 groups (P = 0.945, using a one-way ANOVA). No significant body weight changes were observed after any of the treatments, according to a paired t-test (P = 1.000 for control, P = 0.184 for MPTP alone group, P = 0.529 for MPTP with high-dose selegiline group, and P = 0.423 for MPTP with low-dose selegiline group). MPTP: 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine.

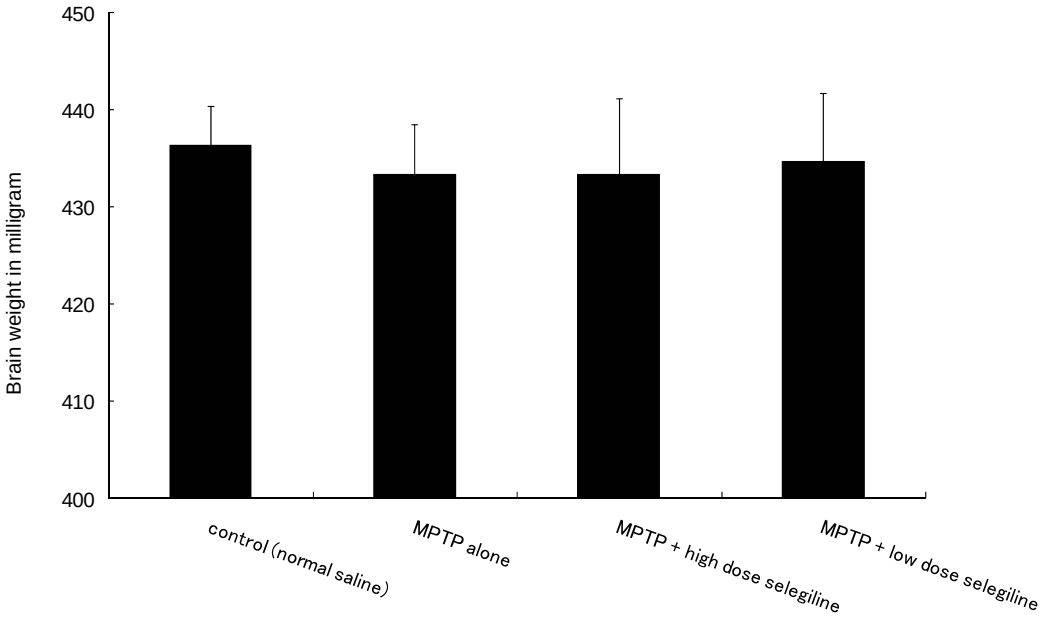

Figure 3. Brain weight differences. No differences in the whole brain weight were observed among the 4 groups (P = 0.920, using a one-way ANOVA).

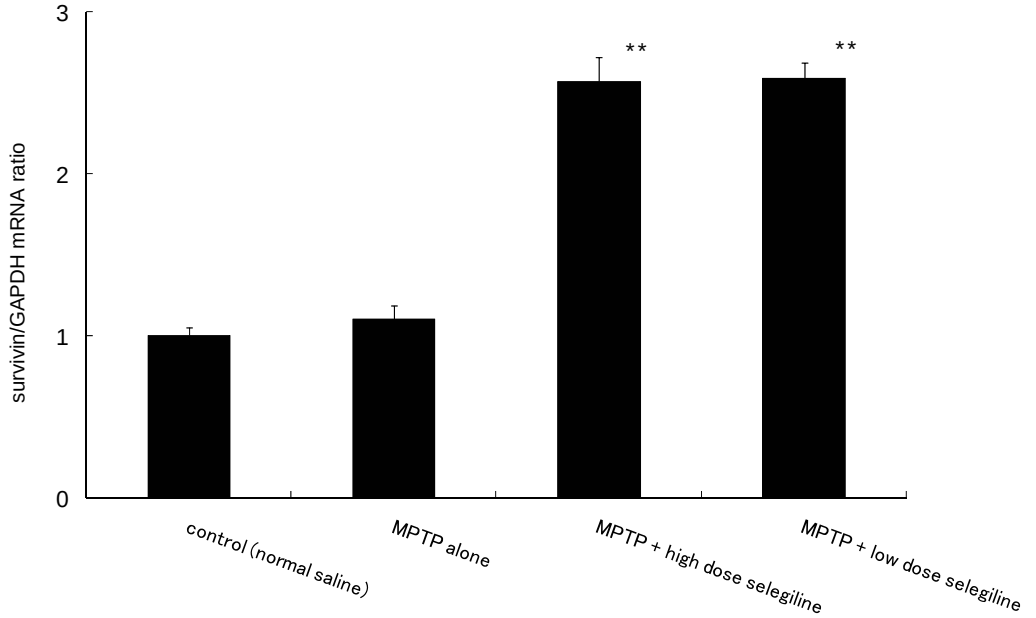

Figure 4. *Survivin* mRNA expression in the corpus striatum. The relative expression of survivin in each group was standardized against that of the control. Survivin expression differed significantly among the 4 groups ($P = 3.60 \times 10^{-6}$, using a one-way ANOVA). The administration of MPTP alone (30 mg/kg of body weight for 7 consecutive days) did not influence survivin expression. However, the administration of both MPTP with high-dose (1.0 mg/kg) and low-dose (0.1 mg/kg) selegiline treatment for 7 consecutive days significantly upregulated survivin expression (**P < 0.01, using a Tukey-Kramer post-hoc test). The degree of upregulation was similar between the MPTP with high-dose selegiline group and the MPTP with low-dose selegiline group. MPTP: 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine.

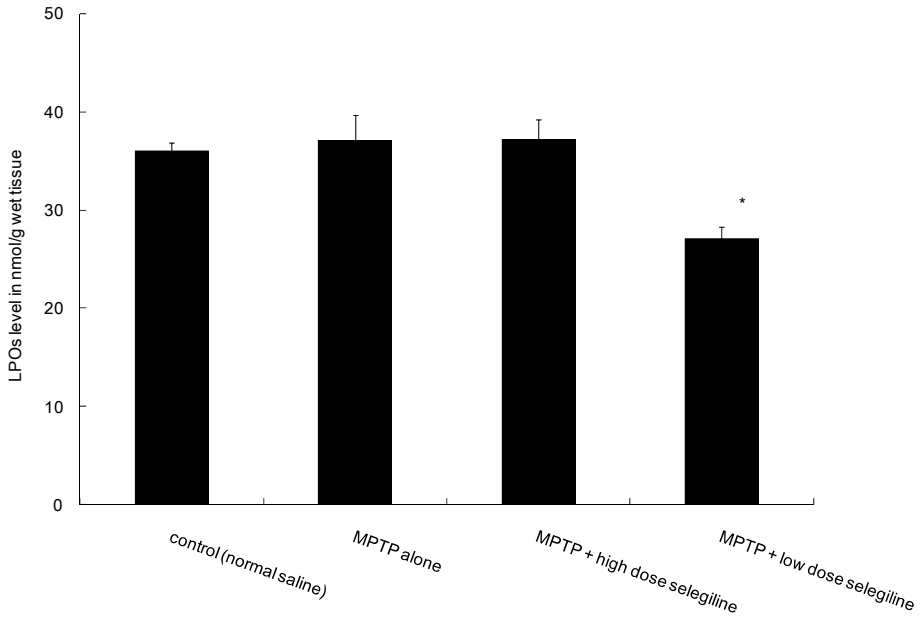

Figure 5. Lipid peroxide levels in the corpus striatum. The lipid peroxide (LPO) levels, which was assessed by measuring malondialdehyde reactive substances, was significantly decreased after the administration of MPTP with low-dose selegiline (0.1 mg/kg) ($P = 0.011$, using a one-way ANOVA and $*P < 0.05$, using a subsequent Tukey-Kramer post-hoc test). No changes in the levels of LPO were found after the administration of MPTP with high-dose selegiline (1.0 mg/kg) or after the administration of MPTP alone. MPTP: 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine.

alone did not influence *survivin* mRNA expression; the expression level in the group treated with MPTP alone was similar to that in the control group. Meanwhile, the administration of either low- or high-doses of selegiline with MPTP markedly upregulated *survivin* mRNA expression. The degree of upregulation was similar in both low- and high-dose groups.

Concentration of lipid peroxides in the corpus striatum

The LPO levels in the CS are shown in Figure 5. Treatment with MPTP alone did not significantly alter the LPO levels. Interestingly, MTPT treatment with low-dose (0.1 mg/kg) selegiline significantly suppressed LPOs formation but high-dose (1.0 mg/kg) treatment did not influence the LPO levels, which remained at the same level as that in the control group and the MPTP alone group.

DISCUSSION

Clinical studies have suggested that MAO-B inhibitors exert neuroprotective properties apart from their primary and adjunctive pharmacological effects on DA [28-31]. The mechanism of neuroprotection can largely be classified into antioxidative properties, antiapoptotic properties, and neurotrophic properties. How MAO-B inhibitors exert their antiapoptotic property has not been well clarified. In past studies, the antiapoptotic effects of selegiline have been shown to be associated with the modulation of gene expression [32-35]. The upregulation of antiapoptotic *Bcl-2* and *Bcl-XI* and the down regulation of proapoptotic *Bad*, *Bax*, *PARP*, and *caspase*3 have been reported [7, 8, 32-37]. Selegiline prevents the progressive reduction of the mitochondrial membrane potential in preapoptotic neurons [32-35]. The antiapoptotic effect of selegiline is associated with the propagylamine moiety, which protects mitochondrial viability and the mitochondrial permeability transition pore by activating *Bcl-2* and down regulating the *Bax* family of proteins [36]. These antiapoptotic effects of selegiline have been shown to be independent from its MAO-B inhibitory property [32-35].

However, the effect of selegiline on IAPs has not yet been investigated. In the present study,

we found that the antiapoptotic property of selegiline is involved in the upregulation of survivin (a member of the IAP family) mRNA expression in the CS during the final apoptotic process. Selegiline is not an absolute selective MAO-B inhibitor, but the higher its administration dose the lower its MAO-B selectivity [30]. In the present study, however, both a high dose (1.0 mg/kg) and a low dose (0.1 mg/kg) of selegiline administered together with MPTP upregulated *survivin* mRNA expression to the same degree (Figure 4). Taking the above facts into account, the effect of selegiline on survivin mRNA expression is likely to be independent from its MAO-B inhibitory property. Crocker *et al.* demonstrated that the over-expression of another IAP family member, X-linked inhibitor of apoptosis protein (XIAP), protected a mouse model of PD that was induced by MPTP administration [38]. These previous results together with the present results suggest that selegiline has a novel effect on survivin, a member of the IAP family, protecting against neuronal apoptosis and promoting neuronal survival. He *et al.* also observed a neural protective effect of selegiline (30 mg/kg) against MPTP in mouse brain; however, whether this effect was caused by an antioxidative or an antiapoptotic mechanism was uncertain [39].

Another noteworthy point of the present study is that a low dose (0.1 mg/kg), but not a high dose (1.0 mg/kg), of selegiline suppressed the formation of LPOs in the CS (Figure 5). In view of the dose-dependent MAO-B inhibitory effect of selegiline [30], the antioxidative function of selegiline seems to be, unlike its antiapoptotic property, associated with MAO-B inhibition. The antioxidative effects of selegiline were previously demonstrated *in vivo* [40-43] and *in vitro* [44-48]. Previous experiments revealed that selegiline at a dose of 10 mg/kg, which is 10-fold higher than the high dose used in the present study, induced superoxide dismutase (SOD) and catalase activities, increased the glutathione (GSH) concentration, and suppressed the formation of LPOs in the brains of rodents [47, 48]. However, Carrilo *et al.* reported that a dose of 0.5 mg/kg was sufficient to induce SOD activities [46]. Selegiline induced SOD and catalase more efficiently than glutathione peroxidase (GSH-Px) [44, 47]. Moreover, the ability of selegiline to increase SOD activities seemed to be tissue and brain region specific [45]. Selegiline, even at a low dose (0.1 mg/kg), might induce antioxidative agents other than SOD, catalase, GSH, and GSH-Px, such as metallothionein, which has been shown to exert neuroprotective effects during 6-hydroxydopamine (6-OHDA)-induced striatal neural injury [49]. Although the most efficient dose of selegiline required to exert an antioxidative effect should be further examined, the antioxidative of selegiline is likely to be promising.

DA and/or DA agonists have been the standard pharmacological therapy for Parkinson's disease [1]. In addition to the original pharmacological property of selegiline, which augments the properties of DA through its effect on monoamine oxidase activity, the available evidence suggests that the pleiotropic properties of selegiline could serve as a novel therapeutic strategy for PD through its antiapoptotic properties via survivin induction in addition to its antioxidative properties.

CONCLUSION

Selegiline administration might serve as a novel pharmacological therapeutic strategy for Parkinson's disease through its antiapoptotic properties via survivin induction as well as its antioxidative properties.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

REFERENCES

1. Fahn, S. 1995, Merritt's Textbook of Neurology, L. P. Rowland (Ed.), Williams and Wilkins, Philadelphia, 713.
2. Dauer, W. and Przedborski, S. 2003, Neuron, 39, 989.
3. Beal, M. F. 2003, Ann. Neurol., 53(Suppl. 3), S39.
4. Siderowf, A. and Stern, M. 2003, Ann. Intern. Med., 138, 651.
5. Chiueh, C. C., Andoh, T., Lai, A. R., Lai, E. and Krishna, G. 2000, Neurotox. Res., 2, 293.
6. Foley, P., Gerlach, M., Youdim, M. B. H. and Riederer, P. 2000, Parkinsonism Relat. Disord., 6, 25.

7. Naoi, M. and Maruyama, W. 2010, Curr. Pharm. Des., 16, 2799.
8. Inaba-Hasegawa, K., Akao, Y., Maruyama, W. and Naoi, M. 2013, J. Neural Transm., 120, 435.
9. Deveraux, Q. L. and Reed, J. C. 1999, Genes Dev., 13, 239.
10. Conway, E. M., Pollefeyt, S., Comelissen, J., DeBaere, I., Steiner-Mosonyi, M., Ong, K., Baens, M., Collen, D. and Schuh, A. 2000, Blood, 95, 1435.
11. Shin, S., Sung, B.-J., Cho, Y.-S., Kim, H.-J., Ha, N.-C., Hwang, J.-I., Chung, C.-W., Jung, Y.-K. and Oh, B.-H. 2001, Biochemistry, 40, 1117.
12. Kiechle, F. L. and Zhang, X. 2002, Clin. Chim. Acta, 326, 27.
13. Kawamura, K., Sato, N., Fukuda, J., Kodama, H., Kumagai, J., Tanikawa, H., Shimizu, Y. and Tanaka, T. 2003, Dev. Biol., 256, 331.
14. Lu, B., Mu, Y., Cao, C., Zeng, F., Schneider, S., Tan, J., Price, J., Chen, J., Freeman, M. and Hallahan, D. E. 2004, Cancer Res., 64, 2840.
15. Jiang, Y., de Bruin, A., Caldas, H., Fangusaro, J., Hayes, J., Conway, E. M., Robinson, M. L. and Altura, R. A. 2005, J. Neurosci., 25, 6962.
16. Iscru, E., Ahmed, T., Coremans, V., Bozzi, Y., Caleo, E. M., D'Hooge, R. and Balschun, D. 2013, Neuroscience, 231, 413.
17. Heikkila, R. E., Cabbat, F. S., Manzino, L. and Duvoisin, R. C. 1984, Neuropharmacology, 23, 711.
18. Gerlach, M., Riederer, P., Przuntek, H. and Youdim, M. B. 1991, Eur. J. Pharmacol., 208, 273.
19. Rįos, C., Alvarez-Vega, R. and Rojas, P. 1995, Pharmacol. Toxicol., 76, 348.
20. Rojas, P. and Rįos, C. 1997, Neurochem. Res., 22, 17.
21. Rojas, P. and Klaassen, C. D. 1999, Neurosci. Lett., 273, 113.
22. Rojas, P., Rojas-Castañeda, J., Vigueras, R. M., Habeebu, S. S., Rojas, C., Rįos, C. and Ebadi, M. 2000, Neurochem. Res., 25, 503.
23. Ono, S. I., Hirai, K. and Tokuda, E. 2009, Biol. Pharm. Bull., 32, 1813.
24. Ono, S. I., Endo, Y., Tokuda, E., Tabata, K., Asami, S., Ito, Y. and Suzuki, T. 2007, Biol. Trace Elem. Res., 113, 93.
25. Tokuda, E., Ono, S. I., Ishige, K., Naganuma, A., Ito, Y. and Suzuki, T. 2007, Toxicology, 229, 33.
26. Ono, S. I., Ishizaki, Y., Tokuda, E., Tabata, K., Asami, S. and Suzuki, T. 2007, Biol. Trace Elem. Res., 115, 147.
27. Ono, S. I., Cai, L. and Cherian, M. G. 1998, Radiat. Res., 150, 52.
28. Ives, N. J., Stowe, R. L., Marro, J., Counsell, C., Macloed, A., Clarke, C. E., Gray, R. and Wheatley, K. 2004, BMJ, 329, 593.
29. Riederer, P. and Lachenmayer, L. 2003, J. Neural Transm., 110, 1273.
30. Youdim, M. B. and Riederer, P. F. 2004, Neurology, 63(Suppl. 2), S32.
31. Schapira, A. H. and Olanow, C. W. 2004, JAMA, 291, 358.
32. Tatton, W. G. and Greenwood, C. E. 1991, J. Neurosci. Res., 30, 666.
33. Tatton, W. G., Seniuk, N. A., Ju, W. H. Y. and Ansari, K. S. 1993, Monoamine oxidase inhibitors in neurological diseases, A. Lieberman, W. Olanow, M. Youdim, and K. Tipton (Ed.), Raven, New York, 217.
34. Wu, R. M., Murphy, D. L. and Chiueh, C. C. 1995, J. Neural Transm. Gen. Sect., 100, 53.
35. Tatton, W. G. and Chalmers-Redman, R. M. 1996, Neurology, 47(Suppl. 3), S171.
36. Youdim, M. B., Maruyama, W. and Naoi, N. 2005, Drugs Today (Barc.), 41, 369.
37. Youdim, M. B., Edmondson, D. and Tipton, K. F. 2006, Nat. Rev. Neurosci., 7, 295.
38. Crocker, S. J., Liston, P., Anisman, H., Lee, C. J., Smith, P. D., Earl, N., Thompson, C. S., Park, D. S., Korneluk, R. G. and Robertson, G. S. 2003, Neurobiol. Dis., 12, 50.
39. He, X. J., Uetsuka, K. and Nakayama, H. 2008, Neurotoxicology, 29, 1141.
40. Koutsilieri, E., Chen, T.-S., Rausch, W.-D. and Riederer, P. 1996, Eur. J. Pharmacol., 306, 181.
41. Mytilineou, C., Radcliffe, P., Leonardi, E. K., Werner, P. and Olanow, C. W. 1997, J. Neurochem., 68, 33.

42. Mytilineou, C., Leonardi, E. K., Radcliffe, P., Heinonen, E. H., Han, S.-K., Werner, P., Cohen, G. and Olanow, C. W. 1998, J. Pharmacol. Exp. Ther., 284, 700.
43. Chetsawang, B., Kooncumchoo, P., Govitrapong, P. and Ebadi, M. 2008, Neurochem. Int., 53, 283.
44. Carrillo, M. C., Kanai, S., Nokubo, M. and Kitani, K. 1991, Life Sci., 48, 517.
45. Carrillo, M. C., Kitani, K., Kanai, S., Sato, Y. and Ivy, G. O. 1992, Life Sci., 50, 1985.
46. Carrillo, M. C., Kanai, S., Sato, Y., Nokubo, M., Ivy, G. O. and Kitani, K. 1993, Life Sci., 52, 1925.
47. Thiffault, C., Aumont, N., Quirion, R. and Poirier, J. 1995, J. Neurochem., 65, 2725.
48. Leret, M. L., San Millán, J. A., Fabre, E., Gredilla, R. and Barja, G. 2002, Toxicology, 170, 165.
49. Ebadi, M., Ramana Kumari, M. V., Hiramatsu, M., Hao, R., Pfeiffer, R. F. and Rojas, P. 1998, Neurol. Neurosci., 12, 103.