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Oxidative Stress in Mitochondria

Decision to Survival and Death of Neurons in Neurodegenerative Disorders

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Abstract

In mitochondria, oxidative phosphorylation and enzymatic oxidation of biogenic amines by monoamine oxidase produce reactive oxygen and nitrogen species, which are proposed to cause neuronal cell death in neurodegenerative disorders, including Parkinson's and Alzheimer's disease. In these disorders, mitochondrial dysfunction, increased oxidative stress, and accumulation of oxidation-modified proteins are involved in cell death in definite neurons. The interactions among these factors were studied by use of a peroxynitrite-generating agent, *N*-morpholino sydnonimine (SIN-1) and an inhibitor of complex I, rotenone, in human dopaminergic SH-SY5Y cells. In control cells, peroxynitrite nitrated proteins, especially the subunits of mitochondrial complex I, as 3-nitrotyrosine, suggesting that neurons are exposed to constant oxidative stress even under physiological conditions. SIN-1 and an inhibitor of proteasome, carbobenzoxy-L-isoleucyl-γ-*t*-butyl-L-alanyl-L-leucinal (PSI), increased markedly the levels of nitrated proteins with concomitant induction of apoptosis in the cells. Rotenone induced mitochondrial dysfunction and accumulation and aggregation of proteins modified with acrolein, an aldehyde product of lipid peroxidation in the cells. At the same time, the activity of the 20S β-subunit of proteasome was reduced significantly, which degrades oxidative-modified protein. The mechanism was proved to be the result of the modification of the 20S β-subunit with acrolein and to the binding of other acrolein-modified proteins to the 20S β-subunit.

Increased oxidative stress caused by SIN-1 treatment induced a decline in the mitochondrial membrane potential, $\Delta\Psi m$, and activated mitochondrial apoptotic signaling and induced cell death in SH-SY5Y cells. As another pathway, p38 mitogen-activated protein (MAP) kinase and exracellular signal-regulated kinase (ERK) mediated apoptosis induced by SIN-1. On the other hand, a series of neuroprotective propargylamine derivatives, including rasagiline [*N*-propargyl-1*(R)*aminoindan] and (–)deprenyl, intervened in the activation of apoptotic cascade by reactive oxygen species–reactive nitrogen species in mitochondria through stabilization of the membrane

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potential, ΔΨm. In addition, rasagiline induced antiapoptotic Bcl-2 and glial cell line-derived neurotrophic factor (GDNF) in SH-SY5Y cells, which was mediated by the ERK–nuclear factor (NF)-κB pathway. These results are discussed in relation to the interaction of oxidative stress and mitochondria in the regulation of neuronal death and survival in neurodegenerative diseases.

Index Entries: Oxidative stress; mitochondria complex I; proteasome; 3-nitrotyrosine; acrolein; Parkinson's disease; apoptosis; rasagiline; transcription factors.

Oxidative-Modified Protein as the Marker of Oxidative Stress

Oxidative stress has been proposed as one of the major causes inducing neuronal death in aging and age-associated disorders *(1)*, and mitochondria produce most of reactive oxygen and nitrogen species (ROS and RNS, respectively) in the cells. The superoxide anion radical is generated by oxidative phosphorylation in mitochondria and reacts with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$), one of the most potent radicals. On the other hand, oxidation of biogenic amines by monoamine oxidase in the mitochondrial outer membrane generates hydrogen peroxide. Mitochondria are now considered to play a pivotal role in apoptosis *(2)*, which is the common death type of neurons in Parkinson's (PD) and Alzheimer's diseases (AD) *(3)*. The role of mitochondria in the process of apoptotic commitment is recognized, and impairment of energy charge and redox, permeability transition (PT), disruption of membrane potential (ΔΨm), and release of cytochrome-*c* are observed prior to fragmentation of nuclear DNA, a hallmark of apoptotic morphological features.

Neurodegenerative disorders are characterized by a decline of specified neurons in selected brain regions, associated with protein deposits specific for each disease. In PD, dopamine neurons in the substantia nigra degenerate progressively with formation of Lewy bodies (LBs). The pathogenesis of PD remains as an enigma, but PD is considered a consequence of various genetic and environmental interactions. The vulnerability of the neurons might be the result of increased generation of ROS and RNS, reduced antioxidant capacity, high contents of iron and dopamine, and mitochondrial dysfunction in nigral dopamine neurons. ROS and RNS generated in mitochondria modify bioactive molecules, such as lipids, proteins, DNA, and carbohydrates, either directly or indirectly with peroxidation products of lipid or carbohydrates. 4-Hydroxynonenal (4-HNE) and acrolein, aldehyde products of lipid peroxidation, are cytotoxic, and aldehyde-modified proteins were increased in dopamine neurons of the nigro-striatum in PD *(4)*, neurofibrillary tangles in AD *(5)*, and the spinal cord in amyotrophic lateral sclerosis (ALS) *(6)*. Figure 1 shows the accumulation of 4-HNE-modified protein in the substantia nigra of parkinsonian brain. Dopamine neurons containing neuromelanin were selectively modified with 4-HNE more markedly in the PD brain than those in the control or in cells other than dopamine neurons. This suggests the essential role of dopamine in increased oxidative stress.

One of the most active RNS, $ONOO^-$, nitrates sulfhydryl and hydroxyl residues in cysteine, methionine, phenylalanine, and tyrosine, and the modification inactivates the membrane function and key enzymes *(7)*. 3-Nitrotyrosine (3-NT) is synthesized by nitration of tyrosine residues in protein and a marker for the oxidative stress induced by $ONOO^-$ in vivo *(8)*. An increase in 3-NT-containing protein (3-NT protein) was observed in atherosclerosis *(9)*, ALS *(6)*, AD *(10)*, and PD *(11)*. Another oxidation product of tyrosine is dityrosine, which is produced from free and protein-bound tyrosine in the presence of hydrogen peroxide and myeroperoxidase and was detected in atherosclerotic plaques and lipofuscin pigments in the aged human brain *(12)*.

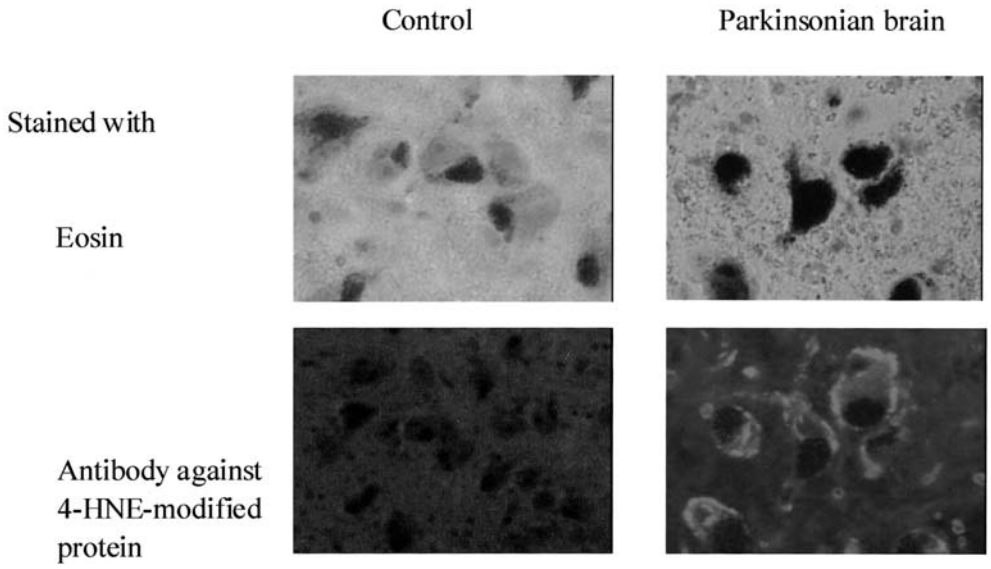

Fig. 1. 4-HNE-modified protein in the substantia nigra of the parkinsonian brain. In dopamine neurons containing neuromelanin, proteins stained with the anti-4-HNE antibody increased in the parkinsonian brain, but were not detected in the age-matched control.

Oxidative modification produces aggregated and crosslinked proteins, which are resistant to proteolytic degeneration and difficult to removed from cells. Accumulation of modified proteins might impact on a variety of cellular functions by changing the enzymatic, regulatory, and transporting potencies of specific proteins, in addition to occupying space in the limited cellular volume. The level of oxidized proteins might reflect the balance between the generation of ROS-RNS and degradation of modified protein, in which the ubiquitin–proteosome system plays a key role *(13),* as discussed in the following sections.

Mitochondrial Complex I Subunits Are Major Targets of $ONOO^-$

In the brain, NO has been thought to be produced in microglia and astrocytes and transported to neurons, where it reacts with superoxide, yielding $ONOO^-$. However, NO is synthesized also *in situ* in the neurons and functions as a neuromodulator *(14).* Using an antibody against 3-NT protein *(15),* nitrated proteins were detected in control brains. In brains from AD and PD patients, the same proteins stained with anti-3NT antibody as in control increased markedly, indicating that $ONOO^-$ preferentially modified selective proteins in neuronal cells under physiological and pathological conditions. Figure 2 shows the presence of 3-NT protein in control SH-SY5Y cells almost at the same levels as in the cells treated with $ONOO^-$-generating *N*-morpholino sydnomine (SIN-1), indicating that the cells are exposed to constant oxidative stress. However, the control cells are intact in growth and proliferation, and under physiological conditions, an active mechanism functions to eliminate modified protein from the cells and to protect cellar function in neuronal cells.

As shown in Fig. 3, the presence of nitrated protein in control cells was confirmed further by the Western blot analysis of the subcellular fractions of SH-SY5Y cells. In mitochondrial fraction, 3-NT protein was detected in the subunits of complex I stained with that against mitochondria complexes I, II, III, and IV,

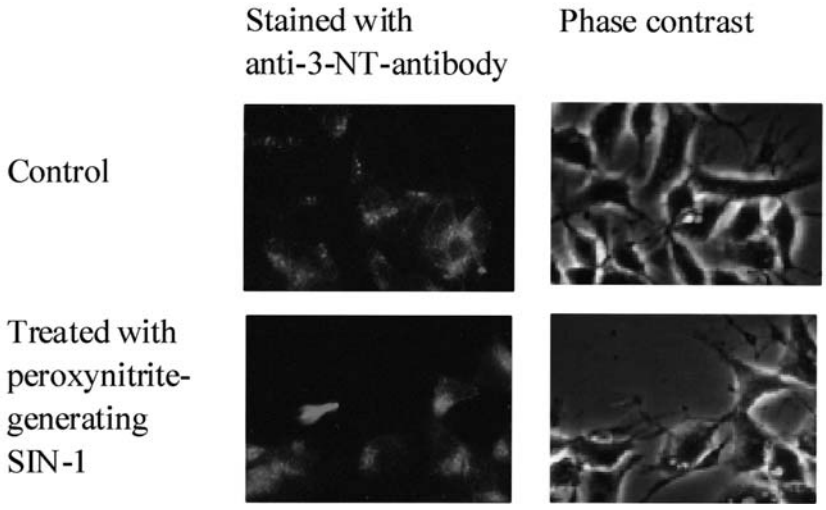

Fig. 2. 3-NT protein detected in control SH-SY5Y cells and cells treated with $ONOO^-$-generating SIN-1. In cytoplasm, 3-NT proteins were detected by staining with anti-3-NT antibody *(15).* Note that the 3-NT protein was detected also in the control.

respectively *(16),* (Fig. 3). These results suggest that complex I subunits are nitrated preferentially and the modification might contribute to mitochondrial dysfunction observed in the nigro-striatum of the parkinsonian brain *(17).*

$ONOO^-$-generating SIN-1 induced apoptosis in SH-SY5Y cells *(14,18–20)* and inhibited ATP synthesis in mitochondria. The inhibition might be the result of the binding of NO and $ONOO^-$ to cytochrome oxidase or inactivation of complexes II and III and ATPase *(21).* However, SIN-1 treatment did not increase 3-NT protein as markedly. These seemingly contradicting results indicate again that the levels of oxidative stress and oxidative-modified protein are regulated by the degradation rate rather than the production of ROS-RNS.

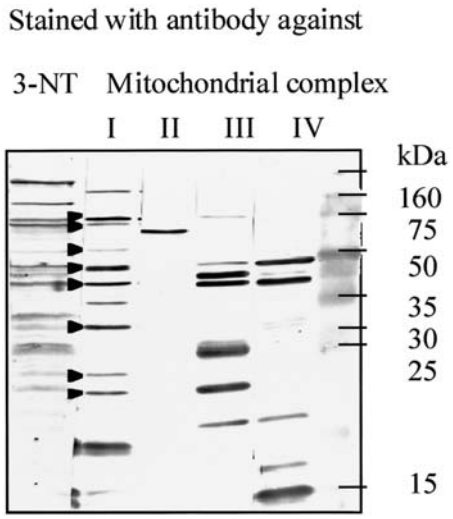

Fig. 3. 3-NT proteins in the subunits of mitochondrial complex I. Mitochondria were prepared from SH-SY5Y cells and subjected to Western blot analysis using antibody against 3-NT protein or mitochondrial complex I, II, III, and IV, respectively.

Proteasome Plays a Key Role in Accumulation of Oxidized Proteins

Insoluble protein aggregates, such as LBs in PD and senile plaques composed of β-amyloid in AD, are hallmarks of neurodegeneration. However, it remains to be clarified whether protein aggregates cause neuronal cell death directly or are the results of deteriorated cellular homeostasis in dying neurons. Protein aggregation is considered a manifestation of

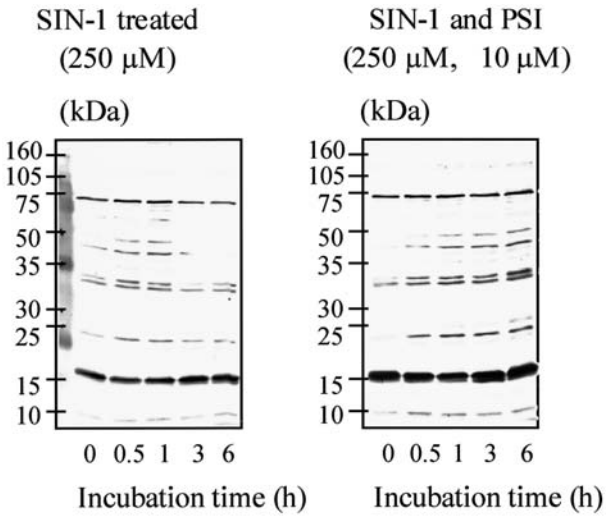

Fig. 4. Effects of $ONOO^-$-generating SIN-1 and a proteasome inhibitor, PSI, on 3-NT levels in SH-SY5Y cells. After incubated for 0.5, 1, 3, and 6 h, the cells were subjected to the immunoblotting analysis with anti-3-NT antibody. PSI increased 3-NT in the same proteins as in control and SIN-1-alone-treated cells.

disturbed cellular protein-folding homeostasis maintained by the ubiquitin–proteasome system. Ubiquitinated proteins and proteasome subunits *(22,23)*, in addition to α-synuclein, Parkin and ubiquitin C-terminal hydrolase-L1 (UCH-1) *(24–27)*, are the components of LBs and are sometimes modified with ROS-RNS.

To clarify the interactions among oxidative stress, dysfunction of the proteasome system, and formation of the inclusion body, the effects of a proteasome inhibitor, carbobenzoxy-L-isoleucyl-γ-*t*-butyl-L-alanyl-L-leucinal (PSI) were examined on the aggregation of oxidative-modified proteins and the cell vulnerability *(20,28)*. PSI increased the amount of 3-NT proteins in SH-SY5Y cells, but the number of 3-NT proteins was almost the same as in the control (Fig. 4). At the same time, the number of apoptotic cells increased significantly by PSI, but that of necrotic cells was not (Fig. 5). These results indicate that inhibition of proteasome activity might play a key role in the accumulation of oxidative-modified protein and the induction of cell death.

Rotenone, a Complex I Inhibitor, Inactivates Proteasome by Oxidative Modification

The activity and protein of complex I of the mitochondrial electron transfer chains reduced in the nigro-striatum of patients with PD *(17)*. The systemic administration of rotenone, an inhibitor of complex I, induced parkinsonism in rodents, and fibrillar cytoplasmic inclusions containing ubiquitin and α-synuclein were detected in dopamine neurons *(29)*. The effects of mitochondrial dysfunction on the proteasome system were studied by use of rotenone *(28)*. Apoptosis was induced in the cells after 4–5 d treatment with rotenone. The oxidative modification of proteins was followed by the use of an antibody against acrolein-modified protein *(30)*. As shown in Fig. 6, the levels of acrolein-modified protein increased markedly by the rotenone treatment. In the lysate of rotenone-treated cells, aggregation of acrolein-modified protein with high molecular mass was also detected.

The ubiquitin–proteasome system is a major site for removal of damaged or modified proteins and also regulatory proteins controlling cell cycle and signal transduction. In the nigro-striatum of the parkinsonian brain, the decreased activity of proteasome was reported, suggesting its involvement in the pathological features *(31)*. Oxidized protein is preferentially degraded in vitro by 20S proteasome in an ATP-independent way. Binding of the regulatory subunit, 19S complex (ATPase, PA700), to both ends of the 20S cylinder produces 26S proteasome with higher catalytic activity than 20S proteasome. 26S proteasome degrades polyubiquitinated proteins and ornithine decarboxylase in an ATP-dependent process.

In SH-SY5Y cells, the rotenone treatment increased ROS-RNS levels detected with 2,7-dichlorofluorescence diacetate only transitionally and slightly, whereas the oxidized protein levels increased progressively, suggesting that protein might function as "the second scavenger" of ROS-RNS and that degradation of modified proteins might be impaired. The

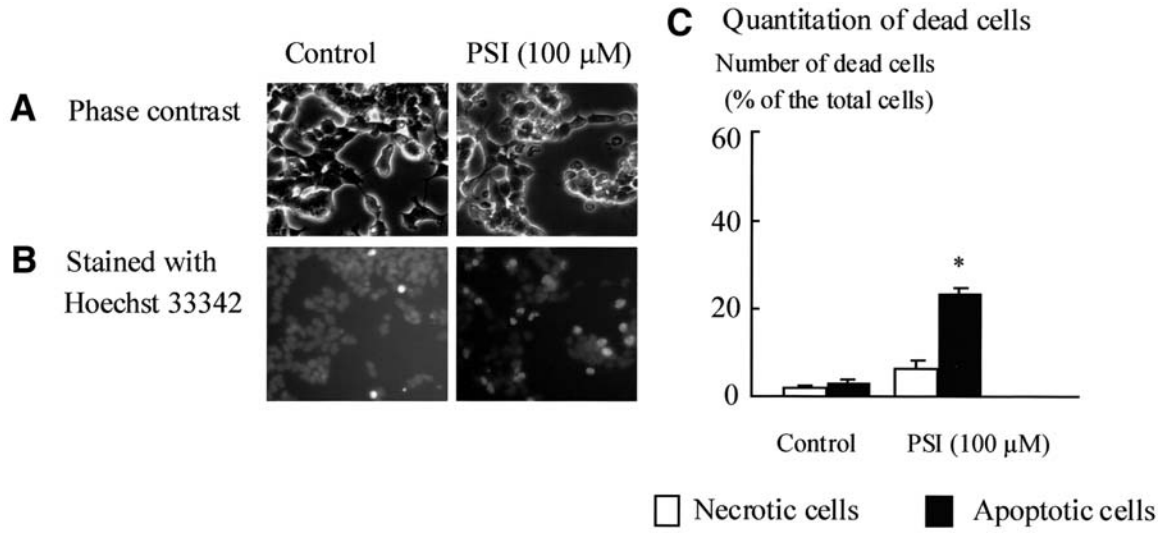

Fig. 5. Cytotoxic effect of PSI treatment on SH-SY5Y cells. The cells were treated with or without PSI for 24 h. **(A)** Phase-contrast microscopy; **(B)** nuclear staining with Hoechst 33342, **(C)** quantitative measurement of apoptotic and necrotic cells. Apoptotic cells were assessed from condensed nuclei stained with Hoechst 33342. The column and the bar represent the mean and SD of the number of dead cells, respectively, expressed as the percentage of the total. The open and the filled columns represent necrotic and apoptotic dead cells, respectively. $*p < 0.05$ from control.

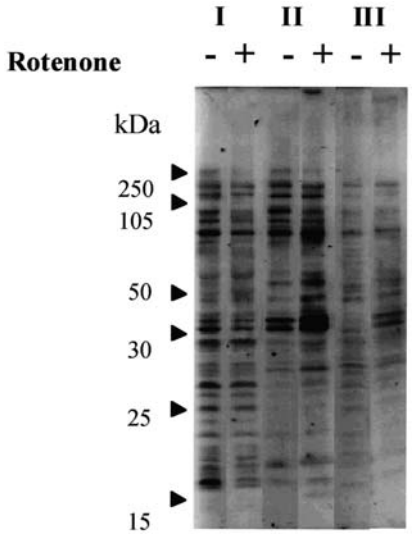

Fig. 6. Acrolein-modified protein in rotenone-treated SH-SY5Y cells. The cells were treated with 5 μ*M* rotenone (+) or without (–) for 3 d and the subcellular fractions were prepared. I: cytoplasm; II: P_1 fraction (nuclei, plasma membrane); III: P_2 fraction (mitochondria). Proteins were immunoblotted with an antibody against acrolein-modified protein. In the P_1 fraction, aggregated acrolein-modified proteins were detected at the top of the gel.

enzymatic activity of proteasome (post-glutamyl peptidase-like activity) measured with a synthetic fluorescent substrate, carbobenzoxy-L-leucyl-L-leucyl-L-glutamic acid α-(4-methyl-coumaryl-7-amide) [Z-Leu-Leu-Glu-MCA], reduced in a time- and dose-dependent way and virtually was not detected after 4 d treatment with rotenone, as shown in Table 1.

The lysate of rotenone-treated cells was immunoprecipitated with antibody against the

Table 1
Effects of Rotenone Treatment on Proteasome Activities in SH-SY5Y Cells

		Proteasome activity in SH-SY5Y cells treated with rotenone (pmole/min/μg protein)	
Rotenone (25 n*M*)	ATP (2 m*M*)	After 6 h	After 96 h
Control	–	0.89 ± 0.11[a]	5.19 ± 0.47
	+	1.76 ± 0.02	8.21 ± 0.11
Sample	–	0.97 ± 0.02	0.96 ± 0.04
	+	2.25 ± 0.02	1.80 ± 0.13

Cytoplasmic fraction was prepared from the cells treated with 25 n*M* rotenone. Postglutamyl peptidase-like proteasome activity was measured fluorometrically using Z-Leu-Leu-Glu-MCA as a substrate with or without the addition of ATP (2 m*M*).

[a] Mean ± SD of four experiments.

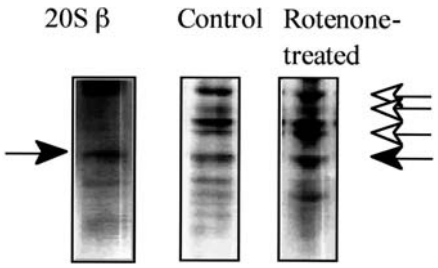

Fig. 7. Modification of the 20S β-subunit of proteasome with acrolein after treatment with rotenone. The cells were treated with 25 μ*M* rotenone (rotenone-treated) or without (control) for 4 d, lyzed, and precipitated with antibody against the 20S β-subunit. The immunoprecipitant was subjected to Western blot analysis with antibody against acrolein-modified protein. The 20S β-subunit in the precipitant was visualized with anti-20S β-antibody (20S β). A filled arrow shows that 20S β was modified with acrolein.

20S β-subunit and the subunit protein was modified by acrolein, as shown by Western blot analysis with anti-acrolein antibody (Fig. 7). Acrolein-modified proteins other than the 20S β-subunit were also detected in the immunoprecipitant, suggesting that the oxidized proteins might serve as substrates of 20S proteasome and the aggregated ones might inhibit the activity. However, it requires further studies to clarify whether the acrolein-modified protein can directly inhibit the proteasome activity, as in the case of 4-HNE proteins *(32).*

Apoptotic Signal Activated by ROS-RNS

Both SIN-1 and NO induce apoptosis in SH-SY5Y cells by opening a megachannel called mitochondrial permeability transition pore (mPTP) with a decline in mitochondrial membrane potential (ΔΨm), as shown in Fig. 8. Opening of mPTP induces matrix swelling and physical disruption of the outer membrane in mitochondria and the release of Ca^{2+}, cytochrome-*c,* and apoptosis-inducing factors into cytoplasm. The mPTP is a protein complex composed of a voltage-dependent anion channel and other components located at the contact site between the mitochondrial outer membrane and inner membrane, and the opening is regulated by Bcl-2 in the outer membrane and the adenine nucleotide translocator in the inner membrane *(2).* Bcl-2 family proteins regulate cell death induced by oxidative stress and other stimuli, either in a preventing way (Bcl-2 and Bcl-xL) or a promoting way (Bax, Bad, and Bid). The antiapoptotic function of Bcl-2 is mediated by maintaining

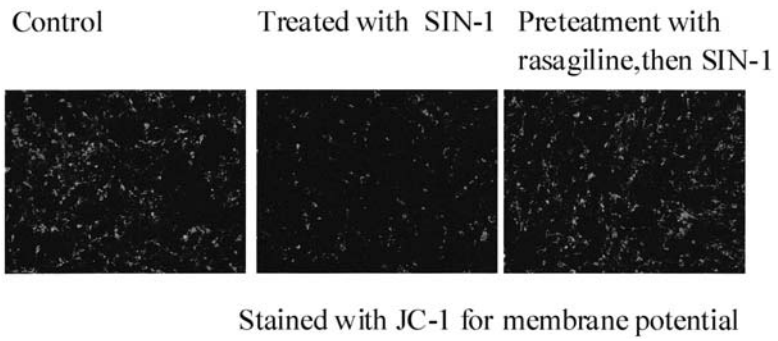

Fig. 8. Reduction in ΔΨm by SIN-1 treatment and the effect of neuroprotective rasagiline. SH-SY5Y cells were treated with SIN-1 (250 μ*M*) for 1 h, with or without pretreatment with 0.1 μ*M* rasagiline, and stained with 5,5′.6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazoylcarbocyanine iodide (JC-1). The level of ΔΨm was determined from red fluorescence, indicating JC-1 aggregates in intact mitochondria.

mitochondria–cytosolic coupling of oxidative phosphorylation, preventing the opening of mPTP and functioning as an antioxidant, either directly or by upregulating other ROS scavengers.

The opening of the mPTP by $ONOO^-$ has been considered to be the result of the nitration of tyrosine residues in the mPTP to release cytochrome-*c (33)* or of cytochrome-*c* itself *(34)*, of the oxidative crosslinking of thiol groups with protein amalgamation and pore formation, or of the lipid peroxidation to generate 4-HE and acrolein with the potential to induce permeability transition. As another mechanism, $ONOO^-$ might modify the Bcl-2 family and change the localization and function, as in the case of Bax *(35)*. The opening of the mPTP activates caspase-3, the executor of apoptosis, as shown in Fig. 9 A, induces translocation of glyceraldehydes-3-phosphate dehydrogenase (GPDH) into nuclei, and, finally, fragmentation of nuclear DNA.

Aside from apoptotic signaling in mitochondria, several different protein kinases, including p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK), were reported to be involved in apoptosis induced by ROS-RNS *(36–38)*. As shown in Fig. 9 B, an inhibitor of p38 MAPK, SB202190, prevented the activation of caspase-3 and apoptosis induced by $ONOO^-$, suggesting that p38 MAPK might be also involved in apoptosis induced by $ONOO^-$. In addition, $ONOO^-$ activated extracellular signal-regulated kinase (ERK) kinase (MEK1), the phosphorylation of which was inhibited by PD98059, a selective inhibitor, and also by superoxide dismutase (SOD) plus catalase, which catalyzes superoxide into H_2O via H_2O_2 *(14,19)*. Interestingly, SB202190 attenuated the activation of ERK kinase, but it remains to be clarified whether p38 MAPK and MEK1 interact with each other and whether the activation of kinases is the consequence of mitochondrial death signaling or vice versa.

Mitochondria and Cell Signaling Mediate Neuroprotective Function of Propargylamines

Neuroprotection is a new therapeutic strategy to slow or halt the progression of PD and other neurodegenerative disorders *(39–42)*. The apoptotic cascade, which is activated sequentially and progresses rather slowly, is proposed as a target of neuroprotection *(3)*. Recently, a series of propargylamine derivatives, rasagiline (a secondary cyclic benzylamine), (–)deprenyl (a tertiary aralkylamine),

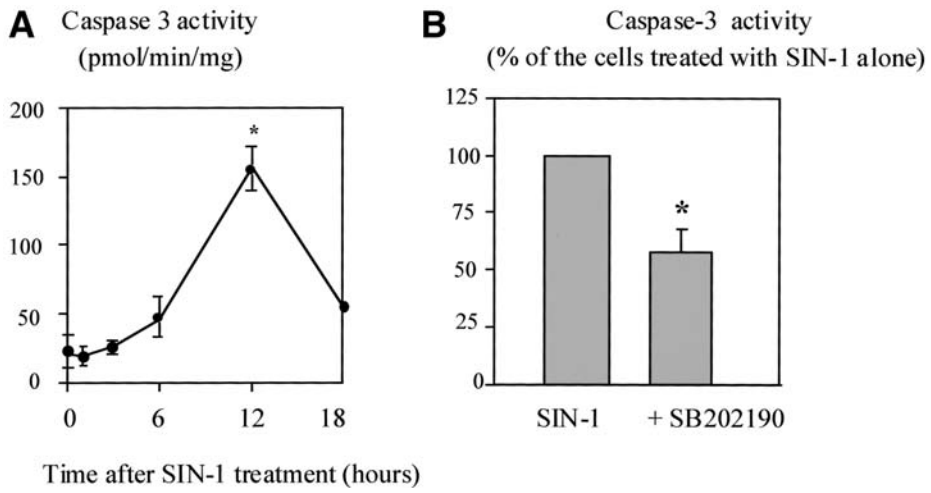

Fig. 9. Activation of caspase-3 by SIN-1 and suppression by a p38 inhibitor, SB202190. **(A)** The cells were treated with SIN-1, and caspase-3 activity in the cell lysate was measured with Asp-Glu-Val-As-4-methyl-cumaryl-7-amide as a substrate. **(B)** the cells were treated with 40 μ*M* SB202190 and then 10 μ*M* SIN-1 for 18 h; then, the activities of caspase-3 were determined. *$p < 0.01$ compared to the control.

and 2-HMP [*N*-(2-hexyl)-*N*-methyl-propargylamine, a tertiary branched alkylamine], have been proved to protect neurons from cell death induced by ROS-RNS, neurotoxins, and other stimuli *(41–47).* At present, rasagiline is the most potent in preventing apoptosis. The hitherto-confirmed mechanism underlying the neuroprotection by propargylamines is shown in Fig. 10. Rasagiline prevents the decline in ΔΨm and swelling of mitochondria (Figs. 8 and 10), the release of cytochrome-*c*, nuclear GAPDH translocation, and fragmentation of nuclear DNA. The antiapoptotic function of rasagiline has been proved to be the result of (1) the stabilization of ΔΨm and suppression of mPT *(47,48)* and (2) the induction of antiapoptotic, prosurvival genes of Bcl-2 *(48–50),* glial cell line-derived neurotrophic factor (GDNF) *(51),* and antioxidative enzymes, catalase and SOD *(52).* The activation of the nuclear factor-κB (NF-κB) pathway has been confirmed to mediate the induction of antiapoptotic genes *(51).* Rasagiline induced the phosphorylation of the inhibitory subunit (IκB) of NF-κB, and the active p65 subunit was translocated into nuclei (Fig. 11A). In addition, an inhibitor of IκB kinase, sulfasalazine, suppressed the activation of NF-κB and also the induction of GDNF *(51)* and *bcl-2* by rasagiline (Fig. 11B). NF-κB is one of the most important transcription factors and is activated by various pathogenic stimuli, including oxidative stress, ischemic insults, and β-amyloid. The induction of antiapoptotic genes depends on the stereochemical structure of rasagiline, suggesting that the binding site in mitochondria might mediate signaling for antiapoptotic gene expression. In addition, we found that ERK kinase was also activated by rasagiline, as an upper stream signal of NF-κB activation. It is interesting that ERK is involved in either proapoptotic signaling through p38 kinase and also in the antiapoptotic pathway through NF-κB. The regulation of these opposite signals should be elucidated to find new drugs suppressing apoptotic cascade and protecting neurons. The stress signaling from mitochondria to nuclei also remains to be elucidated, but the activation of the NF-κB pathway might play a key role, as reported recently *(53).*

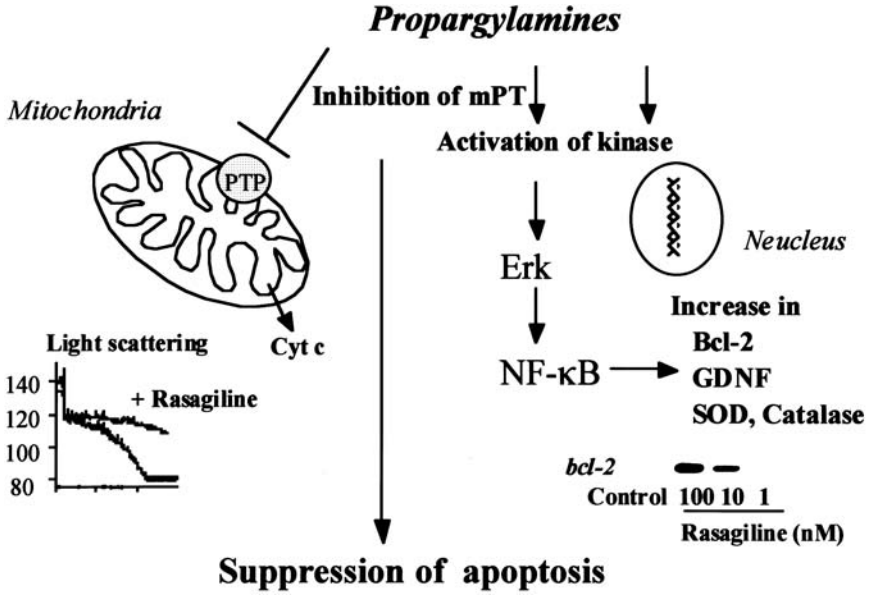

Fig. 10. Mechanisms behind neuroprotective function of propargylamines. Rasagiline prevents opening of the mitochondrial permeability transition (mPT) pore (PTP) and suppresses the mitochondrial swelling, as shown by the light scattering of isolated mitochondria. Rasagiline activates ERK and the NF-κB pathway and induces antiapoptotic Bcl-2, GDNF, and antioxidant enzymes. *bcl-2* mRNA was increased by rasagiline in a dose-dependent way.

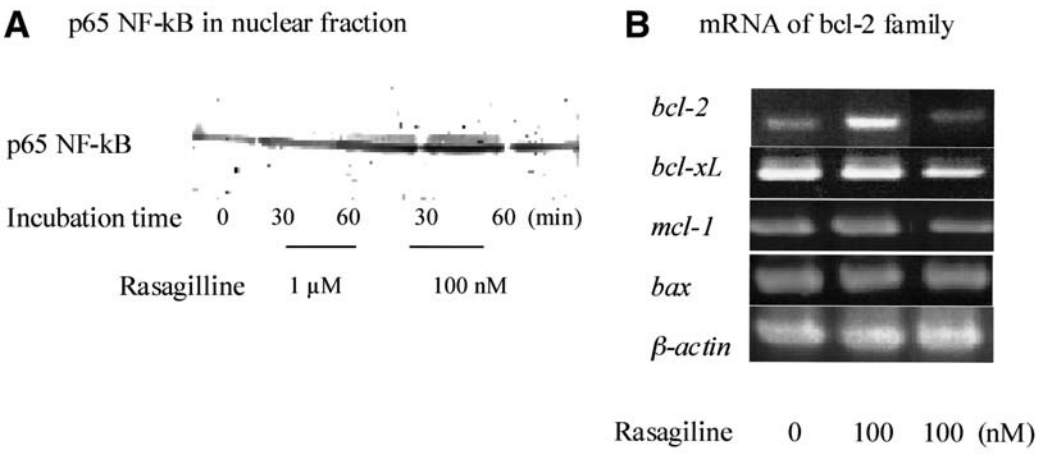

Fig. 11. Activation of NF-κB and induction of antiapoptotic *bcl-2* mRNA by rasagiline. **(A)** Translocation of activated NF-κB in nuclei. SH-SY5Y cells were treated with rasagiline, and in the nuclear fraction, active p65 NF-κB increased with 1 μ*M* and 100 n*M* rasagiline in a time-dependent way. **(B)** Sulfasalazine, an inhibitor of IκB kinase suppressed rasagiline-induced induction of the antiapoptotic *bcl-2* family. After treatment with rasagiline, mRNA was subjected to reverse transcription–polymerase chain reaction analysis for the *bcl-2* family. Sulfasalazine suppressed the increase of *bcl-2, bcl-xL,* and *mcl-1* by rasagiline. Proapoptotic *bax* was not affected by rasagiline.

Conclusion

The results of this article suggest that mitochondrial dysfunction might induce the degeneration of dopamine neurons through the modification and inactivation of the proteasome system and the subsequent aggregation of oxidized proteins. At present, the precise mechanism behind the induction of cell death requires further investigation, but it might be quite relevant to consider that the inactivation of proteasome might play a critical role in activation of apoptotic signal. In neurodegenerative disorders, the environmental and genetic factors might induce a malignant cycle among the mitochondrial dysfunction, increased oxidative stress, and the reduced activity of proteasome in neurons, resulting in the typical pathological features, cell death, and formation of inclusion body. Our recent studies on the ubiquitin–proteasome system in the substantia nigra of human brains further support our hypothesis. In the Parkinsonian brain, the 20S β-subunit precipitated with the specified antibody was modified with acrolein, whereas that from the control was not modified.

Future studies on the mitochondrial signaling, protein oxidation, and proteasome system will give arise a new insight in the involvement of oxidative stress in neuronal cell death. The results will provide us with further development of neuroprotective agents for neurodegenerative disorders.

Acknowledgments

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