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Review

Quinone Formation as Dopaminergic Neuron-specific Oxidative Stress in the Pathogenesis of Sporadic Parkinson's Disease and Neurotoxin-induced Parkinsonism

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by dopaminergic neuron-specific degeneration in the substantia nigra. A number of gene mutations and deletions have been reported to play a role in the pathogenesis of familial PD. Moreover, a number of pathological and pharmacological studies on sporadic PD and dopaminergic neurotoxin-induced parkinsonism have hypothesized that mitochondrial dysfunction, inflammation, oxidative stress, and dysfunction of the ubiquitin-proteasome system all play important roles in the pathogenesis and progress of PD. However, these hypotheses do not yet fully explain the mechanisms of dopaminergic neuron-specific cell loss in PD. Recently, the neurotoxicity of dopamine quinone formation by auto-oxidation of dopamine has been shown to cause specific cell death of dopaminergic neurons in the pathogenesis of sporadic PD and dopaminergic neurotoxin-induced parkinsonism. Furthermore, this quinone formation is closely linked to other representative hypotheses in the pathogenesis of PD. In this article, we mainly review recent studies on the neurotoxicity of quinone formation as a dopaminergic neuron-specific oxidative stress and its role in the etiology of PD, in addition to several neuroprotective approaches against dopamine quinone-induced toxicity.

Key words: dopamine quinone, quinoprotein, Parkinson's disease, oxidative stress, neurotoxin

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by degeneration of dopamine (DA)-containing neurons in the substantia nigra and by the presence of Lewy bodies. A number of efficient therapeutic drugs, including L-DOPA, dopamine agonists, and inhibitors of dopamine-metabolizing enzymes, have been used for the clinical treatment of patients with PD. It has been shown that several genes are mutated or deleted in familial PD, but the etiology of sporadic PD, which accounts for the majority of PD cases, is still obscure. A number of important results have been accumulated from pathological and pharmacological studies on PD and from animal or *in vitro* studies using dopaminergic neurotoxins which cause parkinsonism in animals. These studies have shown that (1) mitochondrial dysfunction, (2) inflammation, (3) oxidative stress, and (4) impairment of the ubiquitin-proteasome system play important roles in the pathogenesis and progress of sporadic and familial PD. However, the mechanisms of dopaminergic neuron-specific cell loss in PD have not been fully clarified. More recently, an additional factor, (5) DA quinone formation, has been investigated with respect to not only L-DOPA-induced neurotoxicity but also the pathogenesis of PD and

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dopaminergic neurotoxin-induced parkinsonism [1-3]. This article focuses on the neurotoxicity of quinone formation as a dopaminergic neuron-specific oxidative stress and its role in the etiology of PD by reviewing studies on patients with sporadic PD and studies employing models of sporadic PD induced by dopaminergic neurotoxins. In addition, neuroprotective approaches against DA quinone toxicity are also addressed.

Dopaminergic Neurotoxin-induced Parkinsonism—Involvement of Mitochondrial Dysfunction, Inflammation, Oxidative Stress, and Proteolytic Stress—

Following the initial reports that a dopaminergic neurotoxin, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), caused irreversible parkinsonism with progressive depletion of DA neurons in the substantia nigra [4, 5], the neurotoxic mechanism of MPTP has been widely investigated and has been used as a representative dopaminergic neurotoxin in animal models of PD. MPTP which across the blood-brain barrier is converted to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase-B in the glial cells. MPP^+ can be selectively up-taken through DA transporters at the nerve endings of nigrostriatal DA neurons, and then inhibit mitochondrial complex I to cause cell death of DA neurons [6, 7]. Furthermore, MPTP-induced parkinsonism is responsive to L-DOPA. It has been reported that an other dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), which has been commonly used to make *in vivo* and *in vitro* models of PD, also induces mitochondrial impairment by inhibiting mitochondrial complexes I and IV [8-10] and reducing cytochrome c [11], although there have been reported that 6-OHDA does not in fact impair mitochondrial function [12, 13]. Therefore, these findings imply the hypothesis of mitochondrial dysfunction: inhibition of mitochondrial respiratory enzymes by certain environmental neurotoxin which up-taken into DA neurons may cause selective neuronal loss of DA neurons in the substantia nigra. Betarbet *et al.* revealed that chronic and systemic infusion of the lipophilic pesticide, rotenone, which is an inhibitor of mitochondrial complex I, causes parkinsonism with hypokinesia and rigidity in rats with highly selective nigrostriatal dopaminergic degeneration and fibrillar cytoplasmic inclusions containing ubiquitin and α-synuclein. These results indicate that chronic exposure to a common pesticide can reproduce the anatomical, neurochemical, behavioral and neuropathological features of PD [14]. The dopaminergic neurotoxicity of rotenone strongly supports the possible involvement of mitochondrial dysfunction in the pathogenesis of PD, although it is unclear how rotenone, which inhibits complex I systemically, produces selective degeneration of DA neurons.

Intracerebral injection of bacterial endotoxin lipopolysaccharide (LPS) has been shown to induce the expression of inflammatory cytokines and their related molecules, i.e., interleukin (IL)-1β, IL-6, IL-12, p35, tissue necrosis factor (TNF)-α, and inducible nitric oxide synthase (iNOS) [15]. Furthermore, intranigral injection of inflammogen LPS has been shown to induce degeneration of nigral DA neurons with accumulation of microglia in rats [16, 17]. These reports showed that the microglial activation and inflammatory reaction induced by chronic exposure to the inflammogen were capable of inducing selective degeneration of nigral dopaminergic neurons. In animal models treated with MPTP, astroglial and microglial reactions and lymphocytic infiltration were found around impaired neurons in the substantia nigra, suggesting that an immune mechanism contributed to the MPTP-induced neuronal damage [18]. As for the neurotoxicity of rotenone, this may be at least partly due to the inflammatory reaction occurring in conjunction with activation of microglia. Marked microglial activation has been observed in the striatum and substantia nigra of rotenone-treated animals, and was prominent before anatomical evidence of dopaminergic lesions [19]. Rotenone stimulated the release of superoxide from microglia, and this release was attenuated by inhibitors of NADPH oxidase. The rotenone-induced neurotoxicity was also reduced by the inhibition of NADPH oxidase or scavenging of superoxide [20]. In primary mesencephalic neuron-glia cultures, dopaminergic neurons from NADPH oxidase-null mice were more resistant to rotenone neurotoxicity [21]. Furthermore, nontoxic or minimally toxic concentrations of the pesticide rotenone (0.5 nM) and LPS (0.5 ng/ml) synergistically induced dopaminergic neurodegeneration in primary mesencephalic mixed cultures. All these findings indicate that rotenone-induced dopaminergic neurotoxicity may be based not only on mitochondrial dysfunction but also on inflammatory reaction and free radical generation through microglial activation, and that inhibition of inflammation might become a promising therapeutic inter-

vention for PD [22]. In other words, further studies on the selectively toxic mechanisms of rotenone may elucidate the multifactorial etiology of PD. In fact, the dopaminergic neurotoxicity induced by MPTP, MPP^+ and 6-OHDA was inhibited and ameliorated by treatment with aspirin, salicylate and meloxicam, which are nonsteroidal anti-inflammatory drugs (NSAIDs) inhibiting cyclooxygenase (COX) [23–26], and minocycline, which is a tetracycline derivative that inhibits microglial activation independently of its antimicrobial properties [27–29].

As mentioned above, oxidative stress with generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) is known to play important roles in the pathogenesis and progress of PD as a common degenerative process. Superoxide generated is leaked from mitochondria in the case of its dysfunction, especially when the activity of mitochondrial respiratory enzymes reduced by neurotoxins, MPTP, 6-OHDA and rotenone. Non-enzymatic and spontaneous auto-oxidation of DA produce superoxide and reactive quinones [30, 31]. In the inflammatory process, superoxide is also generated in activated microglia through NADPH oxidase or xanthine oxidase. The superoxide generated is converted to hydrogen peroxide by superoxide dismutase (SOD), and superoxide also reacts with nitric oxide to consequently generate peroxynitrite, which is a highly RNS that leads protein nitration. Hydrogen peroxide is up-taken into neurons and normally reduced by antioxidants such as glutathione (GSH), glutathione peroxidase (GPx) or catalase. Where transition metals, especially iron, are abundant, however, hydrogen peroxide reacts with metals to form the most cytotoxic ROS, hydroxyl radicals. The generation of ROS [32–34] and compensatory elevation of antioxidative enzymes, SOD, GPx and catalase [35] were reported in the basal ganglia of MPTP-treated animals or MPP^+-treated cultured cells. Furthermore, dopaminergic neurotoxicity induced by MPTP administration was inhibited in Cu/Zn-SOD transgenic mice [36]. Neurotoxic 6-OHDA is up-taken into striatal nerve endings of dopaminergic neurons through DA transporters, and the toxin produces ROS such as superoxide, hydrogen peroxide and hydroxyl radicals when it is converted to reactive quinones by auto-oxidation [37, 38]. We have previously reported that the intracerebroventricular injection of 6-OHDA produced marked increases in Cu/Zn-SOD activity and lipid peroxides in the striatum [39] and that 6-OHDA-induced reduction of nigral DA neurons was prevented in Cu/Zn-SOD transgenic mice [40]. Rotenone also produces the release of superoxide from microglia through activation of NADPH oxidase [20, 21].

The generated hydrogen peroxide activates NF-κB transcription factor, which is related to inflammation [41, 42], and consequently promotes transcription of iNOS, COX, inflammatory cytokines (IL-1β, IL-6, TNF-α) and apoptosis-promoting molecules (p53, Bcl-Xs, Bax) [43]. The inflammatory cytokines activate iNOS expression directly or indirectly through activation of NF-κB and thereby start a vicious cycle. It is known that superoxide and hydrogen peroxide can open mitochondrial permeability transition pores directly or indirectly through the activation of NF-κB and Bax protein [44] to promote the release of cytochrome c and consequent apoptotic cell death. Nitric oxide also inhibits mitochondrial respiratory enzymes to leak superoxide [45]. Thus, mitochondrial dysfunction, inflammation, and oxidative stress by generated ROS or RNS seem to be linked to each other in establishing the pathogenesis of parkinsonism by dopaminergic neurotoxins in nigrostriatal DA neurons and surrounding glial cells (Fig. 1).

Other possible hypothesis, which is proteolytic stress, has been recently focused. Impairment of ubiquitin-proteasome system leading to endoplasmic reticulum (ER) stress, unfolded protein response and consequent aggregation of cytotoxic proteins are involved in the pathogenesis of PD (see reviews [46–48]). This hypothesis of proteolytic stress has arisen from the following evidences: that parkin, mutation of which leads to an autosomal recessive form of PD, is a ubiquitin ligase [49], that α-synuclein, mutation of which causes familial PD, is a major component of inclusion Lewy bodies [50], and that differentiated PC12 cells expressing mutant α-synuclein showed a decrease in proteasome activity [51]. ER stress caused by accumulation of unfolded protein leads to up-regulation of parkin protein to prevent unfolded protein response-induced dopaminergic cell death [52]. Dopaminergic neurotoxins 6-OHDA, MPP^+ and rotenone induced ER stress and unfolded protein response in catecholaminergic PC12 cells [53]. The involvement of the ER-Golgi system in dopaminergic cell death in PD is also supported by our recent study showing that rotenone induced disassembly of the Golgi apparatus in dopaminergic cells [54]. In dopaminergic neuroblastoma SH-SY5Y cells and PC12 cells, 6-OHDA increases the levels of free ubiquitin and ubiquitinated

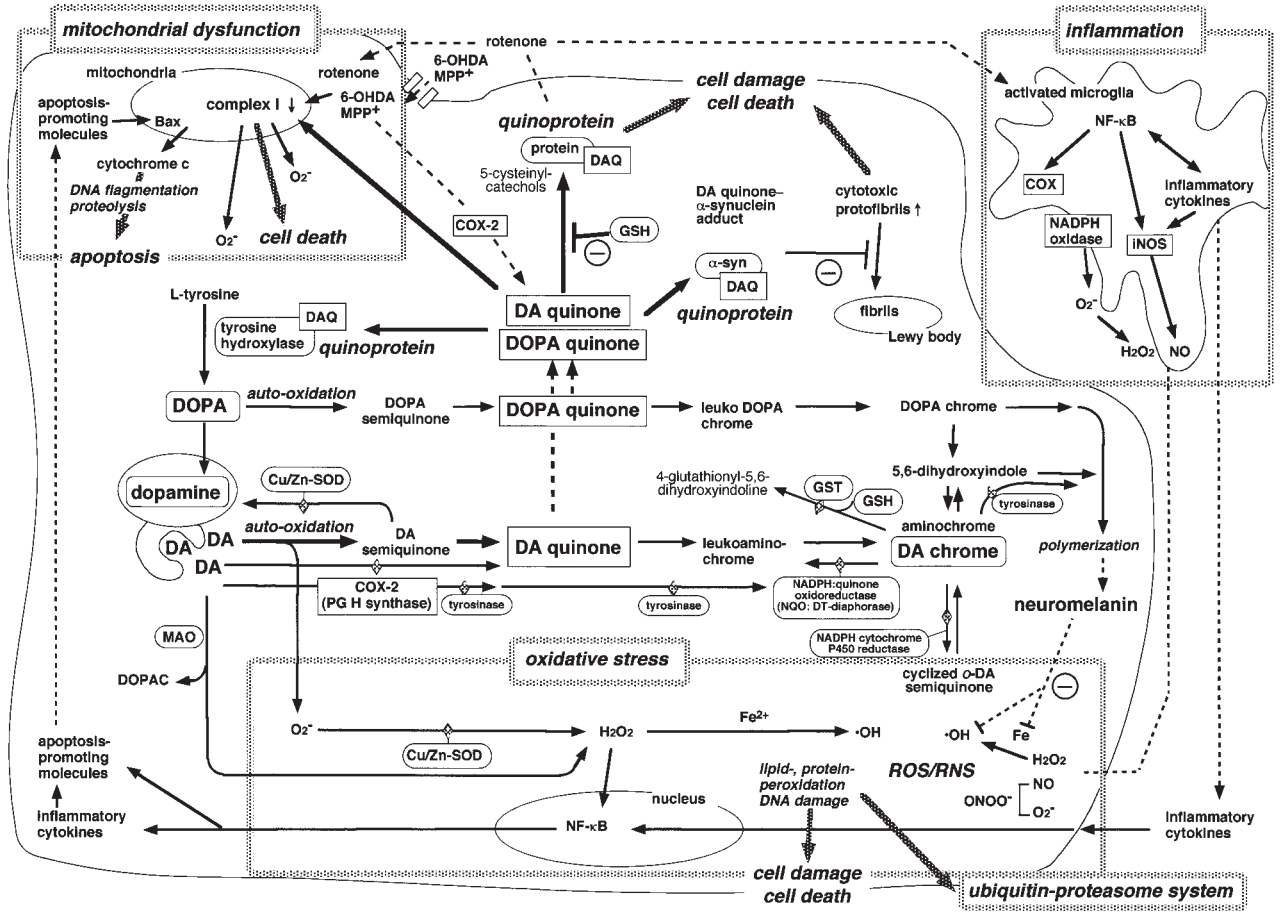

Fig. 1 Neurotoxicity of quinone formation as a dopaminergic neuron-specific oxidative stressor which closely links representative hypotheses, mitochondrial dysfunction, inflammation, oxidative stress, and impairment of the ubiquitin-proteasome system in the pathogenesis and progress of sporadic Parkinson's disease and dopaminergic neurotoxin-induced parkinsonism.

proteins, and proteasome inhibition by MG-132 potentiates toxicity of 6-OHDA [55]. Recently, Höglinger *et al.* reported changes in proteasome activities induced by dopaminergic neurotoxins: rotenone and MPP^+ reduced proteasome activity via ATP depletion, while 6-OHDA increased the activity in response to oxidative stress in primary cultured mesencephalic dopaminergic neurons [13]. Furthermore, severe inhibition of proteasome activity by the proteasome inhibitor epoxomicin or MG-132 synergistically exacerbated dopaminergic neurotoxicity of rotenone, MPP^+ and 6-OHDA, and antioxidant *N*-acetylcysteine and glucose supplementation protected against the synergistic neurotoxicity [13]. These reports suggest that proteasome inhibition enhances vulnerability to subtoxic oxidative stress and energy loss by complex I inhibition. Proteasome inhibition reduces mitochondrial complex I and II activities in SH-SY5Y cells [56], and up-regulates COX-2 and its ubiquitin conjugates [57]. Thus, proteolytic stress in the ubiquitin-proteasome system is linked not only to oxidative stress but also to mitochondrial dysfunction and the inflammatory process. It has been reported that proteasome inhibition leads to formation of ubiquitin/α-synuclein-positive inclusions in PC12 cells or mesencephalic cultured cells [58–60]. Furthermore, unilateral infusion of lactacystin, a proteasome inhibitor, into the substantia nigra or infusion of lactacystin/epoxomicin into the striatum causes selective degeneration of dopaminergic neurons in the substantia nigra with cytoplasmic accumulation of ubiquitin and α-synuclein to form inclusion bodies [60, 61]. The dopaminergic neurotoxic property of overexpressed α-synuclein has been revealed in ani-

mals that overexpress wild-type or mutant α-synuclein [48, 62–66]. It has been suggested that the toxicity of α-synuclein is due, in part, to the increase in transient formation of pathogenic oligomerized α-synuclein protofibrils at the conversion of fibrils to form Lewy bodies [67], as will be discussed further below. In contrast to the aggravating effects of severe proteasome inhibition on the neurotoxicity of rotenone, MPP^+, and 6-OHDA [13] and the neurotoxic property of wild-type or mutant α-synuclein, Sawada *et al.* recently reported that moderate proteasome inhibition by lactacystin or MG-132 blocked MPP^+- or rotenone-induced cell death of primary cultured dopaminergic neurons, despite the appearance of α-synuclein-positive inclusions [68], suggesting that impairment of proteasome plays an important role both in α-synuclein-positive inclusion formation and dopaminergic neuronal death on different lines, and that the formation of inclusion bodies actually exerts neuroprotective, rather than neurotoxic, effects. Thus, although it remains uncertain whether proteolytic stress due to impairment of the ubiquitin-proteasome system is involved in dopaminergic cell death in PD, such stress may be a common process downstream of the pathogenesis of PD.

Supporting Evidence of Mitochondrial Dysfunction, Inflammation, Oxidative Stress and Proteolytic Stress in Patients with Sporadic Parkinson's Disease

The activities of mitochondrial complex III in the striatum and of complex I in the substantia nigra are significantly reduced in the brain of patients with sporadic PD [69–71]. These findings support the mitochondrial dysfunction hypothesis mentioned above. It is well known that the inflammation is involved in the pathogenesis of PD [22, 72–74]. In fact, in the substantia nigra of patients with PD, activated microglia that express iNOS and COX-1 and -2—which are rate-limiting enzymes that catalyze the formation of prostaglandin precursors from arachidonic acid—were found to be accumulated [75, 76]. Furthermore, inflammatory cytokines (IL-1β, IL-6, TNF-α), an NF-κB transcription factor related to gene induction of cytokines, and some growth factors were increased in the striatum and cerebrospinal fluid of patients with PD [74, 77, 78]. Oxidative stress is thought to play an important role in the pathogenesis and progression of PD. This suggested mechanism is supported by evidence in the brain of patients with sporadic PD showing decreases in the levels of GSH, GPx and catalase in the basal ganglia, including the striatum and substantia nigra [79–82], and increases in ferrous ions and iron deposits in the substantia nigra [82, 83]. The most cytotoxic ROS hydroxyl radicals might be generated from hydrogen peroxide through metal-catalyzed Fenton reaction to cause degenerative reactions including lipid or protein peroxidation and DNA fragmentation [84]. Microglia showing up-regulated iNOS and COX expression and protein nitration were found in the substantia nigra of parkinsonian patients [76, 85], suggesting that nitric oxide reacts with superoxide to generate peroxynitrite, which promotes protein nitration. Concerning the ubiquitin-proteasome system, structural and enzymatic defects of 26S/20S proteasome, especially 20S proteasome α-subunits, have been revealed in the substantia nigra of patients with sporadic PD [86–88], and parkin has also been shown to be present in Lewy bodies in the substantia nigra of patients with non-familial PD [89, 90], suggesting that proteolytic stress is also involved in the pathogenesis of non-familial PD or parkinsonism (see reviews [46–48]).

Neurotoxicity of Quinone Formation as Dopaminergic Neuron-specific Oxidative Stress

These representative hypotheses—*i.e.*, that mitochondrial dysfunction, inflammation, and the resulting oxidative stress and impairment of the ubiquitin-proteasome system are common processes in the etiology of PD—can explain the mechanism of neurotoxicity induced by MPTP and 6-OHDA and some parts of pathogenesis in sporadic and familial PD, as mentioned above. However, these hypotheses have not yet fully clarified the mechanisms of dopaminergic neuron-specific cell loss in PD and rotenone-treated parkinsonian animal models. Although rotenone is not up-taken through DA transporters and systemically inhibits mitochondrial complex I, it also causes highly selective nigrostriatal dopaminergic degeneration with fibrillar cytoplasmic inclusions [14]. It has been shown that ROS generation from the mitochondria of damaged neurons and from microglia through the activation of NADPH oxidase [20, 21] induces the selective degeneration of nigral DA neurons that are abundant in iron and vulnerable to oxidative stress [19, 91]. However, this explanation does not fully

clarify the mechanism of dopaminergic neuron-specific cell loss, since ROS distributes diffusely and its toxic effects are not localized. Recently, Sakka *et al.* reported that depletion of DA attenuated rotenone-induced neurotoxicity, suggesting that the existence of endogenous DA itself is involved in the selective loss of dopaminergic neurons induced by rotenone exposure [92].

In contrast to the general oxidative stress induced by ROS/RNS, neurotoxicity of DA quinones or DOPA quinones have recently received attention as dopaminergic neuron-specific oxidative stress known to play a role in the pathogenesis of PD and neurotoxin-induced parkinsonism [3]. There are 2 major pathways in the DA-related oxidative stress (Fig. 1). When an excess amount of cytosolic DA exists outside of the synaptic vesicle, *i.e.*, after L-DOPA treatment, DA is easily metabolized via monoamine oxidase-B or by auto-oxidation to produce cytotoxic ROS, and then forms neuromelanin [1, 93]. In the oxidation of DA by monoamine oxidase, hydrogen peroxide and dihydroxyphenylacetic acid (DOPAC) are generated. On the other hand, non-enzymatic and spontaneous auto-oxidation of DA and L-DOPA produces superoxide and reactive quinones such as DA quinones or DOPA quinones [30, 31]. These quinones are easily oxidized to the cyclized aminochromes DA-chrome (aminochrome) and DOPA-chrome, and then are finally polymerized to form melanin. Furthermore, other pathways of DA oxidation have been reported, and in these pathways, DA quinones are also generated in the enzymatic oxidation of DA by prostaglandin H synthase (COX), lipoxygenase, tyrosinase, and xanthine oxidase [94–97]. Although the auto-oxidation of DA via quinone formation produces general ROS which shows widespread toxicity not only in DA neurons but also in other regions, highly reactive DA quinone and DOPA quinone themselves have been reported to exert predominant cytotoxicity in DA neurons and surrounding neural cells, since these quinones are generated from free cytosolic DA outside the synaptic vesicle or from L-DOPA [93].

The quinones generated from DA or L-DOPA exert cytotoxicity by interacting with various bioactive molecules in or beside dopaminergic neurons. The functional proteins which possess cysteine residues are thought to be the targets of DA quinone and DOPA quinone. These quinones conjugate with the sulfhydryl group of the amino acid cysteine, resulting predominantly in the formation of 5-cysteinyl-DA and 5-cysteinyl-DOPA, respectively [31, 98, 99]. Since the sulfhydryl group on cysteine is often found at the active site of functional proteins, covalent modification of cysteine residues by DA quinone or DOPA quinone to form 5-cysteinyl-catechols would irreversibly alter or inhibit protein function and consequently cause cytotoxicity. This mechanism of neurotoxicity by the formation of 5-cysteinyl-catechols on protein is supported by evidence showing the generation of DA quinone and DOPA quinone, and consequent formation of 5-cysteinyl-catechols, 5-cysteinyl-DA, 5-cysteinyl-DOPA, and 5-cysteinyl-DOPAC, in the cell loss of dopaminergic neuronal cultured cells treated with DA and L-DOPA [100–102]. In particular, it is of interest that tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, is one of the proteins that may be targeted by the DA quinone generated in the brain, since DA quinones covalently modify and inactivate the DA-synthesizing enzyme to subsequently form redox-cycling quinoprotein [103, 104] (Fig. 1).

The formation of catechol-quinones *in vitro* and *in vivo* has been dramatically blocked by treatment with GSH, cysteine, or ascorbate [100, 101, 103, 105], since the reduced sulfhydryl group of cysteine in GSH and free cysteine compete with the sulfhydryl group of cysteine on proteins conjugating with DA or DOPA quinones. We recently revealed the neurotoxic properties of DA- and L-DOPA-related compounds in human neuroblastoma SH-SY5Y cells by generating DA- or DOPA-semiquinone radicals, which were subsequently converted to toxic quinones [106]. DA or L-DOPA possessing 2 hydroxyl groups on the benzene ring showed marked cell toxicity and generation of DA- or DOPA-semiquinone radicals, as detected by *in vitro* ESR spectrometry. The cell death and the formation of these semiquinone radicals induced by DA or L-DOPA were markedly prevented by the addition of Cu/Zn-SOD or GSH, but not by the addition of catalase [106]. SOD can act as a superoxide: semiquinone oxidoreductase to prevent quinone formation [107]. Furthermore, by conjugating with DA quinone, GSH prevents the binding of DA quinone to the sulfhydryl groups of cysteine on proteins as described above. Therefore, the protective effects of SOD and GSH against DA- or L-DOPA-induced cytotoxicity may be the result of the quinone-quenching activities of these antioxidants (Fig. 1). The involvement of quinone formation in DA- or DOPA-induced cytotoxicity is also supported by our recent study that overexpression of Cu/Zn-SOD protects SH-SY5Y cells against DA-induced cytotoxicity accompanied by an increase in their GSH level [108]. If

DA-related neuronal death is caused by the generation of general ROS or RNS, the cytotoxicity of DA would be prevented by antioxidants such as ascorbic acid and α-tocopherol. However, ascorbic acid and α-tocopherol have lesser or no effects against DA-induced cell death in PC12 cells, while the cell death is markedly inhibited by the thiol-containing compounds GSH, *N*-acetylcyteine, and dithiothreitol [109]. Furthermore, these thiol reagents prevent auto-oxidation of DA and consequent melanin formation. Taken together, these findings indicate that quinone formation may play an important role in the neuronal damage induced by dopaminergic neuron-specific oxidative stress.

One of the target proteins for DA quinone is thought to be α-synuclein, which is a major component of the insoluble fibrils of Lewy bodies in patients with PD [2]. The soluble state of α-synuclein is converted to aggregated fibrils via transient formation of pathogenic protofibrils by its oligomerization [67]. Mutant α-synuclein (A30P), which is linked to familial autosomal dominant PD, enhances the rate of protofibril formation but inhibits the conversion of protofibrils to fibrils, thereby increasing the level of toxic protofibrils [110], which lead to the destruction of synaptic vesicular membranes [111]. Conway *et al.* screened 169 compounds that inhibited the conversion of protofibrils to fibrils, and found that all but one of 15 fibril inhibitors were catecholamines related to DA and L-DOPA. Furthermore, DA quinone reacts with α-synuclein to form the DA quinone-α-synuclein adduct, which inhibits fibril formation at oligomerization by stabilizing the protofibrils [2]. Since α-synuclein does not contain cysteine residues, the DA quinone-α-synuclein adduct may be one of the quinoproteins formed by coupling DA quinone to tyrosine residues of α-synuclein and/or by nucleophilic attack of quinone to lysine residues forming a Schiff base [103, 112] (Fig. 1).

In addition to neurotoxic DA quinones, cyclized *o*-semiquinones derived from DA-chromes are involved in the cytotoxicity induced by auto-oxidation of DA [113–117]. In the pathway of melanin formation, the cyclized aminochrome DA-chrome (aminochrome) is generated from DA quinone via leukoaminochrome, and is converted to 5,6-dihydroxyindole and then indole-5,6-quinone, which is consequently polymerized to form melanin. It has been proposed that reduction of DA-chrome catalyzed by NADPH cytochrome P450 reductase produces cyclized *o*-DA semiquinone (leukoaminochrome *o*-semiquinone) radicals which are highly reactive molecules [116, 118]. This cytotoxic effect of cyclized *o*-DA semiquinone derived from DA-chrome is supported by a report showing that unilateral injection of cyclized aminochrome, DA-chrome into the substantia nigra resulted in apomorphine-induced rotation behavior with a significant reduction of nigral DA neurons [119].

The cytotoxicity of quinone formation is closely linked not only to general oxidative stress but also to mitochondrial dysfunction, inflammation, and proteasome impairment (Fig. 1). Quinone formation by DA oxidation reduces mitochondrial function and opens the mitochondrial permeability transition pores in the brain [120]. DHBT-1 (7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2*H*-1,4-benzothiazine-3-carboxylic acid), a further oxidation product of 5-cysteinyl-DA, irreversibly inhibits the activity of mitochondrial complex I [121]. As described above, several NSAIDs protected against dopaminergic cell loss in MPTP-treated parkinsonian models [23, 24, 26]. Furthermore, reduction of nigral DA neurons by MPTP injection was prevented in COX-2 knockout mice or by treatment with a COX-2 inhibitor [122]. One possible dopaminergic neuron-specific target molecule of NSAIDs is thought to be prostaglandin H synthase, which is abundant in the brain [96]. This enzyme, which exerts both COX activity and peroxidase activity on the arachidonic acid cascade, catalyzes the production of prostaglandin G2 from arachidonic acid by its COX activity, and then converts prostaglandin G2 to prostaglandin H2/E2 by its peroxidase activity. In the presence of DA, oxidative reaction of DA to form DA quinone is coupled with the latter step. In other words, the DA quinone is generated not only in auto-oxidation of DA, but also by the enzymatic oxidation of DA by prostaglandin H synthase in a reaction mediated by hydrogen peroxide [96]. The NSAIDs indomethacin and aspirin inhibit this prostaglandin H synthase-mediated oxidation of DA [123]. These findings suggest that the induction of COX and resulting formation of DA quinone play an important role in the degeneration of DA neurons by dopaminergic neurotoxins or excess DA. In fact, prostaglandin E2, which is generated by prostaglandin H synthase, is increased in the substantia nigra of patients with PD [123]. Furthermore, the 5-cysteinyl-DA/homovanillic acid concentration ratio has been shown to be significantly higher in the cerebrospinal fluid of the patients with PD who received L-DOPA therapy [124]. In another study, the 5-cysteinyl-catechols were

significantly higher in the substantia nigra of PD patients than in that of controls [125]. Apoptotic and selective dopaminergic cell death with formation of ubiquitin and α-synuclein-positive inclusion *in vitro* or *in vivo* induced by the proteasome inhibitor lactacystin or epoxomicin was suppressed by reducing endogenous DA, and enhanced by treatment with L-DOPA or the monoamine oxidase inhibitor pargyline [60]. In a related work, DA induced proteasome inhibition in PC12 cells, and this inhibition was attenuated by GSH, monoamine oxidase inhibitors, or a DA uptake inhibitor [126]. These findings suggest a possible role of proteasome inhibition in the toxicity induced by high levels of DA or its quinone in the cytosol. Thus, DA quinone- or DOPA quinone-induced cytotoxicity is thought to be involved not only in the pathogenesis of PD but also in the adverse reactions induced by long-term L-DOPA therapy.

Neuroprotective Approaches against DA Quinone Cytotoxicity

As mentioned above, various studies have introduced neuroprotective approaches against DA quinone toxicity. The DA- or L-DOPA-induced formation of catechol-quinones such as 5-cysteinyl-DA and 5-cysteinyl-DOPA and consequent dopaminergic cell damage *in vitro* and *in vivo* were dramatically prevented by treatment with SOD, GSH, *N*-acetylcyteine, or dithiothreitol, but not by treatment with catalase or α-tocopherol [100, 101, 103, 105, 106, 108, 109]. These protective effects of SOD, GSH, and some thiol reagents against DA- or L-DOPA-induced cytotoxicity may be due to their quinone-quenching activities. SOD exerts protective effects by acting as a superoxide: semiquinone oxidoreductase to prevent quinone formation [106–108]. The sulfhydryl group of free cysteine in GSH and thiol reagents compete with the sulfhydryl group of cysteine on functional proteins conjugating with DA or DOPA quinones [3, 96, 106, 109]. These quinone-quenching reagents, especially *N*-acetylcyteine, which can cross the blood-brain barrier, may be therapeutically useful against the neurotoxicity of quinone formation that causes oxidative stress specifically in the dopaminergic neurons.

Against the DA-chrome-induced neurotoxicity, 2 enzymes, NAD(P)H: quinone oxidoreductase (NQO: DT-diaphorase) and GSH transferase (GST) M2-2, exert neuroprotective effects to prevent the formation of cyclized *o*-DA semiquinone (Fig. 1). NQO: DT-diaphorase (so-called quinone reductase), which is present in the dopaminergic neurons in the substantia nigra [127], catalyzes the two-electron reduction of quinones, reduces DA-chrome to leukoaminochrome by competing with NADPH cytochrome P450 reductase, and consequently inhibits the formation of *o*-semiquinone radicals [113, 115, 128]. Treatment with butylated hydroxyanisole, dimethyl fumarate (DMF), or *tert*-butylhydroquinone, which up-regulates the activity of NQO: DT-diaphorase, protects against cell death related to quinone formation [129–132]. GST M2-2 also prevents the formation of cyclized *o*-semiquinone from DA-chrome by catalyzing the conjugation of DA-chrome with GSH to form 4-glutathionyl-5,6-dihydroxyindoline [133, 134]. In particular, DMF increases not only the activity of NQO: DT-diaphorase but also total intracellular GSH and the activities of GST and GSH reductase [130] to reduce the cytotoxicity associated with DA quinone formation.

The neurotoxicity induced by the dopaminergic neurotoxins MPTP, MPP^+, and 6-OHDA was blocked by treatment with the NSAIDs, aspirin, salicylate and meloxicam, which inhibit COX activity [23–26]. The NSAIDs indomethacin and aspirin inhibited prostaglandin H synthase-mediated oxidation of DA [123]. The final product, prostaglandin E2, which is catalyzed by prostaglandin H synthase, was increased in the substantia nigra of patients with PD [123]. The NSAIDs can also inhibit the induction of inflammatory cytokines and apoptosis-promoting molecules by suppressing of iNOS expression or NF-κB induction. Therefore, NSAIDs could be neuroprotective agents in the treatment of parkinsonian patients, and especially for treating adverse reactions caused by long-term L-DOPA therapy, through their anti-inflammatory action and their reduction of catechol quinone-induced cytotoxicity [3, 135].

Furthermore, the melanin-synthesizing enzyme tyrosinase, which catalyzes both the hydroxylation of tyrosine to L-DOPA and the subsequent oxidation of L-DOPA or DA to form melanin, might be useful for reducing the neurotoxicity induced by DA quinone [3] (Fig. 1), since tyrosinase can rapidly oxidize DA or DA quinone to form melanin [136]. We previously reported that inhibition of tyrosinase dramatically reduced the viability of dopaminergic neuronal cells by increasing the intracellular DA contents, and significantly enhanced DA-induced cell death [137]. Tyrosinase also oxidizes 5,6-dihydroxyindole derived from DA- or DOPA-chrome to indole-5,6-quinone. Furthermore, the replacement of

DA synthesis by tyrosinase has been reported in tyrosine hydroxylase-null mice [138]. Neuromelanin exerts antioxidative properties to inhibit lipid peroxidation, quenches free radicals, and has a strong chelating ability as regards transition metals, preventing metal-induced neurotoxicity [3, 139]. Therefore, enhancing the activity of tyrosinase in the brain may prevent or ameliorate the DA quinone or DA-chrome-induced slow progression of cell damage by rapid oxidation of excess amounts of cytosolic DA and L-DOPA to form neuroprotective melanin, and by its DA-synthesizing property.

Concluding Remarks

In conclusion, it is of interest that the cytotoxicity of quinone formation is closely related not only to general oxidative stress but also to mitochondrial dysfunction and inflammation. In other words, quinone formation by auto-oxidation of DA may play an important role as a DA neuron-specific common degenerative factor that closely links representative hypotheses, mitochondrial dysfunction, the inflammatory process, oxidative stress, and impairment of the ubiquitin-proteasome system in the pathogenesis and progress of PD. Further studies will be required to clarify the role of quinone formation in patients with PD.

References

1. Sulzer D and Zecca L: Intraneuronal dopamine-quinone synthesis: a review. Neurotox Res (2000) 1: 181–195.
2. Conway KA, Rochet JC, Bieganski RM and Lansbury PT Jr: Kinetic stabilization of the α-synuclein protofibril by a dopamine-α-synuclein adduct. Science (2001) 294: 1346–1349.
3. Asanuma M, Miyazaki I and Ogawa N: Dopamine- or L-DOPA-induced neurotoxicity: the role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease. Neurotox Res (2003) 5: 165–176.
4. Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM and Kopin IJ: Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res (1979) 1: 249–254.
5. Langston JW, Ballard P, Tetrud JW and Irwin I: Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science (1983) 219: 979–980.
6. Ramsay RR and Singer TP: Energy dependent uptake of MPP^+, the neurotoxic metabolite of MPTP, by mitochondria. J Biol Chem (1986) 261: 7585–7587.
7. Mizuno Y, Sone N and Saitoh T: Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylptridinium ion on activities of the enzymes in the electron transport in mouse brain. J Neurochem (1987) 48: 1787–1793.
8. Glinka YY and Youdim MB: Inhibition of mitochondrial complexes I and IV by 6-hydroxydopamine. Eur J Pharmacol (1995) 292: 329–332.
9. Glinka Y, Gassen M and Youdim MB: Mechanism of 6-hydroxydopamine neurotoxicity. J Neural Transm Suppl (1997) 50: 55–66.
10. Dabbeni-Sala F, Di Santo S, Franceschini D, Skaper SD and Giusti P: Melatonin protects against 6-OHDA-induced neurotoxicity in rats: a role for mitochondrial complex I activity. FASEB J (2001) 15: 164–170.
11. Mazzio EA, Reams RR and Soliman KF: The role of oxidative stress, impaired glycolysis and mitochondrial respiratory redox failure in the cytotoxic effects of 6-hydroxydopamine *in vitro*. Brain Res (2004) 1004: 29–44.
12. Storch A, Kaftan A, Burkhardt K and Schwarz J: 6-Hydroxydopamine toxicity towards human SH-SY5Y dopaminergic neuroblastoma cells: independent of mitochondrial energy metabolism. J Neural Transm (2000) 107: 281–293.
13. Hölinger GU, Carrard G, Michel PP, Medja F, Lombès A, Ruberg M, Friguet B and Hirsch EC: Dysfunction of mitochondrial complex I and the proteasome: interactions between two biochemical deficits in a cellular model of Parkinson's disease. J Neurochem (2003) 86: 1297–1307.
14. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV and Greenamyre JT: Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci (2000) 3: 1301–1306.
15. Stern EL, Quan N, Proescholdt MG and Herkenham M: Spatiotemporal induction patterns of cytokine and related immune signal molecule mRNAs in response to intrastriatal injection of lipopolysaccharide. J Neuroimmunol (2000) 109: 245–260.
16. Castano A, Herrera AJ, Cano J and Machado A: Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. J Neurochem (1998) 70: 1584–1592.
17. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS and Liu B: Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem (2002) 81: 1285–1297.
18. Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Czlonkowski A and Czlonkowska A: The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication in mouse. Exp Neurol (1999) 156: 50–61.
19. Sherer TB, Betarbet R, Kim JH and Greenamyre JT: Selective microglial activation in the rat rotenone model of Parkinson's disease. Neurosci Lett (2003) 341: 87–90.
20. Gao HM, Hong JS, Zhang W and Liu B: Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. J Neurosci (2002) 22: 782–790.
21. Gao HM, Liu B and Hong JS: Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J Neurosci (2003) 23: 6181–6187.
22. Gao HM, Liu B, Zhang W and Hong JS: Novel anti-inflammatory therapy for Parkinson's disease. Trends Pharmacol Sci (2003) 24: 395–401.
23. Aubin N, Curet O, Deffois A and Carter C: Aspirin and salicylate protect against MPTP-induced dopamine depletion in mice. J Neurochem (1998) 71: 1635–1642.
24. Ferger B, Teismann P, Earl CD, Kuschinsky K and Oertel WH: Salicylate protects against MPTP-induced impairments in dopaminergic neurotransmission at the striatal and nigral level in mice. Naunyn Schmiedebergs Arch Pharmacol (1999) 360: 256–261.
25. Mohanakumar KP, Muralikrishnan D and Thomas B: Neuroprotection

by sodium salicylate against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. Brain Res (2000) 864: 281–290.
26. Teismann P and Ferger B: Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease. Synapse (2001) 39: 167–174.
27. Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP and Paul SM: Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci USA (2001) 98: 14669–14674.
28. He Y, Appel S and Le W: Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. Brain Res (2001) 909: 187–193.
29. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H and Przedborski S: Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci (2002) 22: 1763–1771.
30. Tse DC, McCreery RL and Adams RN: Potential oxidative pathways of brain catecholamines. J Med Chem (1976) 19: 37–40.
31. Graham DG: Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. Mol Pharmacol (1978) 14: 633–643.
32. Sinha BK, Singh Y and Krishna G: Formation of superoxide and hydroxyl radicals from 1-methyl-4-phenylpyridinium ion (MPP^+): reductive activation by NADPH cytochrome P450 reductase. Biochem Biophys Res Commun (1986) 135: 583–588.
33. Chacon JN, Chedekel MR, Land EJ and Truscott TG: Chemically induced Parkinson's disease: intermediates in the oxidation of 1-methyl-4-phenylpyridinium ion. Biochem Biophys Res Commun (1987) 144: 957–964.
34. Smith TS and Bennett JJ: Mitochondrial toxins in models of neurodegenerative diseases. I: *In vivo* brain hydroxyl radical production during systemic MPTP treatment or following microdialysis infusion of methylpyridinium or azide ions. Brain Res (1997) 765: 183–188.
35. Cassarino DS, Fall CP, Swerdlow RH, Smith TS, Halvorsen EM, Miller SW, Parks JP, Parker WJ and Bennett JJ: Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease. Biochim Biophys Acta (1997) 1362: 77–86.
36. Przedborski S, Kostic V, Jackson-Lewis V, Naini AB, Simonetti S, Fahn S, Carlson E, Epstein CJ and Cadet JL: Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. J Neurosci (1992) 12: 1658–1667.
37. Heikkila RE and Cohen G: 6-Hydroxydopamine: evidence for superoxide radical as an oxidative intermediate. Science (1973) 181: 456–457.
38. Cohen G and Heikkila RE: The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. J Biol Chem (1974) 249: 2447–2452.
39. Ogawa N, Asanuma M, Kondo Y, Hirata H, Nishibayashi S and Mori A: Changes in lipid peroxidation, Cu/Zn-superoxide dismutase and its mRNA following an intracerebroventricular injection of 6-hydroxydopamine in mice. Brain Res (1994) 646: 337–340.
40. Asanuma M, Hirata H and Cadet JL: Attenuation of 6-hydroxydopamine-induced dopaminergic nigrostriatal lesions in superoxide dismutase transgenic mice. Neuroscience (1998) 85: 907–917.
41. Meyer M, Schreck R and Baeuerle PA: H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. EMBO J (1993) 12: 2005–2015.
42. Simpson CS and Morris BJ: Activation of nuclear factor κB by nitric oxide in rat striatal neurones: differential inhibition of the p50 and p65 subunits by dexamethasone. J Neurochem (1999) 73: 353–361.
43. O'Neill LAJ and Kaltschmidt C: NF-κB: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci (1997) 20: 252–258.
44. Zoratti M and Szabo I: The mitochondrial permeability transition. Biochim Biophys Acta (1995) 1241: 139–176.
45. Lizasoain I, Moro MA, Knowles RG, Darley UV and Moncada S: Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. Biochem J (1996) 314: 877–880.
46. McNaught KS, Olanow CW, Halliwell B, Isacson O and Jenner P: Failure of the ubiquitin-proteasome system in Parkinson's disease. Nat Rev Neurosci (2001) 2: 589–594.
47. Chung KK, Dawson VL and Dawson TM: The role of the ubiquitin-proteasomal pathway in Parkinson's disease and other neurodegenerative disorders. Trends Neurosci (2001) 24: S7–14.
48. McNaught KS and Olanow CW: Proteolytic stress: a unifying concept for the etiopathogenesis of Parkinson's disease. Ann Neurol (2003) 53: S73–84; discussion S84–76.
49. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K and Suzuki T: Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet (2000) 25: 302–305.
50. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL and Helfand SL: Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science (1997) 276: 2045–2047.
51. Tanaka Y, Engelender S, Igarashi S, Rao RK, Wanner T, Tanzi RE, Sawa A, Dawson VL, Dawson TM and Ross CA: Inducible expression of mutant α-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. Hum Mol Genet (2001) 10: 919–926.
52. Imai Y, Soda M and Takahashi R: Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. J Biol Chem (2000) 275: 35661–35664.
53. Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D and Greene LA: Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. J Neurosci (2002) 22: 10690–10698.
54. Diaz-Corrales FJ, Asanuma M, Miyazaki I and Ogawa N: Rotenone induces disassembly of the Golgi apparatus in the rat dopaminergic neuroblastoma B65 cell line. Neurosci Lett (2004) 354: 59–63.
55. Elkon H, Melamed E and Offen D: 6-Hydroxydopamine increases ubiquitin-conjugates and protein degradation: implications for the pathogenesis of Parkinson's disease. Cell Mol Neurobiol (2001) 21: 771–781.
56. Sullivan PG, Dragicevic NB, Deng JH, Bai Y, Dimayuga E, Ding Q, Chen Q, Bruce-Keller AJ and Keller JN: Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. J Biol Chem (2004) 279: 20699–20707.
57. Rockwell P, Yuan H, Magnusson R and Figueiredo-Pereira ME: Proteasome inhibition in neuronal cells induces a proinflammatory response manifested by upregulation of cyclooxygenase-2, its accu-

mulation as ubiquitin conjugates, and production of the prostaglandin PGE2. Arch Biochem Biophys (2000) 374: 325–333.
58. Rideout HJ, Larsen KE, Sulzer D and Stefanis L: Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. J Neurochem (2001) 78: 899–908.
59. McNaught KS, Mytilineou C, Jnobaptiste R, Yabut J, Shashidharan P, Jennert P and Olanow CW: Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. J Neurochem (2002) 81: 301–306.
60. Fornai F, Lenzi P, Gesi M, Ferrucci M, Lazzeri G, Busceti CL, Ruffoli R, Soldani P, Ruggieri S, Alessandri MG and Paparelli A: Fine structure and biochemical mechanisms underlying nigrostriatal inclusions and cell death after proteasome inhibition. J Neurosci (2003) 23: 8955–8966.
61. McNaught KS, Bjorklund LM, Belizaire R, Isacson O, Jenner P and Olanow CW: Proteasome inhibition causes nigral degeneration with inclusion bodies in rats. Neuroreport (2002) 13: 1437–1441.
62. Feany MB and Bender WW: A Drosophila model of Parkinson's disease. Nature (2000) 404: 394–398.
63. Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A and Mucke L: Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science (2000) 287: 1265–1269.
64. Pendleton RG, Parvez F, Sayed M and Hillman R: Effects of pharmacological agents upon a transgenic model of Parkinson's disease in Drosophila melanogaster. J Pharmacol Exp Ther (2002) 300: 91–96.
65. Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ and Bjorklund A: Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J Neurosci (2002) 22: 2780–2791.
66. Lo Bianco C, Ridet JL, Schneider BL, Deglon N and Aebischer P: alpha -Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci USA (2002) 99: 10813–10818.
67. Goldberg MS and Lansbury PT Jr: Is there a cause-and-effect relationship between alpha-synuclein fibrillization and Parkinson's disease? Nat Cell Biol (2000) 2: E115–119.
68. Sawada H, Kohno R, Kihara T, Izumi Y, Sakka N, Ibi M, Nakanishi M, Nakamizo T, Yamakawa K, Shibasaki H, Yamamoto N, Akaike A, Inden M, Kitamura Y, Taniguchi T and Shimohama S: Proteasome mediates dopaminergic neuronal degeneration, and its inhibition causes alpha-synuclein inclusions. J Biol Chem (2004) 279: 10710–10719.
69. Bindoff LA, Birch-Machin M, Cartlidge NEF, Parker WD Jr and Turnbull DM: Mitochondrial function in Parkinson's disease. Lancet (1989) 2: 49.
70. Mizuno Y, Ohta S, Tanaka M, Takamiya S, Suzuki K, Sato T, Oya H, Ozawa T and Kagawa Y: Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. Biochem Biophys Res Commun (1989) 163: 1450–1455.
71. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P and Marsden CD: Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem (1990) 54: 823–827.
72. Nagatsu T: Parkinson's disease: changes in apoptosis-related factors suggesting possible gene therapy. J Neural Transm (2002) 109: 731–745.
73. Hunot S and Hirsch EC: Neuroinflammatory processes in Parkinson's disease. Ann Neurol (2003) 53: S49–58.
74. Hunot S, Hartmann A and Hirsch EC: The inflammatory response in the Parkinson brain. Clin Neurosci Res (2001) 1: 434–443.
75. McGeer PL, Itagaki S, Boyes BE and McGeer EG: Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology (1988) 38: 1285–1291.
76. Knott C, Stern G and Wilkin GP: Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci (2000) 16: 724–739.
77. Hunot S, Brugg B, Ricard D, Michel PP, Muriel M-P, Ruberg M, Faucheux B, Agid Y and Hirsch EC: Nuclear translocation of NF-κB is increased in dopaminergic neurons of patients with Parkinson disease. Proc Natl Acad Sci USA (1997) 94: 7531–7536.
78. Nagatsu T, Mogi M, Ichinose H and Togari A: Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm Suppl (2000) 58: 277–290.
79. Ambani LM, Van Woert MH and Murphy S: Brain peroxidase and catalase in Parkinson's disease. Arch Neurol (1975) 32: 114–118.
80. Kish SJ, Morito C and Hornykiewicz O: Glutathione peroxidase activity in Parkinson's disease. Neurosci Lett (1985) 58: 343–346.
81. Perry TL and Yong VW: Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. Neurosci Lett (1986) 67: 269–274.
82. Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K and Youdim MBH: Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. J Neurochem (1989) 52: 515–520.
83. Dexter DT, Wells FR, Lees AJ, Agid Y, Jenner P and Marsden CD: Increase nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. J Neurochem (1989) 52: 1830–1836.
84. Olanow CW and Cohen G: The pathogenesis of Parkinson's disease; in The Scientific Basis for the Treatment of Parkinson's Disease, Olanow CW and Lieberman AN eds, The Parthenon Publishing Group, Lancs, UK (1992) pp 59–76.
85. Good PF, Hsu A, Werner P, Perl DP and Olanow CW: Protein nitration in Parkinson's disease. J Neuropathol Exp Neurol (1998) 57: 338–342.
86. McNaught KS and Jenner P: Proteasomal function is impaired in substantia nigra in Parkinson's disease. Neurosci Lett (2001) 297: 191–194.
87. McNaught KS, Belizaire R, Jenner P, Olanow CW and Isacson O: Selective loss of 20S proteasome alpha-subunits in the substantia nigra pars compacta in Parkinson's disease. Neurosci Lett (2002) 326: 155–158.
88. McNaught KS, Belizaire R, Isacson O, Jenner P and Olanow CW: Altered proteasomal function in sporadic Parkinson's disease. Exp Neurol (2003) 179: 38–46.
89. Choi P, Ostrerova GN, Sparkman D, Cochran E, Lee JM and Wolozin B: Parkin is metabolized by the ubiquitin/proteosome system. Neuroreport (2000) 11: 2635–2638.
90. Schlossmacher MG, Frosch MP, Gai WP, Medina M, Sharma N, Forno L, Ochiishi T, Shimura H, Sharon R, Hattori N, Langston JW, Mizuno Y, Hyman BT, Selkoe DJ and Kosik KS: Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. Am J Pathol (2002) 160: 1655–1667.
91. Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR and Greenamyre JT: An *in vitro* model of Parkinson's disease: linking mitochondrial impairment to altered α-synuclein metabolism and oxidative damage. J Neurosci (2002) 22: 7006–7015.
92. Sakka N, Sawada H, Izumi Y, Kume T, Katsuki H, Kaneko S,

Shimohama S and Akaike A: Dopamine is involved in selectivity of dopaminergic neuronal death by rotenone. Neuroreport (2003) 14: 2425–2428.
93. Sulzer D, Bogulavsky J, Larsen KE, Behr G, Karatekin E, Kleinman MH, Turro N, Krantz D, Edwards RH, Greene LA and Zecca L: Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. Proc Natl Acad Sci USA (2000) 97: 11869–11874.
94. Korytowski W, Sarna T, Kalyanaraman B and Sealy RC: Tyrosinase-catalyzed oxidation of dopa and related catechol(amine)s: a kinetic electron spin resonance investigation using spin-stabilization and spin label oximetry. Biochim Biophys Acta (1987) 924: 383–392.
95. Rosei MA, Blarzino C, Foppoli C, Mosca L and Coccia R: Lipoxygenase-catalyzed oxidation of catecholamines. Biochem Biophys Res Commun (1994) 200: 344–350.
96. Hastings TG: Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. J Neurochem (1995) 64: 919–924.
97. Foppoli C, Coccia R, Cini C and Rosei MA: Catecholamines oxidation by xanthine oxidase. Biochim Biophys Acta (1997) 1334: 200–206.
98. Fornstedt B, Rosengren E and Carlsson A: Occurrence and distribution of 5-S-cysteinyl derivatives of dopamine, dopa and dopac in the brains of eight mammalian species. Neuropharmacology (1986) 25: 451–454.
99. Ito S and Fujita K: Conjugation of dopa and 5-*S*-cysteinyldopa with cysteine mediated by superoxide radical. Biochem Pharmacol (1982) 31: 2887–2889.
100. Lai CT and Yu PH: Dopamine- and L-β-3,4-dihydroxyphenylalanine hydrochloride (L-Dopa)-induced cytotoxicity towards catecholaminergic neuroblastoma SH-SY5Y cells. Effects of oxidative stress and antioxidative factors. Biochem Pharmacol (1997) 53: 363–372.
101. LaVoie MJ and Hastings TG: Peroxynitrite- and nitrite-induced oxidation of dopamine: implications for nitric oxide in dopaminergic cell loss. J Neurochem (1999) 73: 2546–2554.
102. Pardo B, Mena MA, Casarejos MJ, Paino CL and De Yébenes JG: Toxic effects of L-dopa on mesencephalic cell cultures: protection with antioxidants. Brain Res (1995) 682: 133–143.
103. Kuhn DM, Arthur RE Jr, Thomas DM and Elferink LA: Tyrosine hydroxylase is inactivated by catechol-quinones and converted to a redox-cycling quinoprotein: possible relevance to Parkinson's disease. J Neurochem (1999) 73: 1309–1317.
104. Xu Y, Stokes AH, Roskoski R Jr and Vrana KE: Dopamine, in the presence of tyrosinase, covalently modifies and inactivates tyrosine hydroxylase. J Neurosci Res (1998) 54: 691–697.
105. Hastings TG, Lewis DA and Zigmond MJ: Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. Proc Natl Acad Sci USA (1996) 93: 1956–1961.
106. Haque ME, Asanuma M, Higashi Y, Miyazaki I, Tanaka K and Ogawa N: Apoptosis-inducing neurotoxicity of dopamine and its metabolites via reactive quinone generation in neuroblastoma cells. Biochim Biophys Acta (2003) 1619: 39–52.
107. Cadenas E, Mira D, Brunmark A, Lind C, Segura-Aguilar J and Ernster L: Effect of superoxide dismutase on the autoxidation of various hydroquinones—a possible role of superoxide dismutase as a superoxide: semiquinone oxidoreductase. Free Radic Biol Med (1988) 5: 71–79.
108. Haque ME, Asanuma M, Higashi Y, Miyazaki I, Tanaka K and Ogawa N: Overexpression of Cu-Zn superoxide dismutase protects neuroblastoma cells against dopamine cytotoxicity accompanied by increase in their glutathione level. Neurosci Res (2003) 47: 31–37.
109. Offen D, Ziv I, Sternin H, Melamed E and Hochman A: Prevention of dopamine-induced cell death by thiol antioxidants: possible implications for treatment of Parkinson's disease. Exp Neurol (1996) 141: 32–39.
110. Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE and Lansbury PT Jr: Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA (2000) 97: 571–576.
111. Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC and Lansbury PT Jr: Vesicle permeabilization by protofibrillar α-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry (2001) 40: 7812–7819.
112. Kuhn DM and Arthur R Jr: Dopamine inactivates tryptophan hydroxylase and forms a redox-cycling quinoprotein: possible endogenous toxin to serotonin neurons. J Neurosci (1998) 18: 7111–7117.
113. Baez S, Linderson Y and Segura-Aguilar J: Superoxide dismutase and catalase enhance autoxidation during one- electron reduction of aminochrome by NADPH-cytochrome P-450 reductase. Biochem Mol Med (1995) 54: 12–18.
114. Drukarch B and van Muiswinkel FL: Drug treatment of Parkinson's disease. Time for phase II. Biochem Pharmacol (2000) 59: 1023–1031.
115. Paris I, Dagnino-Subiabre A, Marcelain K, Bennett LB, Caviedes P, Caviedes R, Azar CO and Segura-Aguilar J: Copper neurotoxicity is dependent on dopamine-mediated copper uptake and one-electron reduction of aminochrome in a rat substantia nigra neuronal cell line. J Neurochem (2001) 77: 519–529.
116. Segura-Aguilar J, Metodiewa D and Welch CJ: Metabolic activation of dopamine *o*-quinones to *o*-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. Biochim Biophys Acta (1998) 1381: 1–6.
117. Smythies J: The neurotoxicity of glutamate, dopamine, iron and reactive oxygen species: functional interrelationships in health and disease: a review-discussion. Neurotox Res (1999) 1: 27–39.
118. Solano F, Hearing VJ and Garcia-Borron JC: Neurotoxicity due to *o*-quinone: neuromelanin formation and possible mechanisms for *o*-quinone detoxification. Neurotox Res (2000) 1: 153–169.
119. Diaz-Veliz G, Mora S, Dossi MT, Gomez P, Arriagada C, Montiel J, Aboitiz F and Segura-Aguilar J: Behavioral effects of aminochrome and dopachrome injected in the rat substantia nigra. Pharmacol Biochem Behav (2002) 73: 843–850.
120. Berman SB and Hastings TG: Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J Neurochem (1999) 73: 1127–1137.
121. Li H and Dryhurst G: Irreversible inhibition of mitochondrial complex I by 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid (DHBT-1): a putative nigral endotoxin of relevance to Parkinson's disease. J Neurochem (1997) 69: 1530–1541.
122. Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S, Vila M, Jackson-Lewis V and Przedborski S: Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. Proc Natl Acad Sci USA (2003) 100: 5473–5478.
123. Mattammal MB, Strong R, Lakshmi VM, Chung HD and Stephenson AH: Prostaglandin H synthetase-mediated metabolism of dopamine: implication for Parkinson's disease. J Neurochem (1995) 64: 1645–1654.
124. Cheng FC, Kuo JS, Chia LG and Dryhurst G: Elevated 5-*S*-cysteinyldopamine/homovanillic acid ratio and reduced homovanillic acid in cerebrospinal fluid: possible markers for and potential insights into the pathoetiology of Parkinson's disease. J Neural Transm (1996) 103: 433–446.

125. Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC and Halliwell B: Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J Neurochem (1998) 71: 2112–2122.
126. Keller JN, Huang FF, Dimayuga ER and Maragos WF: Dopamine induces proteasome inhibition in neural PC12 cell line. Free Radic Biol Med (2000) 29: 1037–1042.
127. Schultzberg M, Segura-Aguilar J and Lind C: Distribution of DT diaphorase in the rat brain: biochemical and immunohistochemical studies. Neuroscience (1988) 27: 763–776.
128. Segura-Aguilar J and Lind C: On the mechanism of the Mn3(+)-induced neurotoxicity of dopamine: prevention of quinone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. Chem Biol Interact (1989) 72: 309–324.
129. Munday R, Smith BL and Munday CM: Effects of butylated hydroxyanisole and dicoumarol on the toxicity of menadione to rats. Chem Biol Interact (1998) 108: 155–170.
130. Duffy S, So A and Murphy TH: Activation of endogenous antioxidant defenses in neuronal cells prevents free radical-mediated damage. J Neurochem (1998) 71: 69–77.
131. Choi HJ, Kim SW, Lee SY and Hwang O: Dopamine-dependent cytotoxicity of tetrahydrobiopterin: a possible mechanism for selective neurodegeneration in Parkinson's disease. J Neurochem (2003) 86: 143–152.
132. Hara H, Ohta M, Ohta K, Kuno S and Adachi T: Increase of antioxidative potential by *tert*-butylhydroquinone protects against cell death associated with 6-hydroxydopamine-induced oxidative stress in neuroblastoma SH-SY5Y cells. Mol Brain Res (2003) 119: 125–131.
133. Baez S, Segura-Aguilar J, Widersten M, Johansson AS and Mannervik B: Glutathione transferases catalyse the detoxication of oxidized metabolites (*o*-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. Biochem J (1997) 324: 25–28.
134. Segura-Aguilar J, Baez S, Widersten M, Welch CJ and Mannervik B: Human class Mu glutathione transferases, in particular isoenzyme M2-2, catalyze detoxication of the dopamine metabolite aminochrome. J Biol Chem (1997) 272: 5727–5731.
135. Asanuma M, Miyazaki I and Ogawa N: Neuroprotective effects of nonsteroidal anti-inflammatory drugs on neurodegenerative diseases. Current Pharmaceutical Design (2004) 10: 695–700.
136. Miranda M and Botti D: Harding-passey mouse-melanoma tyrosinase inactivation by reaction products and activation by L-epinephrine. Gen Pharmacol (1983) 14: 231–237.
137. Higashi Y, Asanuma M, Miyazaki I and Ogawa N: Inhibition of tyrosinase reduces cell viability in catecholaminergic neuronal cells. J Neurochem (2000) 75: 1771–1774.
138. Rios M, Habecker B, Sasaoka T, Eisenhofer G, Tian H, Landis S, Chikaraishi D and Roffler-Tarlov S: Catecholamine synthesis is mediated by tyrosinase in the absence of tyrosine hydroxylase. J Neurosci (1999) 19: 3519–3526.
139. Zecca L, Zucca FA, Wilms H and Sulzer D: Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends Neurosci (2003) 26: 578–580.