THE BIPYRIDYL HERBICIDE PARAQUAT-INDUCED TOXICITY IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS: RELEVANCE TO DOPAMINERGIC PATHOGENESIS

A Dissertation

by

WONSUK YANG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Toxicology

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ABSTRACT

The Bipyridyl Herbicide Paraquat-Induced Toxicity in

Human Neuroblastoma SH-SY5Y Cells:

Relevance to Dopaminergic Pathogenesis. (August 2005)

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Paraquat (PQ) is a cationic non-selective bipyridyl herbicide widely used in agriculture to control weeds and grasses. Epidemiologic studies indicate that exposure to pesticides can be a risk factor in the incidence of Parkinson`s disease (PD). A strong correlation has been reported between exposure to paraquat and PD incidence in Canada, Taiwan, and United States. This correlation is supported by animal studies showing that paraquat produces toxicity in dopaminergic neurons of the rat and mouse brain. However, it is unclear how paraquat triggers toxicity in dopaminergic neurons. Based on the previous reports, it was hypothesized that paraquat may induce oxidative stress and proteasomal dysfunction-mediated toxicity in dopaminergic neurons. To explore this possibility, dopaminergic SH-SY5Y human neuroblastoma cells were treated with paraquat, and several biomarkers of oxidative stress or proteasomal dysfunction were investigated. First, a specific dopamine transporter inhibitor GBR12909 significantly protected SY5Y cells against the toxicity of paraquat, indicating that paraquat exerts its toxicity by a mechanism involving the dopamine transporter (DAT). Second, paraquat

increased the levels of reactive oxygen species (ROS) in SY5Y cells, but decreased the levels of glutathione. Third, paraquat inhibited glutathione peroxidase activity, but did not affect glutathione reductase activity. On the other hand, paraquat increased GST activity by 24 hr, after which GST activity returned to the control value at 48 hr. Fourth, paraquat decreased mitochondrial transmembrane potential (MTP). Fifth, paraquat produced the increases in malondialdehyde (MDA) and protein carbonyls, as well as DNA fragmentation, indicating oxidative damage to major cellular components. Sixth, paraquat decreased proteasomal activity, the activities of mitochondrial complex I and V, and intracellular ATP levels, but increased the activities of caspase 3 and 9, indicating that proteasomal inhibition is linked to mitochondrial dysfunction accompanied by the activation of apoptotic signaling pathway. Seventh, paraquat increased the protein levels of heme oxygenase-1 (HO-1), p53, Bax, α-synuclein and ubiquitinated proteins. Eighth, paraquat induced nuclear condensation. Taken together, these findings support the hypothesis that paraquat produces oxidative stress and proteasomal dysfunction-mediated toxicity in SY5Y cells. Thus, current findings suggest that paraquat may induce the pathogenesis of dopaminergic neurons through oxidative stress and proteasomal dysfunction.

DEDICATION

To my parents, Kunho Yang and Soonja Lee

To my wife`s parents, Haetak Kwak and Sungja Lee

To my younger brother Jaesuk Yang

To my wife`s younger brother and sister, Keejun Kwak and Yoonmi Kwak

To my wife Hwajin Kwak and my two kids, Seohyun Yang and Yoona Yang

For their encouragement, love, support, and patience.

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I would like to thank my committee members for their help. Dr. Welsh allowed me to use an inverted light microscope to capture the morphology of SY5Y cells. Dr. Donnelly made me obtain critical toxicology concepts through his class. Dr. Miranda always showed me how to do research as a scientist.

I would like to thank our lab people for their help. Dr. Venkatraj helped me isolate genomic DNA from SY5Y cells for DNA fragmentation analysis. Dr. Qian assisted me in capturing nuclear morphology of SY5Y cells with a fluorescence microscope.

I would like to thank my friends for their help. Dr. Jeong-Eun Lee very kindly provided me with a lot of chemicals for my experiments. Dr. Jinwoong Bok discussed research with me and gave me important ideas all the time.

I would like to thank Mr. Hyeongil Kwak for helping me use the microplate reader. I would like to thank Mrs. Daniel Kimberly for academic administrative help.

TABLE OF CONTENTS

Page

CHAPTER Page

CHAPTER Page

LIST OF FIGURES

FIGURE Page

LIST OF ABBREVIATIONS

AD: Alzheimer`s diseasae

ALS: Amyotrophic lateral sclerosis

AP-1: activator protein 1

ARJP: autosomal recessive juvenile Parkinsonism

BBB: blood-brain barrier

BDNF: brain-derived neurotrophic factor

BSO: L-buthionine sulfoximine

COMT: catechol-*O*-methyltransferase

CREB: cAMP response element binding protein

DAT: dopamine transporter

DCF-DA: 2',7'-dichlorofluorescein diacetate

D2R: dopamine receptor 2

GDNF: glial-derived neurotrophic factor

GPx: glutathione peroxidase

GR: glutathione reductase

GST: glutathione S-Trandferase

HD: Huntington`s disease

HO-1: heme oxygenase-1

JNK: c-jun kinase

LDH: lactate dehydrogenase

MAO A/B: monoamine oxidase A/B

MCB: monochlorobimane

MDA: malondialdehyde

MG132: *N*-[(Phenylmethoxy) carbonyl]-L-leucyl-*N*-[(1*S*)-1-formyl-3-methylbutyl]-L-leucinamide

MPTP: 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine

MPP+: 1-methyl-4phenylpyridium ion

MTP: mitochondrial transmembrane potential

MTT: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide

NOS: nitric oxide synthase

Nurr 1: nuclear receptor-related factor 1

PD: Parkinson`s disease

ROS: reactive oxygen species

SNpc: substantia nigra pars compacta

TH: tyrosine hydroxylase

TMRE: tetramethylrhodamine ethyl ester

UCH-L1: ubiquitin C-terminal hydrolase L1

VMAT 2: vesicular monoamine transporter 2

CHAPTER I

INTRODUCTION

Incidence, clinical symptoms and pathological features of Parkinson`s disease (PD)

Parkinson`s disease (PD) was first described by James Parkinson in 1817. PD is second only to Alzheimer`s disease (AD) as the most common idiopathic neurodegenerative disorder affecting roughly 1-2% of the current population over the age of 65, further increasing in incidence at older ages (de Rijk et al., 1995). Its incidence has been reported to range from 4.5 to 21 new cases per 100,000 populations per year and its prevalence to vary from 18 to 328 per 100,000 people (Tanner, 2003). PD is more common among men than women, although gender-specific differences show more variability worldwide than increasing age (Tanner & Yaov, 1999). The clinical symptoms of PD are tremor, rigidity, bradykinesia, gait disturbance, postural instability, excessive sweating, depression, and loss of facial expression (Fahn, 2003). PD is characterized pathologically by a specific and massive loss (50-70%) of dopaminergic neurons in the A9 area of human brain known as the *substantia nigra pars compacta* (SNpc), which is accompanied by a dramatic reduction of striatal dopamine levels (Lang & Lozano, 1998). An additional important pathological feature of PD is the

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presence of intracytoplasmic inclusions called Lewy bodies in neuronal cell body and Lewy neurites in neuronal processes of the remaining dopaminergic neurons (Forno, 1996).

Mechanisms of dopaminergic neuronal death

The mechanisms involved in the progressive degeneration of nigrostriatal dopaminergic neurons are of major interest in PD research. Recently, particular attention has focused on several conceptually distinct mechanisms, including oxidative stress, mitochondrial dysfunction, excitotoxicity, neurotrophic factors, neuroinflammation, viral infection, proteasomal dysfunction, and apoptosis. It may be possible that they all interact and amplify each other, leading to dopaminergic neuronal death in PD brains (Dunnett & Bjorklund, 1999). Each of thses potential mechanisms of dopaminergic neuronal death in PD will be briefly reviewed in the following sections.

Oxidative stress

Reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, and hydroxyl radicals are normally generated through cellular metabolism and most of them are neutralized by cellular antioxidant GSH and antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Dringen, 2000). Oxidative stress occurs as a consequence of the imbalance between the production of ROS and antioxidant capacity. The brain is specifically at risk for oxidative damage (Figure 1). Furthermore, dopaminergic neurons are inherently predisposed to oxidative

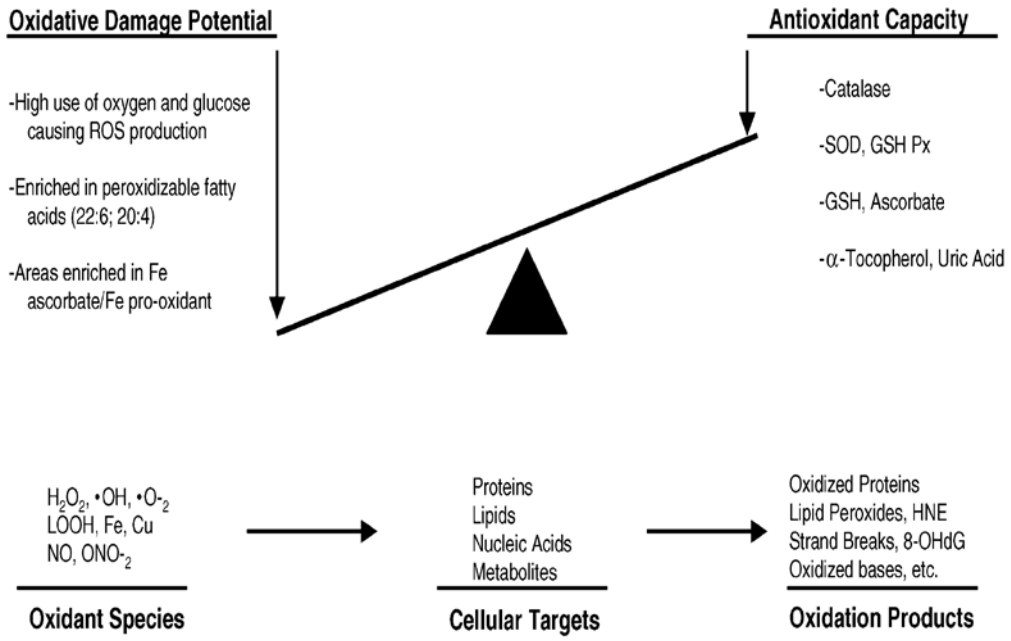

Figure 1. Schematic presentation illustrating how brain is at risk of oxidative damage (Floyd & Hensley, 2002). ROS are produced and interact with cellular targets producing unique oxidation products which in turn, may exert oxidative stress upon the tissue, too. Abbreviations and ROS include H_2O_2, hydrogen peroxide; $OH^{\cdot}$, hydroxyl free radical; $O_2^{\cdot -}$, superoxide; LOOH, lipid hydroperoxide; Fe, iron ion; Cu, copper ion; NO, nitric oxide; ONO^{2-}, peroxynitrite; SOD, superoxide dismutase; GSHPx, glutathione peroxidase; GSH, glutathione; HNE, 4-hydroxy-2-nonenal; and 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

stress because the metabolism of dopamine produces toxic dopamine quinones, superoxide anions, hydroxyl radicals, and hydrogen peroxide (Stokes et al., 1999; Blum et al., 2001). Indeed, studies of PD brains demonstrate that dopaminergic neurons are under oxidative stress condition as evidenced by the accumulation of iron (Good et al., 1992), the decrease of GSH (Pearce et al., 1997), and the increase of protein carbonyl (Alam et al., 1997a). In addition, the increase of malondialdehyde (MDA) (Dexter et al., 1994), 4-hydroxynonenal (4-HNE) (Yoritaka et al., 1996), and the increase of 8-hydroxyguanosine (Alam et al., 1997b) were observed in the surviving dopaminergic neurons.

Mitochondrial dysfunction

In addition to oxidative stress, mitochondria play a critical role in the health and survival of cells by providing ATP that fuels the maintenance, repair, and turnover of cellular components. Five respiratory chain complexes of mitochondria regulate the synthesis of ATP (Figure 2). One of those is complex I, which controls the transfer of one electron from NADPH to the coenzyme Q and the transfer of two protons to the mitochondrial intermembrane space. These protons are then used by complex V to synthesize ATP from ADP (Beal, 1992). Significantly, a selective 30-40% decrease in complex I activity of the mitochondrial respiratory chain has been found in SNpc of PD patients (Schapira et al., 1990). This defect has also been found in platelets and skeletal muscle of PD patients (Mann et al., 1992). Strong support for a mitochondrial DNA-encoded defect comes from studies showing that complex I defects from PD platelets are

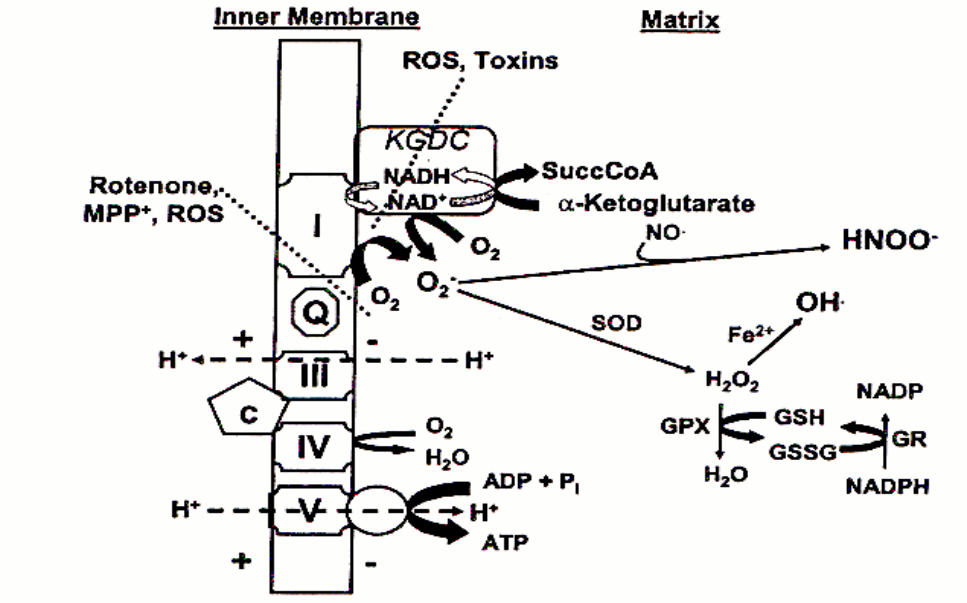

Figure 2. Mitochondrial generation and detoxification of reactive oxygen species (Fiskum et al., 2003). The site of mitochondrial ROS production most widely implicated in Parkinson's disease is complex I of the electron transport chain. Indirect evidence for involvement of complex I includes the observations that neurotoxicants capable of inducing Parkinson's symptoms and neuropathology *in vivo*, such as MPP^+ and rotenone, are inhibitors of complex I and stimulate ROS generation *in vitro*. However, these same agents result in inhibition of the overall enzyme activity of α-ketoglutarate dehydrogenase complex (αKGDC), a multisubunit complex that can also catalyze superoxide ($O_2^{\cdot-}$) and consequently H_2O_2 production. The ROS metabolites most likely to mediate oxidative injury to mitochondria and other cellular constituents are hydroxyl radical ($OH^{\bullet}$) and peroxynitrite ($HNOO^-$). These agents can cause oxidative damage and inhibition of mitochondrial enzyme activities, including those of complex I and αKGDC. This inhibition can lead to metabolic failure through impairment of electron transport-dependent generation of the proton-motive force that drives the synthesis of ATP at complex V (F_1F_0 ATP synthetase). Complete detoxification of superoxide depends on the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR) together with glutathione and a sufficiently reduced redox state of NAD(P)H to drive the reduction of glutathione and consequently the reduction of H_2O_2 to H_2O.

transferable into mitochondrial-deficient cell lines (Gu et al., 1998). These defects are associated with the additional generation of free radicals, the impairement of mitochondrial calcium buffering, the decrease of ATP synthesis, and the opening of permeability transition pore resulting in apoptosis (Nicholls, 2002).

Excitotoxicity

Glutamate-induced excitotoxicity is thought to represent a major mechanism in ischemia (Rothman & Olney, 1986) and epilepsy (Olney et al., 1986), and might play a role in PD (Rodriguez et al., 1998). Excess glutamate changes the permeability of cells to calcium ions (Ca^{2+}) by acting on and through *N*-methyl--aspartate (NMDA) receptors ultimately leading to neuronal death (Mody & MacDonald, 1995). Calcium-dependent NMDA neurotoxicity is based on both excessive nitric oxide formation (Jenner, 2003b) and mitochondrial dysfunction (Schinder et al., 1996). First, the extensive influx of calcium ions causes an activation of nitric oxide synthase, which converts L-arginine to citrulline and nitric oxide. Excess NO is in part responsible for glutamate neurotoxicity. It is likely that the neurotoxic actions of NO are mediated by peroxynitrite ($ONOO^-$). Peroxinitrite is the reaction product from NO and superoxide anion (Dawson & Dawson, 1996). Second, the excess Ca^{2+} entry accompanying NMDA-receptor activation is largely accumulated by the intracellular mitochondria, with effects on mitochondrial membrane potential, ATP synthesis, glycolysis, and ROS generation, leading to failures in cytoplasmic Ca^{2+} homeostasis and thus promoting cell death (Nicholls & Budd, 1998). Dopaminergic nigrostriatal neurons are rich in glutamate receptors and receive an

extensive glutamatergic innervation from the cortex and the subthalamic nucleus (Olanow & Tatton, 1999). Whereas the substantia nigra receives rich glutamatergic inputs, it has been speculated that glutamate-induced excitotoxicity may be involved in cell death in PD (Dunnett & Bjorklund, 1999). In support of a role for glutamate mediated excitotoxicity in PD, it has been reported that NMDA antagonists protect against loss of dopaminergic neurons resulting from 1,2,3,6-methyl-phenyl-tetrahydropyridine (MPTP) treatment in rats (Turski et al., 1991) and primates (Greenamyre et al., 1994). In addition, human studies indicate that the glutamatergic system may contribute to PD. The evaluation of glutamate binding sites in control and PD-diseased brains by autoradiography has revealed a reduction in NMDA-receptors as well as in -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding sites, whereas the metabotrophic binding sites seemed to be unaltered in the SNpc (Difazio et al., 1992). A decreased platelet glutamate uptake has also been evidenced in PD patients (Ferrarese et al., 2001).

Neurotrophic factors

In the normal adult central nervous system (CNS), neurotrophic factors are constitutively expressed at low levels, but abnormally high or low levels are associated with the brain pathology. Sublethal neuronal damage in adult rats induces reactive astrocytes with the upregulation of neurotrophic factors such as ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF) (Chadi et al., 1994; Asada et al., 1995). Reactive astrocytes are found in SNpc regions of PD brains (McGeer et al., 1988),

but an in situ hybridization study found no detectable levels of glial-derived neurotrophic factor (GDNF) mRNA in brains obtained from PD patients or age-matched controls (Hunot et al., 1996). In contrast, low levels of BDNF and basic fibroblast growth factor (bFGF) are found in the surviving dopaminergic neurons of PD brains (Tooyama et al., 1994; Mogi et al., 1999; Howells et al., 2000). Thus, dopaminergic neuronal death may be associated with the decrease and loss of neurotrophic factors because neurotrophic factors are known to protect dopaminergic neurons from toxic insults. Three lines of evidence support this hypothesis. First, brain-derived neurotrophic factor (BDNF) increases the survival of cultured dopaminergic neurons and protects them from exposure to MPTP (Hyman et al., 1991). Second, GDNF and ciliary neurotrophic factor (CNTF) protect SNpc neurons in rats from transection of nigrostriatal axons (Hagg and Varon, 1993; Lin et al., 1993). Third, GDNF has been shown to increase the survival and sprouting of dopaminergic neurons in dopamine-lesioned rodents and primates (Tomac et al., 1995; Gash et al., 1995) and to reverse parkinsonian features in MPTP-treated primates (Lapchak et al., 1997).

Neuroinflammation

There is increasing evidence that neuroinflammation may contribute to dopaminergic pathogenesis (Figure 3). The substantia nigra has the highest density of microglia in the brain (Kim et al., 2000) and reactive microglia have been found in the striatum and substantia nigra of PD patients (McGeer et al., 1988). Significantly, marked increases of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), inter

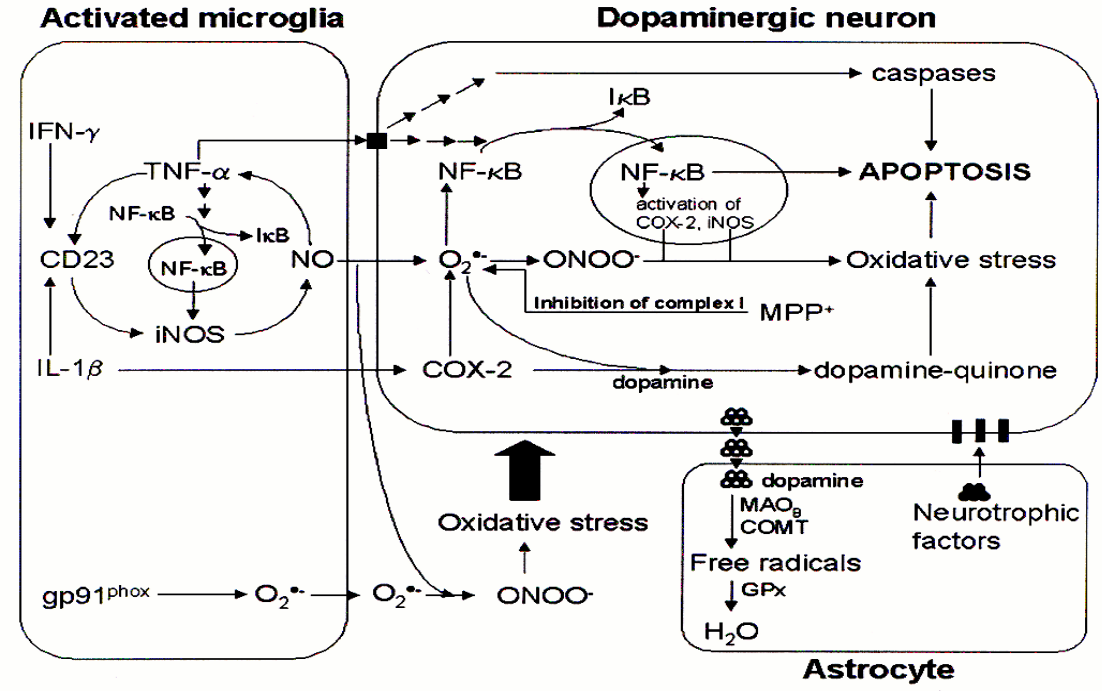

Figure 3. Potential involvement of glial cells in the pathogenesis of Parkinson's disease (Teismann et al., 2004). Activated microglial cells may contribute to dopaminergic neurodegeneration by releasing cytotoxic compounds such as cytokines (*TNF-α*, *IFN-γ*, *IL-1β*) which can induce CD23 expression and trigger iNOS expression with subsequent NO release. Cytokines may exert a direct effect on dopaminergic neurons by activating caspases, NF-κB mediated transduction pathways that lead to the activation of deleterious enzymes (*COX-2*, *iNOS*) resulting in oxidative stress or, alternatively, by direct induction of COX-2 within dopaminergic neurons. NO is membrane permeable and can diffuse to neighboring dopaminergic neurons where it can react with superoxide $O_2^{\cdot -}$ to form peroxynitrite ($ONOO^-$). COX-2 or direct inhibition of complex I of the respiratory chain are possible pathways of $O_2^{\cdot -}$ origin. Additionally activated microglia can release $O_2^{\cdot -}$ due to activated NADPH-oxidase (shown $gp91^{phox}$ upregulation). All of the described machinery leads ultimatively to the demise of the neuron. Astrocytes might have a protective effect by releasing neurotrophic factors, or metabolizing dopamine by monoamino oxidase-B (MAO_B) and catechol-*O*-methyl transferase (COMT), or eliminating free radicals by GPx.

feron-γ (IFN-γ), interleukin-1β (IL-1β), and interleukin–6 (IL-6) have been found in the substantia nigra, the striatum and the cerebrospinal fluid (CSF) of PD patients (Nagatsu et al., 2000). Furthermore, cyclooxygenase-2 (COX-2) is upregulated in dopaminergic neurons of PD patients (Teismann et al., 2003). Also, high levels of COX-2 and inducible nitric oxide synthase (iNOS) are found in glial cells of PD brains (Knott et al., 2000). Each enzyme is known to produce nitric oxide, superoxide anions and peroxinitrite, ultimately leading to the damage of surrounding dopaminergic neurons (Teismann & Schulz, 2004).

Viral infection

Some clinical reports and epidemiological data suggest that a virus may play a role in the etiology of PD. Indeed, an encephalitis virus induces postencephalitic parkinson-like symptoms in the patients (Duvoisin and Yahr, 1965; Kusano et al., 1966). In addition, the AIDS virus causes dopamine deficits in the patients (Sarder et al., 1996). Furthermore, influenza A virus has been suspected to contribute to the incidence of PD (Takahashi and Yamada, 2001). In support of these observations in humans, animal studies indicate that Theiler`s virus and mouse hepatitis have a predilection for and can severely damage the substantia nigra (Fishman et al., 1985; Simas et al., 1996). Significantly, Oliver et al. (1997) showed that Theiler`s virus specfically infects and destroys dopaminergic neurons in the substantia nigra of stereotaxically inoculated mouse.

Proteasomal dysfunction

Protein degradation is one of the essential mechanisms in the regulation of cellular proteins involved in cell cycle, development, growth, cell signaling, and antigen processing (De Martino & Slaughter, 1999). The ubiquitin-26S proteasome system (UPS) is a major pathway involved in detoxification and targeting of damaged proteins for degradation (Ciechanover & Brundin, 2003). Therefore, the dysfunction of UPS might cause the accumulation and aggregation of toxic proteins in the cell. Low chymotryptic-, tryptic-, and post-acidiclike hydrolyzing 20S/26S proteasomal activities have been found in the substantia nigra but not in the cortex or striatum of PD patients (McNaught & Jenner, 2001). Furthermore, the levels of α subunits not but β subunits of 20S are markedly reduced in dopaminergic neruons of SNpc (McNaught et al., 2002). Also, the proteasome activator 19S/PA700 is decreaed in SNpc of PD pateints (McNaught et al., 2003). Thus, these human brain studies suggest that the impairment of UPS might contribute to the formation of Lewy bodies, resulting in the dopaminergic pathogenesis of PD (Figure 4).

Apoptosis

Apoptosis is a morphologically and biochemically defined mode of cell death characterized by nuclear and cytoplasmic condensation, loss of mitochondrial transmembrane potential, DNA fragmentation, dilation of endoplasmic reticulum, blebbing of plasma membrane, formation of apoptotic bodies, and lack of an inflammatory reaction (Majno & Joris, 1995). It is triggered by the intrinsic suicidal

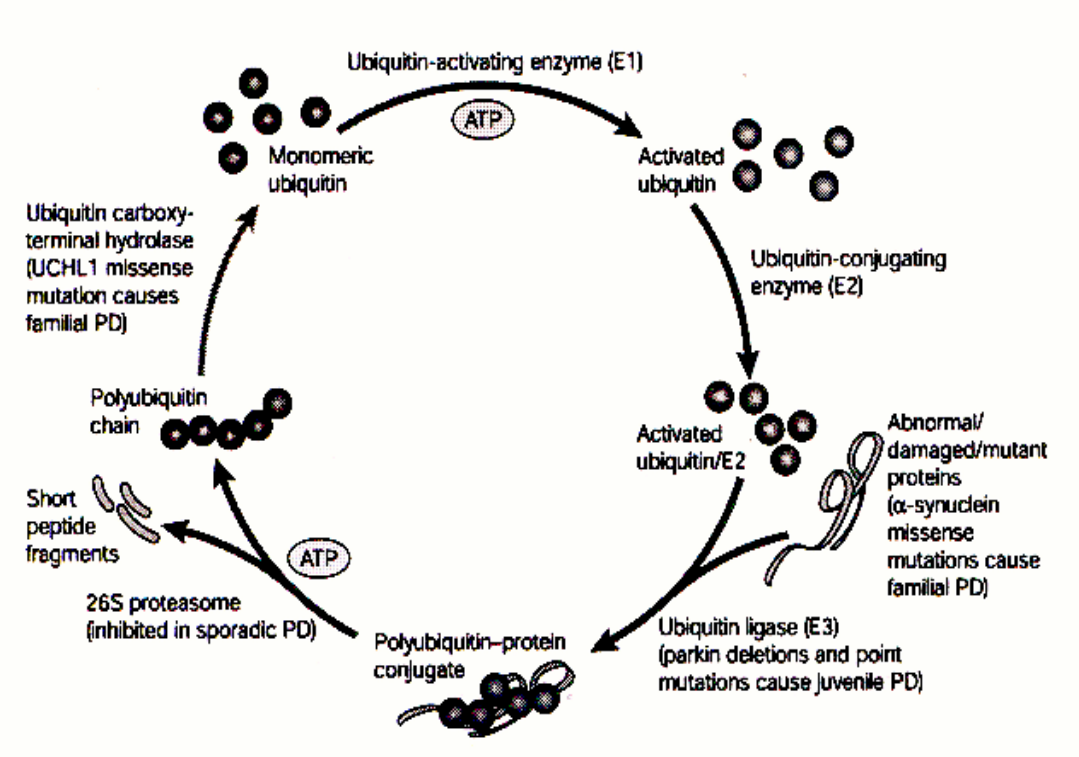

Figure 4. Degradation of abnormal proteins by the ubiquitin–proteasome system and impairments that lead to the development of Parkinson's disease (McNaught et al., 2001). The first step is ATP-dependent identification and labeling of toxic proteins with multiple ubiquitin molecules (ubiquitination) as a signal for ATP-dependent degradation by the 26S proteasome. The second step is the attachment of polyubiquitinated proteins to the 19S proteasome and subsequent degradation of those by the 20S proteasome. The final step is the recovery (de-ubiquitination) and subsequent recycling of ubiquitin molecules from polyubiquitin chains that are released from proteins immediately before their translocation into the proteasome. Many studies have revealed defects in several components of the ubiquitin–proteasome system in sporadic Parkinson's disease (PD). These alterations could be significant in the initiation, development and/or progression of the neurodegenerative process in PD, and indicate that impaired protein clearance due to misfolding or proteasomal defects might be a common theme underlying the different etiologies of PD.

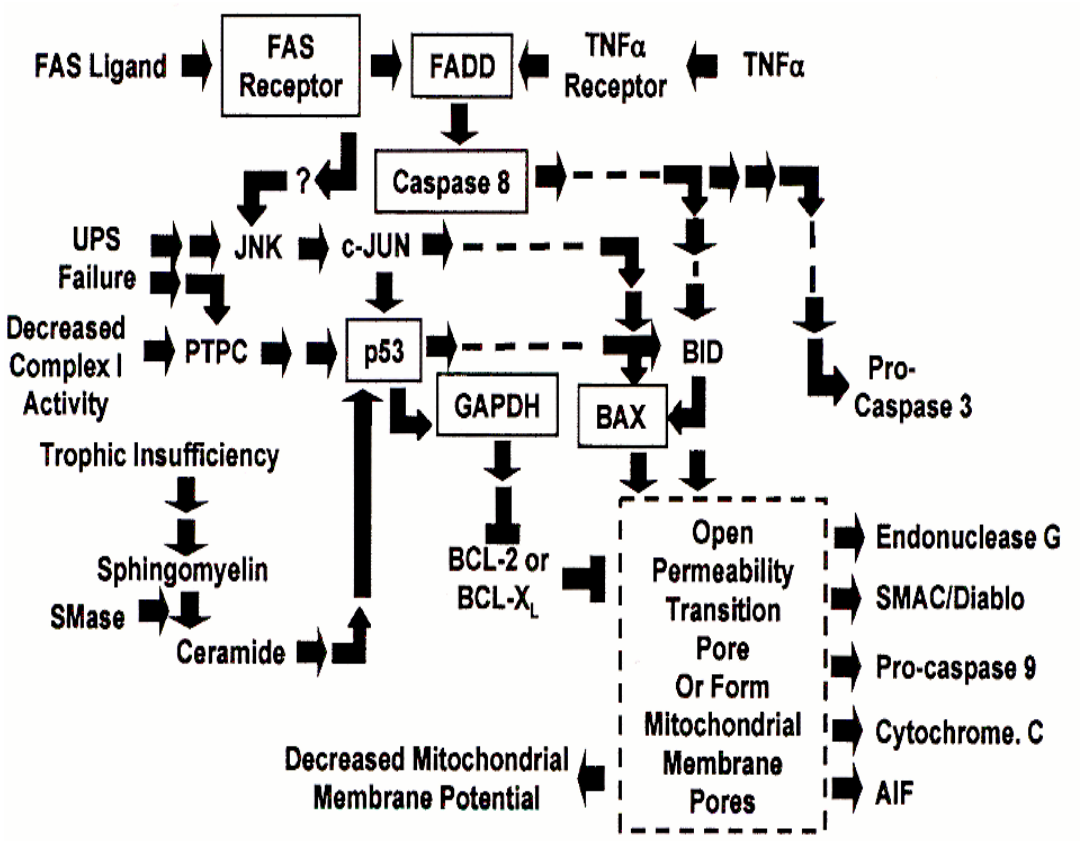

Figure 5. Schematic for premitochondrial signaling for apoptosis in the PD nigra (Tatton et al., 2003). The schematic illustrates the key role of the proapoptotic protein BAX in the induction of increased mitochondrial membrane permeability that allows the release of the different factors that signal for apoptotic degradation. Two possibly interdependent signaling pathways have been shown to activate BAX and/or its antagonists, BCL-2, and BCL-XL. The signaling elements that have been shown by immunocytochemistry of PD postmortem nigra are enclosed in boxes: (1) the p53-GAPDH-BAX pathway; and 2) the FAS receptor-FADD-caspase 8-BAX pathway. UPS (ubiquitin-proteasomal system); SMase (sphingomyelinase)

machinery of the cell, as well as environmental stimuli, including irradiation, oxidative stress, viruses, and withdrawal of neurotrophic support (Wyllie et al., 1980). There have been several reports indicating that apoptotic neuronal death is a major pathway of dopaminergic pathogenesis in PD (Figure 5). In fact, DNA fragmentation (Mochizuki et al., 1997; Tatton et al., 1998), activated forms of caspase-8, -9, -3 (Andersen, 2001), the upregulation of Bax (Tatton, 2000), p53 (de la Monte et al., 1998), and NF-kB (Hunot et al., 1997) have been demonstrated in dopaminergic neurons of PD patients.

Etiologic factors

PD can be classified into sporadic and familial PD, which account for approximately 90 or 10% of total PD cases, respectively (Tanner et al., 1999). As a result, it has been debated whether environmental factors or genetic factors are primary contributors in the incidence of PD among human populations. Currently, it is commonly believed that PD is triggered by the gene-environment and aging-environment interaction (Le Couter et al., 2002; Tsang & Soong, 2003).

Aging

Aging has long been considered to be the most unequivocal etiologic factor of PD because PD is rare before 50 years of age and its incidence increases with age thereafter. Aging is the progressive and irreversible decline of the different physiological functions of the organism during the last part of its life and it is a complex process that is under genetic control and influenced by environment (Albers & Beal, 2000). Aging-dependent

increases of protein carbonyl, malondialdehyde (MDA), and 8-hydroxy-2-deoxyguanosine are found in human brain (Oliver et al., 1987; Mecocci et al., 1993). Correspondingly, these indicators of damage are also reported in the substantia nigra of PD brain (Jenner, 2003). In addition, studies show that an aging-related change in xenobiotic metabolism might contribute to PD incidence (Irwin et al., 1992; Steventon et al., 2001).

Genetic factors

There has been considerable interest in the potential role of genetic factors in the etiology of PD (Golbe, 1993). Approximately 5-10% of PD patients have a familial form of PD with an autosomal-dominant pattern of inheritance. Large pedigrees have been identified where members in different generations suffer from PD. In addition, the incidence of PD is greater in family members than in age-matched controls (Plante-Bordeneuve et al., 1995). A twin study revealed no difference in concordance between monozygotic and dizygotic twins of PD patients aged 60 years or older but a significantly increased incidence was observed in monozygotic twins who developed PD at less than 50 years of age (Tanner et al., 1999). These findings suggest that genetic factors are important in early-onset patients but are not likely to play a major role in sporadic PD. To date, mutations have been identified in α-synuclein, nuclear receptor-related 1 (Nurr 1), DJ-1, ubiquitin C-terminal hydrolase L1 (UCH-L1), neurofilament medium subunit protein (NF-M), and parkin among PD patients. In addition, a number of studies suggest that functional polymorphisms in genes of dopamine metabolism and

transport, iron homeostasis, inflammation, mitochondrial abnormalities, and exogenous or endogenous toxin metabolism might play a role in PD incidence.

α-synuclein gene was identified in a large Italian–American family with autosomal dominant early-onset PD (Golbe et al., 1990). Linkage to markers on chromosome region 4q21–q23 was demonstrated and a missense mutation A53T in exon 4 of the gene encoding α-synuclein identified. This mutation was also found in affected members of three Greek families with PD (Polymerropoulos et al., 1997) and cellular localization studies revealed that α-synuclein abnormally deposited in Lewy bodies, the major pathologic hallmark of idiopathic PD (Spillantini et al., 1998). A second mutation (A30P) was later found in a small German family with PD (Kruger et al., 1998). It had been thought that mutations in the α-synuclein gene might be rare because extensive studies in multiple ethnic PD families and sporadic cases did not find mutations in this gene (Chan et al., 1998; Warner et al., 1998). However, a third mutation (E46K) has now been found in a Spanish family (Zarranz et al., 2004), and α-synuclein gene triplication identified in two independent families (Singleton et al., 2003; Farrer et al., 2004). These studies emphasize that either mutations or an increase in the amount of cellular α-synuclein is important for the pathogenesis of PD.

α-synuclein is a 140 amino acid soluble protein with unknown function, which is abundant in neurons and especially concentrated in presynaptic terminals. α-synuclein is a molecular chaperone mediating multiple protein–protein and protein–lipid interactions (Kim et al., 2000). α-synuclein binds to multiple proteins (Figure 6A) such as synphilin-1 (Engelender et al., 1999), tubulin (Alim et al., 2002), microtubule-associated protein

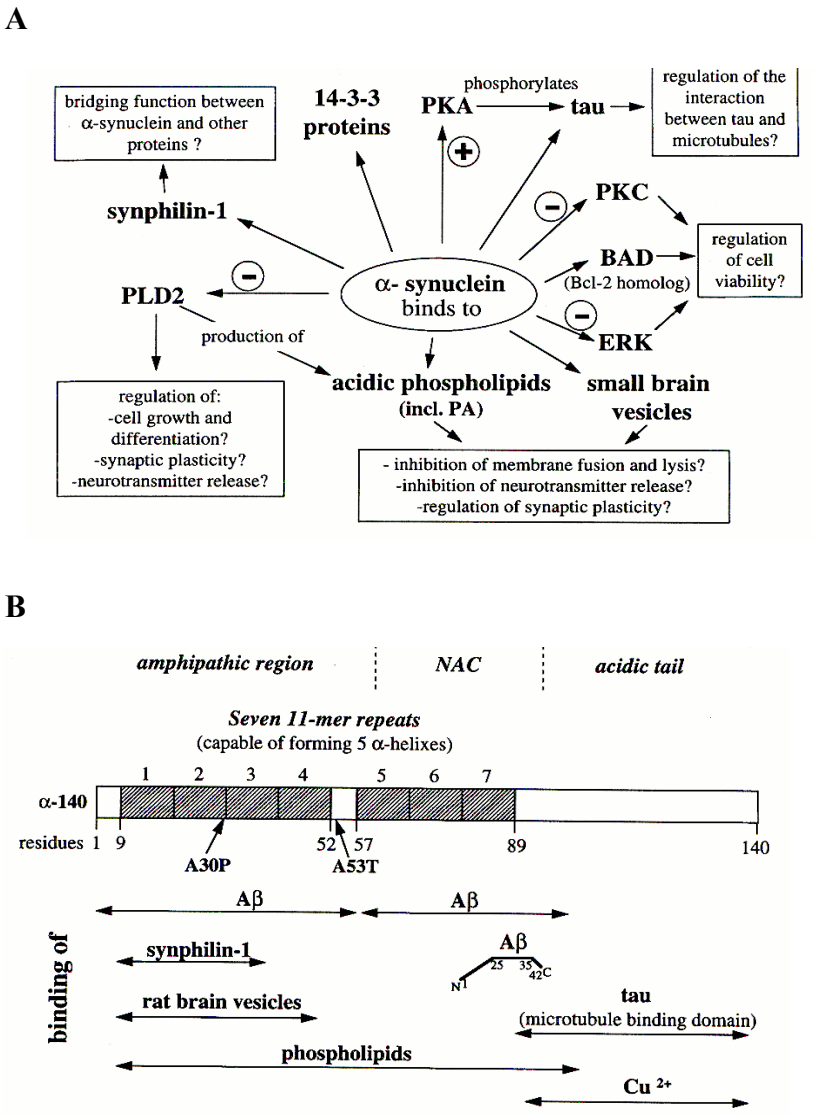

Figure 6. α-synuclein is a major protein component of Lewy bodies in dopaminergic neurons of PD patients (Lücking & Brice, 2000). A. Putative functions of α-synuclein. The binding partners of α-synuclein are marked by arrow. Positive (+) and negative (-) indicate the activation or inhibition by α-synuclein. PA (phosphatidic acid); PLD2 (phospholipase D2); PKC (protein kinase C); PKA (protein kinase A); ERK (extracellular-regulated kinase). B. Structure of α-synuclein and its domains interacting with the binding partners. α-synuclein has three major domains. The seven 11-mer repeats that are able to form five helixes are numbered and hatched. The two PD-linked mutations (A30P and A53T) are indicated. The sites of interaction between α-synuclein and the representative partners are indicated by double-headed arrows.

tau (MAPT) (Jensen et al., 1999), 14-3-3 protein, protein kinase C (PKC), Bcl-2-associated death proteins, extracellular regulated kinases (Ostreova et al., 1999), and unphosphorylated tyrosine hydroxylase (TH) (Perez et al., 2002). The 102-103 N-terminal residues of α-synuclein are responsible for lipid binding (Figure 6B).

α-synuclein has limited structure in the absence of lipid and adopts a helical structure in the presence of phospholipid (Conway et al., 1998). The α-synuclein mutants (A53T and A30P) linked to early-onset PD show enhanced oligomerization of amyloid intermediates (Conway et al., 2000). These mutants have altered lipid-binding properties, which may modulate amyloid deposition in vivo (Perrin et al., 2000).

Genetic polymorphisms in the promoter of α-synuclein have been identified as PD susceptibility markers. The genetic variability in the α-synuclein promoter is thought to influence its transcriptional regulation. NACP-Rep1 is a polymorphic complex microsatellite located approximately 10kb upstream of the translation start site of the α-synuclein gene. Four successive dinucleotide repeat regions give rise to five alleles that differ in size by two nucleotides (Xia et al., 1996). The repeat composition in NACP-Rep1 contributes to a significant variation in α-synuclein expression level (Chiba-Falek et al., 2001) and susceptibility to sporadic PD (Farrer et al., 2001). Two other common polymorphisms, 116C/G and 668T/C substitution, in the promoter region of the α-synuclein gene have also been investigated. There is significantly higher expression of the 668C/116G haplotype than the other two haplotypes (668T/116G or 668T/116C) in the population (Holzmann et al., 2003). However, these polymorphisms are not significantly associated with PD (Pastor et al., 2001). The 668C/116G haplotype is more

common in some populations of PD patients, suggesting some environmental and/or polygenetic interaction. In a single study, an alternative polymorphism (770C/A) is considered to be part of a risk haplotype for PD (Holzmann et al., 2003). These studies suggest that variation in the regulation and expression of α-synuclein gene is important determinants in the risk of developing PD.

The I93M mutation in the ubiquitin carboxy terminal hydrolase-L1 (UCH-L1) gene was identified in a single German PD family with a reduced penetrance inheritance pattern (Leroy et al., 1998). Both patients presented with typical resting tremor at around age of 50. Pathologic confirmation of idiopathic PD is yet to be made. This gene mutation is not found in multiple ethnic PD families and sporadic PD patients, and is therefore an extremely rare cause of disease or possibly a harmless substitution (Hasegawa et al., 2001; Shi and Tao 2003). However, recent proteomic evidence reveals that full-length UCH-L1 is a major target of oxidative damage and UCH-L1 is downregulated in the brains of patients with either Alzheimer's disease or PD (Choi et al., 2004). UCH-L1 hydrolyzes small C-terminal adducts of ubiquitin to generate the ubiquitin monomer, making it an important component of the ubiquitin–proteasome system. Besides hydrolase activity, UCH-L1 also exhibits dimerization-dependent ubiquityl ligase activity (Liu et al., 2002), suggesting multiple functions in this system. Expression of UCH-L1 is highly specific to neurons and to cells of the diffuse neuroendocrine system (Doran et al., 1983). It represents 1 to 2% of total soluble brain protein and is found in Lewy bodies and other protein aggregations (Wilkinson et al., 1989). UCH-L1 gene spans 10 kb and has nine coding exons and a high GC content

between exons 1 and 3 (Day et al., 1990). A polymorphism in exon 3 of the UCH-L1 gene (S18Y) is associated with a reduced susceptibility to PD in some populations. The S18Y polymorphism is relatively rare in the European population (allele frequency is 14–20%) but common in the Japanese (39–54%) and Chinese (~50%) populations (Levecque et al., 2001; Lincoln et al., 1999; Maraganore et al., 1999; Momose et al., 2002; Satoh & Kuroda, 2001; Toda et al., 2003). In addition to PD, UCH-L1 has been implicated in spinocerebellar ataxia (SCA), in which a UCH-L1 mutant is a genetic enhancer of degeneration in animal models, and Huntington's disease, in which the UCH-L1 S18Y polymorphism is linked to age of onset (Ciechanover & Brundin, 2003; Kim et al., 2003). Although these neurodegenerative diseases also have protein deposits, the protein deposits are considered neuroprotective in these genetic disorders with overwhelming evidence that cellular apoptosis is responsible for the neurodegeneration (Evert et al., 2000; Ciechanover & Brundin, 2003).

Autosomal recessive juvenile Parkinsonism (ARJP) is a relatively rare syndrome with a very early onset, a slow clinical course extending over decades, and a loss of dopamine neurons in the ventral substantia nigra but no Lewy bodies or Lewy neurites at autopsy. It has only one of the two pathological hallmarks of idiopathic PD. By positional cloning in a Japanese ARJP patient with a deletion of 6q, the closely linked marker D6S305 isolated a gene, which was designated as parkin (Kitada et al., 1998; Matsumine et al., 1997). Homozygous parkin mutations are found in nearly half the patients presenting with ARJP and perhaps 5% of young adults with PD (Lucking et al., 1998). To date, over 1000 single nucleotide variants have been identified in the national

center for biotechnology information (NCBI) database. Some parkin gene polymorphisms are not related to ARJP or idiopathic PD (Oliveira et al., 2003), while some heterozygous parkin mutations are considered as susceptibility alleles. Those mutations lying in exon 7 of the parkin gene (Arg256Cys, Arg275Trp, Cys253Trp, Cys253Tyr, and Asp280Asn) are especially predisposing to the late-onset form of PD due to a variable functional reduction in parkin (Oliveira et al., 2003). Lewy body pathology has been found in a single ARJP case with an exon 7 substitution of Arg275Trp and deletion of exon 3 (Farrer et al., 2001). Parkin, however, colocalizes with α -synuclein in the brain and is found in Lewy bodies in sporadic PD (Schlossmacher et al., 2002). A hypothesis of partial loss of parkin function associated with PD and Lewy body formation has been proposed (Hardy & Cookson, 2003). Parkin is expressed primarily in the nervous system and is one member of a family of E3 ubiquitin ligases, which attach short ubiquitin chains to proteins and tag them for degradation through the proteasome pathway. Four identified substrates for the ubiquitin-ligase function of parkin include 22 kilodalton glycosylated form of α -synuclein (Shimura et al., 2001), parkin-associated endothelin receptor-like receptor, (Imai et al., 2001), CDCrel-1 involved in cytokinesis that may influence synaptic vesicle function (Zhang et al., 2000) and synphilin-1 (Chung et al., 2001).

DJ-1 was recently identified as causal for ARJP (Bonifati et al., 2003). The locus for this form was first localized to 1p36 in a genetically isolated community in the Netherlands (Van Duijn et al., 2001). The DJ-1 gene contains eight exons spanning 24 kb encoding a 189 amino acid (20 kDa) protein. The first two exons (1A and 1B) are

noncoding and alternatively spliced. A large homozygous chromosomal deletion removing 4 kb of genomic DNA, including the exon encoding the start codon, has been identified in one Dutch kindred, while in an Italian family, affected individuals are homozygous for the L166P point mutation. In both pedigrees, only homozygous individuals are affected. Recently, six additional mutations of DJ-1 gene have been discovered in ARJP patients. In particular, M26I, IVS6-1G/C, c.56delC, and c.67G/A are thought to be causative and these pathogenic mutations account for approximately 1% of early onset PD (Abou-Sleiman et al., 2003; Golbe & Mourdian, 2004). There have been no pathological studies to date. Unlike the other monogenetic PD genes, polymorphisms in the DJ-1 gene do not appear to predispose to late onset sporadic PD (Morris et al., 2003). In the brain, DJ-1 expression is ubiquitous, with higher levels of the transcript in subcortical regions, such as the caudate nucleus, thalamus, substantia nigra, and hippocampus, which are more often affected in PD (Bonifati et al., 2003). DJ-1 is localized to both the nucleus and cytoplasm of different cell types, and is particularly prominent in astrocytes in human brain tissue. Unlike α-synuclein and parkin, DJ-1 is not an essential component of Lewy bodies and Lewy neurites (Bandopadhyay et al., 2004; Rizzu et al., 2004). DJ-1 has multiple protein–protein interactions and multiple possible functions. For example, first, it interacts with c-*myc* and increases cell transformation in the presence of myc or h-ras (Nagakubo et al., 1997). Second, it interacts with the protein inhibitor of activated signal transducer and activator of transcription (STAT) [PIASx] (Takahashi et al., 2001). PIASx interacts with the androgen receptor, inhibiting gene expression. DJ-1 binds to PIASx, thereby positively

regulating androgen receptor-controlled genes. PIASx is an E3-like enzyme that adds SUMO-1 (ubiquitin-related protein) to target proteins. Third, it can be sumoylated at K130. Fourth, it has been found to shift to a more acidic isoform (p*I* 5.8) after treatment of cells with the herbicide paraquat, causing α-synuclein upregulation and aggregation by oxidizing proteins (Manning-Bog et al., 2002; Mitsumoto & Nakagawa, 2001).

Nuclear receptor-related factor 1 (Nurr1 or NR4A2) is a requisite gene in the differentiation and maintenance of dopamine neurons (Law et al., 1992). The Nurr 1 is a member of the nuclear orphan receptor superfamily that also functions as a zinc finger transcription factor (Law et al., 1992). Due to its function, previous studies had already suggested Nurr1 to be a candidate gene for PD (Zetterstrom et al., 1997). Recently, two heterozygous mutations (−291T del and −245 T to G) were revealed in 10 out of 107 individuals with familial PD, but not in 94 individuals with sporadic PD or in 221 age-matched unaffected controls (Le et al., 2003). Both mutations affect a noncoding exon (exon 1) of the gene and lead to a marked decrease in Nurr1 mRNA levels. The clinical phenotype of patients with mutated Nurr1 is concordant with typical late-onset PD. Radiological or neuropathological data are not yet available (Deckker et al., 2003). The Nurr1 gene contains eight exons and spans 8.3kb (Ichinose et al., 1999). The frequency of the 7048insG polymorphism in intron 6 of the Nurr1 gene is higher in sporadic PD than in controls. The significance of heterozygosity for the polymorphism between patients and controls is disputed (Tan et al., 2003; Zheng et al., 2003). Nurr1 involves the regulation of corticotropin-releasing hormone and the regulation of gene transcription encoding tyrosine hydroxylase (TH) and dopamine transporter (DAT) (Le

et al., 2003). The transcriptional activation of Nurr1 is regulated by NF-κB and cAMP response element-binding protein (CREB) (McEvoy et al., 2002).

The neuronal intermediate filament cytoskeleton consists of neurofilament (NF) proteins. NFs are composed of a triplet of proteins of various molecular weights, i.e., high, medium (NF-M), and low (Al-Chalabi & Miller, 2003). Together with other axonal components such as microtubules, they are important for axonal transport and stability. Rearrangement of NF components is charateristic of cellular pathology linked with neurodegeneration, including neurofibrillary tangles, neuritic plaques, and Lewy bodies and neuritis (Al-Chalabi & Miller, 2003). A down-regulation of mRNA encoding NF-M and the accumulation of hyperphosporylated neurofilaments are the common features of Alzheimer's disease and PD (Julien & Mushynski, 1998; Wang et al., 2001). A missense mutation of NF-M G1747A has been identified in a single French–Canadian family with a reduced penetrance of 25% at age 44 (Lavedan et al., 2002). The proband had an onset of PD at 16 years of age, suggesting that abnormal NF-M may directly induce PD. NF-M gene screening in large populations has excluded the possibility that the G1747A variant (Gly336Ser) is a polymorphism associated with PD (Lavedan et al., 2002). Further detailed mutational analyses of the NF-M gene in 322 families and sporadic PD patients found a Pro725Gln substitution in a sporadic case and a deletion in position 829 in a patient with familial PD. However, this NF-M sequence did not cosegregate with PD in this family (Kruger et al., 2003). Two other common polymorphisms in NF-M do not appear to predispose to PD, although there was a significant twofold increase in their frequencies in PD compared to controls (Kruger et al., 2003). These studies suggest that

rare variants in the NF-M gene increase the susceptibility to PD and suggest that cytoskeletal elements play a role in the disease process.

Dopamine is metabolized by dopamine β -hydroxylase (DBH), catechol-*O*-methyltransferase (COMT) and monoamine oxidases (MAO) to produce norepinephrine, 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxy-4-hydroxyphenylethanol (MOPET) respectively, and eventually homovanillic acid (HVA) (Figure 7). A DBH gene promoter polymorphism (C-1021T) has been demonstrated to regulate plasma DBH activity. Genetically determined low serum DBH activity (genotype T/T) has a protective role against PD (Healy et al., 2004). COMT degrades dopamine extraneuronally. A common polymorphism in COMT, Val158Met, which results in a form of the enzyme with low activity, increases the relative risk for PD (Yoritaka et al, 1997; Wu et al., 2001). Stratification analysis suggests that the COMT-Val158Met polymorphism is associated with PD in younger female subjects (Goudreau et al., 2002). MAO is classified as A or B on the basis of differential substrate specificity and differential sensitivity to inhibitors (Weinshilboum, 1983). MAO-B inhibition is associated with enhanced activity of dopamine, as well as with decreased production of hydrogen peroxide, a source of ROS. There is an increased risk of PD with longer length GT dinucleotide repeats in intron 2 of the MAO-B gene as well as with the intron 13G MAO-B genotype (Chekoway et al., 1998; Costa et al., 1997; Mellick et al., 2000).

Cytochrome *P450* (CYP) enzymes are a large group of monooxygenase enzymes responsible for the metabolism of toxic hydrocarbon. Nicotinamide adenine dinucleotide

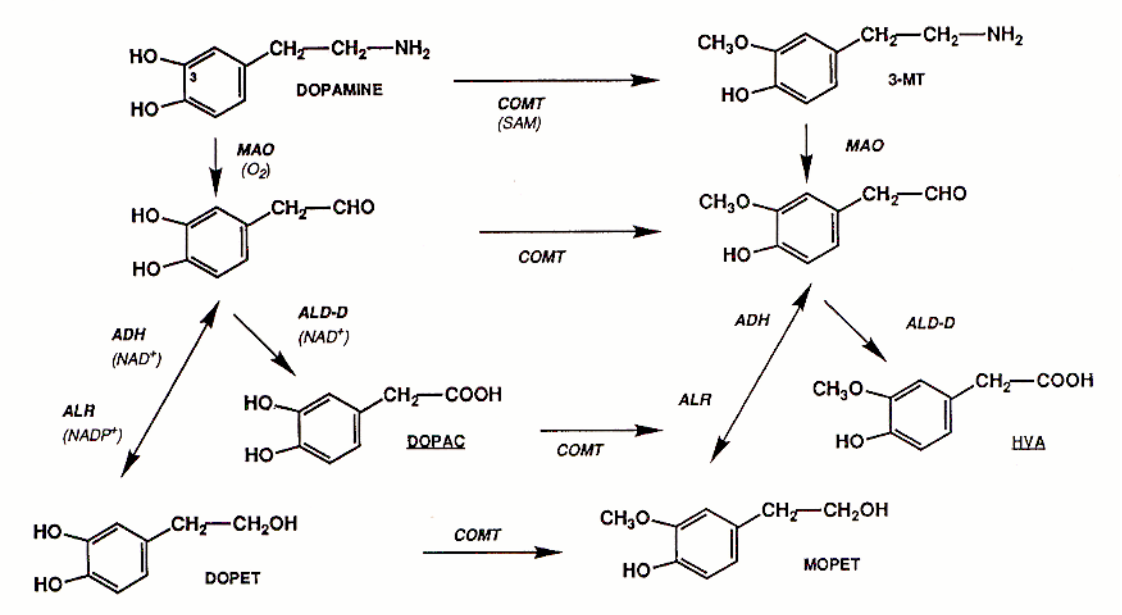

Figure 7. Metabolic pathways of dopamine (Elsworth & Roth, 1997; Stokes et al., 1999). Several different pathways of dopamine metabolism exist in dopaminergic neurons. In noradrenergic neurons, dopamine serves as precursor for norepinephrine (NE) and epinephrine (EPI). Dopamine can be metabolized by monoamine oxidase (MAO), catechol-*O*-methyltransferase (COMT), aldehyde dehtdrogenase (ALD-D), aldehyde reductase (ALR), and alcohol dehydrogenase (ADH). In brain, dopamine is mainly metabolized into DOPAC and HVA, with less formation of DOPET and MOPET. Dopamine metabolism produces dopamine quinone and reactive oxygen species (ROS), both of which are very toxic in cells.

phosphate (NADPH) provides the reducing power for this chemical reaction and O_2 used as a substrate.

The name cytochrome P450 comes from its wavelength of light (450 nm) that is absorbed by the isoenzymes. The cytochrome *P450* (CYP) enzymes are located on the smooth endoplasmic reticulum of cells throughout the body, but the highest concentrations are found in the liver and small intestine. These enzymes are responsible for the oxidative (phase I) metabolism of a wide number of compounds, including drugs, environmental toxicants, dietary components, and endogenous components such as steroids and prostaglandins. Because PD may have an environmental cause, cytochrome *P450s*, which detoxify many potential neurotoxic xenobiotics, have been investigated exhaustively. Many studies have focused on CYP2D6 and CYP1A1 with controversial and inconclusive results (Amstrong et al., 1992; Riedl et al., 1998; Chan et al., 2002).

Glutathione *S*-transferases (GST) are phase II detoxification enzymes, and are involved in the detoxification of endogenous or exogenous toxins, which may play a role in the pathogenesis of PD. GST is also an important cellular antioxidative enzyme against catecholamine-derived quinones as a consequence of catecholamine metabolism. Oxidative stress is widely thought to contribute significantly to the pathogenesis of PD, and GST activity is reduced in the substantia nigra of PD (Sian et al., 1994). Four genotypes (GSTT1, GSTM1, GSTP1, and GSTZ1) of GSTs have been tested in several PD association studies (Rahbar et al., 2000; Harada et al., 2001; Kelada et al., 2003). The distribution of the GSTP1 genotypes differ significantly between patients and controls exposed to pesticides (Menegon et al., 1998). The polymorphisms of the

GSTM1 and GSTT1 loci arise from the complete deletion of each gene, and can substantially affect the metabolism of some substances (Pemble et al., 1994; Seidegard & Pero, 1988). The polymorphisms at the GSTP1 (Ile104Val and Ala113Val) and GSTZ1 (Lys32Glu and Arg42Gly) loci result in amino acid substitutions that may affect substrate selectivity and stability. Genotype GSTP1 may play a role in sporadic cases of PD without pesticide exposure, but neither GSTM1 nor GSTT1 is associated with PD (Kelada et al., 2003). The GSTO1 genotype influences the age at onset of PD, with the less common Asp allele for GSTO1 SNP7, which is associated with a later onset of PD (Li et al., 2003). This effect is thought to be due to a decreased efficacy in IL-1 posttranslational processing, thereby dampening inflammatory responses and neurodegeneration (Li et al., 2003).

Environmental factors

Most PD occurs as sporadic and idiopathic cases, which have no recognizable genetic defects. As mentioned earlier, a recent twin study showed that concordance rates of PD incidence were similar in monozygotic and dizygotic pairs. This finding suggests that heredity is not a major etiologic factor of sporadic PD (Tanner et al., 1999). Instead, several epidemiologic studies have shown that environmental factors including farming, rural living, well-water drinking, and exposure to heavy metals and pesticides (Gorell et al., 1998; Priyadarshi et al., 2001; Lai et al., 2002) might play an important role in PD incidence. In particular, some exogenous chemicals have been associated with Parkinsonism, including iron, manganese, copper, rotenone, maneb, dieldrin, paraquat,

and polychlorinated biphenyls (PCBs). In addition, tetrahydroisoquinolines (TIQ) and β-carbolines (β-C) have been suspected as both endogenous and exogenous neurotoxins, which might contribute to PD incidence. The potential contributions of each of these environmental factors to PD will be discussed in the next sections.

Metals

Metals have been investigated as potential risk factors on the basis of their accumulation in the substantia nigra and their participation in harmful oxidative reactions. However, metal-induced symptoms are significantly different from idiopathic PD. For example, the Parkinsonism caused by manganese does not respond to L-DOPA treatment and the primary target of manganese toxicity seems to be the globus pallidus rather than the nigrostriatal system (Pal et al., 1999). In idiopathic PD, experimental work with metals has mostly focused on the potential role of iron and other transition elements. Iron is unevenly distributed throughout the brain, but it reaches particularly high concentrations in the basal ganglia (Zecca et al., 1994). Increased iron in the brains of PD patients was first reported by Earle (1968) and subsequent studies have confirmed this accumulation in the substantia nigra pars compacta (Sofic et al., 1988; Dexter et al., 1989b). Dexter et al. (1990) have also measured decreased levels of ferritin in the nigral tissue of patients with Parkinson's disease. Ferritin is the primary intracellular protein capable of keeping iron bound in a non-reactive status. Thus, iron accumulation together with decreased binding capability may enhance the risk for iron-mediated toxic reactions in PD. In particular, the Fenton reaction could generate the highly toxic hydroxyl radical

in the presence of iron and hydrogen peroxide, thus leading to oxidative stress and possibly neurodegeneration.

If metals such as iron play a role in nigrostriatal injury, the source and mechanisms of their accumulation remain to be identified, and the possibility that environmental exposures to metals may represent risk factors for idiopathic PD should be considered. Epidemiological evidence supporting this possibility is suggestive but still inconclusive (Semchuck et al., 1993; Seidler et al., 1996). In a population-based case-control study, the potential role of metals as risk factors for PD was evaluated in the occupational setting in which the effects of metals would be expected to be magnified by greater and prolonged exposures (Gorell et al., 1997). Increased risk for PD was only found in workers exposed to metals for more than 20 years, suggesting a long latency and/or slowly progressive mechanism of accumulation and toxicity. Also, enhanced risk was shown as a result of combined metal exposures, i.e. lead–copper, lead–iron and iron–copper, raising the possibility that metal may act additively in contributing to nigrostriatal degeneration. Based on these data, the effects of prolonged exposures and interactions should become a focus of future studies investigating the relationship between exposure to metals and PD incidence.

Pesticides

Since World War II, numerous pesticides including insecticides, herbicides, fungicides, rodenticides, and fumigants have been widely used. Exposure to pesticides may be due to direct contact with the skin, intake of contaminated food and water, or

aspiration of sprayed pesticides or fumigants. In the last decade, a number of epidemiologic studies of the relationship between exposure to pesticides and PD incidence have been conducted around the world (Seidler et al., 1996; Gorell et al., 1998). Most of these studies reported a positive association of PD with overall exposure to pesticides. The odds ratio (OR) for positive association ranged from 1.02 to 7.0. For example, Priyadarshi`s group performed a meta-analysis of 19 studies published between 1989 and early 1999 to examine the association between PD incidence and exposure to pesticide. They found a significant heterogenicity among studies and calculated a combined OR of 1.94 (Priyadashi et al., 2000). Their findings showed a positive correlation between PD and the duration of pesticide exposure. Exposure to pesticide may influence the mortality of patients with PD. In California, mortality with PD as the underlying cause of death has been shown to be higher in agricultural pesticide-use counties than in non-use counties (Ritz & Yu, 2000).

Maneb is a widely used fungicide, which is known to damage the dopaminergic system. Exposure to maneb can induce neurologic alterations in humans, such as postural tremor, cerebellar signs, and bradykinesia (Ferraz et al., 1988). Chronic exposure to maneb is known to induce parkinson-like symptoms (Meco et al., 1994). Based on the neurochemical and behavioral changes and its apparently selective disruption of the nigrostriatal system, maneb is assumed to be able to cross the blood-brain barrier (Thiruchelvam et al. 2002). Maneb contains a major active fungicidal component manganese ethylene-bis-dithiocarbamate (Mn–EBDC), and belongs to the dithiocarbamate (DTC) fungicide family. In experimental models, maneb appears to

decrease locomotor activity (Morato et al. 1989), potentiates MPTP effects on locomotor activity and catalepsy (Takahashi et al. 1989), and modulates the toxicity of paraquat.

However, commercial maneb contains not only Mn–EBDC but also many other minor reagents without clearly defined functions. Other constituents of maneb, rather than the fungicidal Mn–EBDC, may be responsible for maneb-mediated neurotoxicity, as is the case of MPTP-contaminated synthetic heroin. Alternatively, the neurotoxic effect might be attenuated by the combined action of several constituents of maneb. Furthermore, Mn–EBDC, being relatively stable in vitro, could potentially degenerate to manganese and EBDC in vivo, both of these compounds being potentially neurotoxic. Indeed, manganese is known to be relatively non-toxic to the adult organism except to the brain, where it causes PD-like symptoms when inhaled, even at moderate amounts over longer periods of time (Carpenter, 2001; Gerber et al. 2002).

Occupational exposure to manganese occurs mainly in mining, alloy production, processing, ferro-manganese operations, welding, and work with agrochemicals. Among the neurologic effects is an irreversible parkinsonian-like syndrome, manganism (Levy & Nassetta, 2003; Takeda, 2003). However, although the neurological signs of manganism have received close attention because they resemble several clinical disorders collectively described as extrapyramidal motor system dysfunction and in particular, idiopathic PD and dystonia, there are well-established distinct dissimilarities between idiopathic PD and manganism. Therefore, whether manganese plays an etiologic role in idiopathic PD remains to be determined (Aschner, 2000). On the other hand, the non-manganese moiety of maneb, EBDC component has been suggested to

contribute to toxicity. This conclusion follows from the finding that both mancozeb (Mn–Zinc–EBDC) and zineb (Zinc–EBDC) produce neurotoxicity in cell cultures (Soleo et al. 1996). Indeed, EBDC per se enhances MPTP-induced neurotoxicity (McGrew et al. 2000). The direct involvement of manganese ethylene-bis-dithiocarbamate (Mn–EBDC) in selective dopaminergic neurodegeneration was recently demonstrated in the adult male Sprague-Dawley rats, in which Mn–EBDC was directly delivered to the lateral ventricles (Zhang et al., 2003). This model has shown that Mn–EBDC is able to induce extensive striatal dopamine efflux comparable with that induced by MPP^+. Furthermore, Mn–EBDC preferentially inhibits mitochondrial complex III. As mitochondrial dysfunction is pivotal in the pathogenesis of PD, these results support the proposal that exposure to pesticides such as maneb, or other naturally occurring compounds that inhibit mitochondrial function, may contribute to PD development (Zhang et al., 2003).

Dieldrin is an organochlorine insecticide that has been widely used to control soil pests such as termites, grasshoppers, locusts, beetles and textile pests around the world until the mid-1970s, and is still used in several developing countries. A recent study indicates that dairy products and meats are the primary sources of human exposure to dieldrin, and the daily intake level of dieldrin through these contaminated foods was estimated at 0.059 g per average person (Doong et al., 1999). Whereas the half-life of dieldrin in human blood is estimated to be around 300 days (de Jong, 1991), prolonged exposure to dieldrin through food may greatly increase the accumulation of dieldrin in the brain and other lipophilic tissues. Evidence from several lines of research, ranging from studies of postmortem pathology to cultured neurons, has implicated dieldrin in the

etiology of Parkinson's disease in humans. Fleming`s group first measured the amounts of various organochlorine pesticides in human postmortem brain samples from Parkinson's disease patients, Alzheimer's disease patients, and age-matched controls. Dieldrin was detected in 6 of 20 brains from Parkinson's disease patients, but in none of the 14 age-matched control brains (Fleming et al., 1994). Corrigan`s group has also found significantly higher levels of dieldrin in the caudate nucleus from PD patients as compared to controls (0.515 μg/g versus 0.283 μg/g lipid) (Corrigan et al., 1998). Chronic exposure to dieldrin in a mesencephalic cell cultures shows that dopaminergic neurons are more susceptible than other neurons to dieldrin toxicity (Sanchez-Ramos et al., 1998). In addition, in vivo studies reported massive dopamine depletions in brains following chronic exposure to dieldrin (Sharma et al., 1976; Heinz et al., 1980). These results suggest that chronic exposure to highly lipophilic dieldrin (Suwalsky et al., 1997) could selectively destroy dopaminergic neurons in substania nigra pars compacta (SNpc) and could be a risk factor for Parkinson's disease. In addition to dieldrin, heptachlor is another organochlorine insecticide of particular interest for PD incidence. Heptachlor has been found to cause the alteration of dopamine transporter (DAT) expression, which might enhance the vulnerability of dopaminergic neurons to degeneration (Miller et al., 1999).

Rotenone is a naturally occurring toxin and commonly used insecticide. Unfortunately, clinical evidence for parkinson-like symptoms attributed to rotenone exposure is lacking. However, rotenone exposure might to be relevant to the decreased activity of mitochondrial complex I in the substantia nigra of PD patients (Schapira,

1994) because rotenone is a mitochondrial toxin that selectively inhibits the mitochondrial complex I at the same site as MPP^+. In contrast to MPP^+, rotenone is highly lipophilic and does not depend on dopamine transporters for cellular entry and furthermore, is not sequestered into synaptic terminals. In 1985, it was reported that stereotaxic administration of both MPP^+ and rotenone caused damage to the dopaminergic nigrostriatal pathway in animals (Heikkila et al., 1985). Later it was found that infusion of rotenone alone also induces those effects (Sherer et al., 2003). Inhibition of complex I by rotenone results in highly selective nigrostriatal dopaminerergic degeneration (involving caspase 3-mediated apoptosis) (Ahmadi et al., 2003) and α-synuclein positive cytoplasmic aggregates in nigral neurons (Uversky et al., 2001). Furthermore, an increased striatal dopamine turnover (Thiffault et al., 2000) and reduced tyrosine hydroxylase (TH) levels in the caudate putamen of rodents were observed (Alam & Schmidt, 2002).

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are members of the organic class of halogenated aromatic hydrocarbons that also include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorinated dibenzofuran (Tilson & Kodavanti, 1997). PCBs are synthetic compounds that, despite their ban in 1977, are ubiquitous in the environment due to their improper disposal and resistance to degradation intrinsic to their chemical properties. As a result, PCBs are taken up by marine animals, whereby they preferentially bioaccumulate and biomagnify in higher trophic levels of the food chain

(Senthilkumar et al., 2001). Evidence for a role of PCBs in neurodegeneration was provided in a study that revealed a selective accumulation of PCBs in the caudate nucleus in Parkinson's disease brain tissues (Corrigan et al., 1998). Many previous studies have characterized the effects of PCBs on brain neurotransmitter systems and have implicated the dopaminergic system as a preferential and sensitive target of PCB exposure. For example, the selective depletion of dopamine was found in the caudate nuclei and lateral olfactory bulbs of rats exposed acutely to a mixture of Aroclor 1254, a PCB mixture (Seegal et al., 1985; Chrishti et al., 1996). There have also been studies with PC12 cells that demonstrated a larger time and concentration dependent increase in dopamine depletion with Aroclor 1254 (Seegal et al., 1989). Thus, it was suggested that PCBs might inhibit dopamine synthesis via the inhibition of TH activity, the rate-limiting enzyme involved in the synthesis of dopamine. However, when PCBs were added to soluble TH preparations from rat brain, none of the PCB congeners had any effect on its activity, indicating that PCBs indirectly inhibits dopamine synthesis (Choksi et al., 1997).

Tetrahydroisoquinolines (TIQs) and β-carboline (β-C) derivatives

Some of heterocyclic alkaloid molecules have been suspected as potential endogenous and exogenous parkinsongenic agents, and tetrahydroisoquinoline (TIQ) and β-carboline (β-C) derivatives have been the most studied. TIQs and β-Cs are naturally occurring alkaloids present in a variety of foods (Makino et al., 1988). However, they can also be generated within the brain by reactions involving biogenic amines, such as

condensation of the amine group of dopamine with the reactive carbonyl groups of aldehydes and α-ketoacids (Collins & Origitano, 1983). Interestingly, both TIQs and β-Cs have been detected in the human brain (Niwa et al., 1987; Matsubara et al., 1993) as well as in the cerebrospinal fluid of patients with Parkinson's disease (Kotake et al., 1995; Matsubara et al., 1995). They have also been reported to cause nigrostriatal damage in experimental animals, including nonhuman primates (Nagatsu & Yoshida, 1988; Matsubara et al., 1998).

However, the identification and characterization of TIQs and β-Cs as etiologic neurotixicants involed in Parkinson's disease has provoked a number of important questions. For instance, the selective toxicity of these compounds to the dopaminergic system is not yet proven, because TIQs, β-Cs and their metabolites are weak substrates for DAT (Drucker et al., 1990; Sayre et al., 1991; Kawai et al., 1998). Furthermore, in vivo studies show that TIQs and β-Cs are less effective than MPTP, raising the concern that natural exposure to these compounds may not induce toxicity in dopaminergic neurons. Nevertheless, it can be still hypothesized that long-term exposure to low levels of TIQs and β-Cs damage the nigrostriatal system, particularly in the aging brain. It is also possible that combinations of TIQs and β-Cs may contribute to neurodegeneration through novel synergistic mechanisms. Finally, a different generation of TIQs and β-Cs within specific brain areas may account for selective toxicity to dopaminergic neurons (Gearhart et al., 2000).

Paraquat (PQ)

Paraquat (methyl viologen, 1, 1'-dimethyl-4, 4' bipyridium dichloride) is widely used as a cationic non-selective bipyridyl herbicide (Figure 8) to control weeds and grasses in many agricultural and non-agricultural areas (Cremlyn, 1991). Paraquat is not mobile and biologically active after it has been sprayed in the field, because it is strongly bound to soil particles or is decomposed into a non-toxic product by soil bacteria and sunlight (Roberts et al., 2002). However, active paraquat is highly toxic to humans and many cases of acute poisoning and death have been reported over the past few decades (Onyon & Volans, 1987; Bismuth et al., 1990).

The most frequent routes of exposure to paraquat, either accidentally or intentionally, in humans and animals are by ingestion or through direct skin contact. In addition, people may be exposed to residues of paraquat through the diet, and thus, the US EPA established maximum residue limits (or tolerances) of paraquat in foods. For example, 0.1 ppm is for sorghum, 0.25 ppm is for soybeans, and 0.5 ppm is for hops and ruminant kidney. If ingested, paraquat induces a burning sensation of the mouth and throat, followed by gastrointestinal irritation, subsequently resulting in abdominal pain, loss of appetite, nausea, vomiting and diarrhea (Vale et al., 1987). Direct contact with paraquat solutions or aerosol mists may cause skin burns and dermatitis (Spiewak, 2001). Paraquat splashed in the eyes can irritate, burn or cause corneal damage and scarring of the eyes. Due to its low vapor pressure and the formation of large droplets, the inhalation of paraquat spray used in the open environment has not been shown to cause any significant systemic toxicity. However, the inhalation of paraquat in confined space is

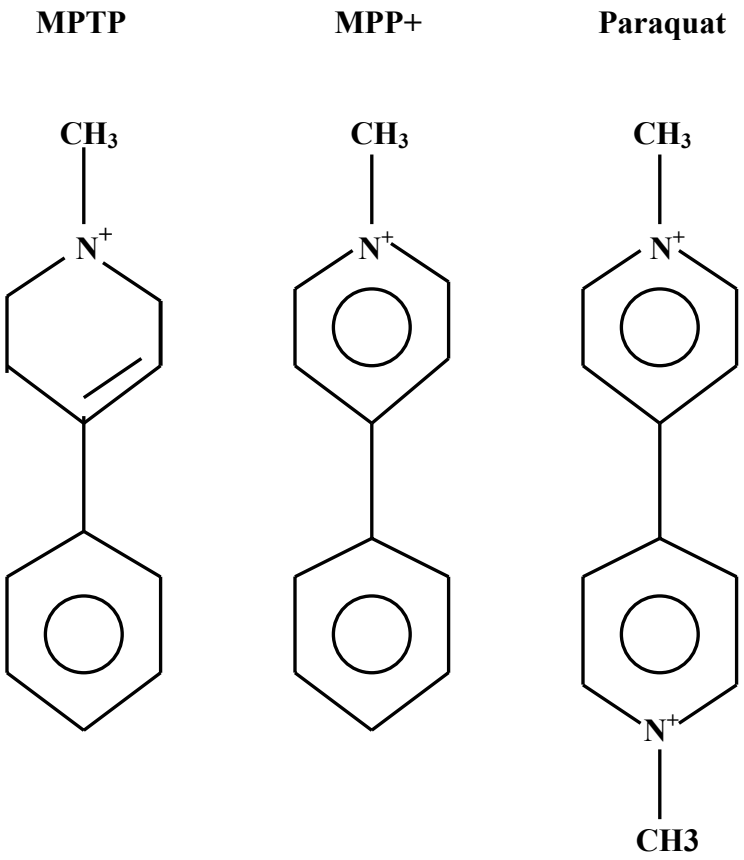

Figure 8. Chemical structures of MPTP, MPP+, and paraquat (Shimizu et al., 2001; Kalivendi et al., 2003). The possibility that paraquat might contribute to PD incidence is based on the observation that its chemical structure closely resembles it of MPP+. MPP+ is the toxic metabolite of a syntheic heroin MPTP, which has been found to cause parkinsonian symptoms in drug users.

known to be associated with fatal pulmonary disease (Howard, 1983).

Regardless of its route of administration in mammalian systems, paraquat is rapidly distributed in most tissues, with the highest concentration found in the lungs andand kidneys (Rose et al., 1976). The compound accumulates slowly in the lung, where paraquat is accumulated through polyamine transporters in the Clara cells and alveolar type I and II epithelial cells (Rose et al., 1974). The paraquat-induced lung injury is morphologically characterized by an early destructive phase, in which the alveolar type I and type II epithelial cells are damaged; and a second proliferative phase defined by alveolitis, pulmonary edema and infiltration of inflammatory cells (Smith & Heath, 1975). Excretion of paraquat, in its unchanged form, is biphasic, owing to lung accumulation, and occurs largely in the urine and, to a limited extent, in the bile. In general, the biotransformation of paraquat is poor in all species studied and the excreted compound is unchanged (Chan et al., 1997; Yang et al., 2000).

Severe damage to brain has been observed in the patients who died of paraquat poisoning (Grant et al., 1980; Hughes, 1988). Of particular concern, paraquat is suggested as a potential etiologic factor in Parkinson`s disease (PD) (Andersen, 2003), because its chemical structure is similar to 1-methyl-4phenylpyridium ion (MPP+), the active metabolite of MPTP. Significantly, paraquat-induced Parkinsonism has been observed in a 32-year old citrus farmer who used paraquat for 15 years in the United States (Sanchez-Ramos et al., 1987). In addition, a strong correlation has been reported between exposure to paraquat and the incidence of PD in Canada (Hertzman et al., 1990). In Taiwan, where paraquat is commonly sprayed on rice fields, the odds ratio for PD

incidence was 6.4 among subjects who had been exposed to this herbicide for more than 20 years (Liou et al., 1997). These epidemiologic reports and case studies are supported by animal studies showing that paraquat produces characteristic pathological features of PD in the rat and mouse brain, including the increase of lipid peroxidation (Tawara et al., 1996), the aggregation of α-synuclein (Manning-Bog et al., 2002), the depletion of striatal dopamine (Liou et al., 1996), the reduction of motor activity (Brooks et al., 1999), and the selective loss of dopaminergic neurons (McCormack et al., 2002).

Study aims

In this study, the human neuroblastoma SK-N-SH-SY5Y cell line (hereafter designated SY5Y) was used as an in vitro model. This cell line is a subclone of the SK-N-SH cell line, which was originally derived from bone marrow of a female tumor patient in the early 1970s (Ross et al., 1983). The cell line has a stable nearly diploid karyotype and its doubling time is 48 hours (Biedler et al., 1978). In the undifferentiated state, SY5Y cell line shows two distinctive morphologies, a small spiny cell with multiple neurites and a large epithelial-like cell. In the differentiated state, the SY5Y cells stop proliferation and extend long neurites accompanied by pseudoganglia assembly and neurite bundle recruitment (Perez-Polo et al., 1979). Thus, this cell line has been selected as a model to study neuronal differentiation and neurite outgrowth induced by nerve growth factor (NGF) (Ridge et al., 1996). In addition, it is used as a cholinergic and adrenergic model system because it develops a cholinergic or adrenergic

phenotype in response to retinoic acid (RA) (Adem et al., 1987) or 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) (Pahlman et al., 1981), respectively.

Significantly, the SY5Y cell line expresses several dopaminergic characteristics including tyrosine hydroxylase (TH), dopamine transporter (DAT), vesicular monoamine transporter-2 (VMAT-2), dopamine receptor 2 (D2R), and monoamine oxidase-A/B (MAO-A/B) (Klegeris and McGeer, 2000; Gómez-Santos et al., 2002; Maňáková et al., 2004; Presgravesn et al., 2004). In addition, dopamine and dopamine metabolites are detected in this cell line (Legros et al., 2004). Thus, the SY5Y cell line has been widely selected as a cellular PD model to study dopaminergic pathogenesis (Lai & Yu, 1997; Lee et al., 2000; Masayo et al., 2003; Shavali et al., 2004). In particular, this cell line has been used to characterize pathological functions of genes involved in the dopaminerigc pathogenesis of PD such as α-synuclein, ubiquitin carboxy terminal hydrolase-L1 (UCH-L1), parkin (or ubiquitin E3 ligase), and DJ-1 (Ardley et al., 2004; Taira et al., 2004; Machida et al., 2005). Furthermore, toxic mechanisms of parkinsogenic chemicals, including dopamine, 6-hydroxydopamine (6-OHDA), MPP+, salsolinol, and rotenone have been extensively investigated to elucidate dopaminergic pathogenesis in this cell line (Dennis and Bennett, 2003; Ben-Shachar et al., 2004; Wanpen et al., 2004; Tirmenstein et al., 2005; Wang and Xu, 2005).

In addition to SY5Y cell line, several other cell lines such as PC12, MN9D, MES23.5, N27, B65 and SN4741 are currently used to study dopaminergic pathogenesis (Son et al., 1999; Diaz-Corrales et al., 2004; Lo et al., 2004; Kweon et al., 2004; Zhou et al., 2004; Zhou and Freed, 2004). However, dopaminergic phenotypes are not fully

characterized and confirmed in these cell lines and they are derived from animals, not humans. For these reasons, SY5Y cell line was selected as an in vitro model in this study.

The mechanisms of paraquat toxicity include the formation of ROS mediated by cytochrome P450 reductases and subsequent damage of ROS to cellular macromolecules (Farrington et al., 1973). In the presence of oxygen and ferric ions, the paraquat radical is rapidly reoxidized into paraquat and thus, this redox cycling reaction of paraquat can produce a large amount of ROS, which is known to cause lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and protein aggregation (Figure 9). Indeed, ROS generated by paraquat causes apoptosis in the developing brain and the adult rat hippocampus (Melchiorri et al., 1998; Barone et al., 2000). In addition, paraquat induces both the upregulation of α-synuclein and the accelerated formation of α-synuclein fibrils in dopaminergic neurons (Manning-Bog et al., 2002; Uversky et al., 2002).

These toxic properties of paraquat give rise to two fundamental questions with regard to dopaminergic pathogenesis. First, does paraquat cause oxidative stress-mediated toxicity in dopaminergic neurons? This toxic event is highly possible because ROS generated by paraquat metabolism might cause and aggravate oxidative stress conditions in dopaminergic neurons. Inherently, dopaminergic neurons are exposed to ROS induced by dopamine metabolism (Asanuma et al., 2003). Second, does paraquat cause proteasomal dysfunction-mediated toxicity in dopaminergic neurons? Most unwanted proteins are degraded by proteasome dependent pathway. Thus, proteasomal dysfunction might directly result in the cellular accumulation of toxic proteins. From this

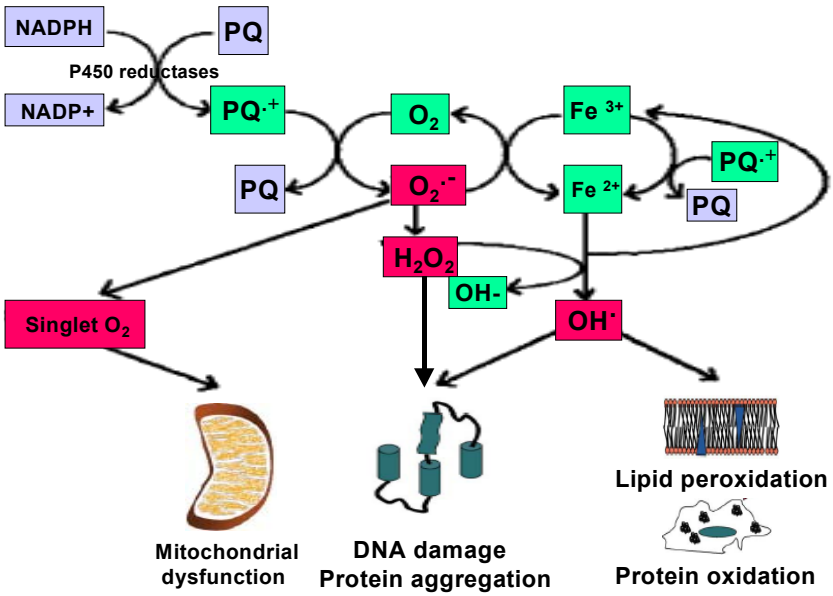

Figure 9. Proposed mechanisms of ROS-induced neuronal damage by paraquat (Yang & Sun, 1998). This figure is modified compared to the original one. Paraquat is first metabolized into paraquat radicals by P450 reductases and NADPH. Then, paraquat radicals react with oxygen to generate superoxide radicals ($O_2^{\cdot-}$), which can dismutate simultaneously or enzymatically to hydrogen peroxides (H_2O_2). H_2O_2 is readily reduced to form toxic hydroxyl radicals ($OH^{\cdot}$) by the conversion of iron from its ferrous (Fe^{2+}) to ferric (Fe^{3+}) state. The generated superoxide is able to reduce a bound transition metal such as copper or iron in its complex to the corresponding cuprous or ferrous state. Subsequently, the reduced bound metals can react locally with hydrogen peroxides to produce the additional hydroxyl radicals. The generated ROS might cause mitochondrial dysfunction, lipid peroxidation, protein oxidation and aggregation, and DNA damage, ultimately resulting in cell death.

viewpoint, the formation of intraneuronal aggregates (Manning-Bog et al., 2002) might reflect the disruption of the proteasome by paraquat. Thus, studies were undertaken to determine whether paraquat produces the biochemical pathology of oxidative stress and proteasomal dysfunction observed in dopaminergic neurons of PD brain.

To this aim, two hypotheses were tested.

The first hypothesis was that paraquat induces oxidative stress-mediated toxicity in SY5Y cells. The following objectives were carried out to test oxidative stress:

1. ROS and GSH were measured because they are initial biomarkers of oxidative stress condition.
2. Activities of GSH-related enzymes were measured because the change of ROS and GSH might be associated with dysregulation of their activities. For example, GPx and GST mainly consume GSH to detoxify ROS or exogenous toxicants, respectively. GR involves redox cycling of GSH to maintain phyiological levels of GSH in cells.
3. Mitochondrial transmmbrane potential was measured because oxidative stress is known to disrupt the normal gating of permeability transition pore resulting in the decrease of mitochondrial transmembrane potential.
4. Lipid peroxidation, protein oxidation, and DNA fragmentation were investigated to confirm paraquat-induced oxidative damage to intracellular macromolecules.
5. The protein levels of HO-1 were investigated because HO-1 is highly upregulated in response to oxidative stress.

The second hypothesis tested was that paraquat induces proteasomal dysfunction-mediated toxicity in SY5Y cells. The following objectives were carried out to address this hypothesis:

1. Proteasomal activity was measured because oxidative stress by toxicants might inhibit proteasomal activity directly or indirectly.
2. Activities of mitochondrial complex I and V were measured because the decrease of complex I activity has been suggested to contribute to proteasomal dysfunction in PD.
3. ATP levels were measured to correlate activities of complex I and V with ATP synthesis or to correlate the change of ATP levels with proteasomal function because 19S proteasomal subunits consume ATP to bind to polyubiquitinated proteins for protein degradation.
4. The protein levels of proteasomal subunits were investigated because loss or decrease of certain proteasomal subunits can cause structural instability leading to proteasomal dysfunction.
5. The protein levels of α-synuclein and ubiquitinated proteins were investigated because proteasomal dysfucntion has shown to induce the accumulation of those.
6. The protein levels of p53 and Bax were investigated because their turnover is directly mediated by proteasome-dependent pathway and proteasomal dysfunction has shown to cause the accumulation of both proteins.

7. Activities of capase-3/-9 were investigated because proteasomal dysfunction has shown to increase their activities and they are downstream intermediates of mitochondrial-dependent cell death pathway regulated by p53 and Bax.
8. Nuclear morphology was investigated because proteasomal dysfunction has shown to induce nuclear condensation as an indicator of apoptotic cell death.

The overall aim of this study was to correlate the relationship between paraquat toxicity and dopaminergic pathogenesis.

CHAPTER II

MATERIALS AND METHODS

Sources of materials

T-75 flasks and culture dishes (60 × 15 mm) were purchased from Corning Inc (NY, USA). 24-well and 96-well plates were purchased from Beckton Dickson (Franklin Lakes, NJ, USA). Fetal bovine serum (FBS) was purchased from Gemini (Woodland, CA, USA). 2',7'-Dichlorofluorescein diacetate (DCF-DA), monochlorobimane (MCB), and tetramethylrhodamine ethyl ester (TMRE) were purchased from Molecular Probes Inc (Eugene, OR, USA). The CellTiter 96 non-radioactive assay kit and DNA marker were purchased from Promega (Madison, WI, USA). A Glutathione peroxidase assay kit was purchased from Calbiochem (San Diego, CA, USA). Glutathione S-transferase and ATP assay kits were purchased from Cayman (Ann Arbor, MI, USA). A DNA purification kit was purchased from Gentra (Minneapolis, MN, USA). A Caspase-3 or -9 colorimetric assay kit was purchased from BioVision (Mount view, CA, USA). A polyclonal heme oxygenase-1 (HO-1) antibody was purchased from Stressgen (Victoria, BC, Canada). A polyclonal tyrosine hydroxylase (TH) antibody was purchased from Zymed (San Francisco, CA, USA). A polyclonal proteasome 20S α- or β-subunit antibody was purchased from Calbiochem (San Diego, CA, USA). 20S proteasome assay kit was purchased from Boston Biochem (Cambridge, MA, USA). A polyclonal proteasome 19S subunit antibody was purchased from Affinity Bioreagents (Golden, CO, USA). A polyclonal p53 or Bax antibody was purchased from Cell Signaling Technology

(Beverly, MA, USA). A monoclonal α-synuclein, ubiquitin, β-actin antibody and anti-mouse second antibody were purchased from Sigma (St Louis, MO, USA). An anti-rabbit second antibody was purchased from Santa Cruz (Santa Cruz, CA, USA). Western Lightning chemiluminescent reagent was purchased from Perkinelmer Life Sciences (Boston, MA, USA). Dulbecco`s modified Eagle`s medium (DMEM), F-12 HAM, glutathione reductase assay kit, and all other chemicals were purchased from Sigma (St Louis, MO, USA).

Cell culture and chemical treatment

The human neuroblastoma SH-SY5Y cell line from American Type Culture Collection was cultured in Dulbecco's modified Eagle's medium (DMEM) containing F-12 (1:1) and 10% fetal bovine serum (FBS) at 37°C in humidified 5% CO_2 and 90% O_2. SH-SY5Y cells have been used to characterize different effects of organophosphorous compounds (Ehrich et al., 1994; Ehrich & Correll, 1998; Cho & Tiffany-Castigloni, 2004), and polycyclic aromatic hydrocarbons (Tang et al., 2003). In addition, the cell line is used as a model to study dopaminergic pathogenesis (Takahashi et al., 1994; Chen et al., 1997; Hasegawa et al., 2003), because it expresses some of representative dopaminergic phenotypes including tyrosine hydroxylase (Shaul et al., 2003), dopamine transporter (Manakova et al., 2004), and dopamine metabolism (Legros et al., 2004). Stock cultures of SY5Y cells (passages 26 to 30) were routinely maintained in T-75 flasks. Except as noted, 5×10^5 cells were first seeded in culture dishes (60 × 15 mm), and then grown until they were 70% - 80% confluent for 3 days before chemical

treatment. To study oxidative stress and proteasomal dysfunction-mediated toxicity, cells were treated with paraquat for different time periods (0 - 48 hr). As a positive control, a natural insecticide rotenone was used to confirm the increase of ROS, the dissipation of mitochondrial transmembrane potential, the change of intracellular ATP level and 20S proteasome α- or β-subunit level. Also, caspase 3 or 9 activity, DNA fragmentation, and nuclear condensation were assayed in SY5Y cells exposed to rotenone (Nakamura et al., 2000; Wang et al., 2002; Molina-Jimenez et al., 2003; Shamoto-Nagai et al., 2003; Newhouse et al., 2004; Watabe and Nakai, 2004). Further, a specific inhibitor of γ-GCS, L-buthionine sulfoximine (BSO) and hemin were employed to test for GSH depletion (Cao et al., 2004) or the induction of heme oxygenase-1 (HO-1) (Nakaso et al., 2003), respectively in SY5Y cells. A specific proteasome inhibitor MG 132 was also used to assay the increase of p53, Bax, α-synuclein and ubiquitinated proteins (Biasini et al., 2004; Nakaso et al., 2004).

Trypan blue exclusion

Trypan blue exclusion was performed as previously described (Storch. et al., 2000; Cho and Tiffany-Castiglioni, 2004). Cells grown in culture dishes (60 × 15 mm) were treated with paraquat (0.05 - 1.0 mM) or co-treated with paraquat (0.05 - 1.0 mM) and 1 μM GBR12909 for 48 hr. After treatment, cells were washed and harvested with sterile PBS. The cell suspension was mixed at the ratio of 4:1 with 0.4 % trypan blue solution and incubated for 5 min. The viable cells were counted in a hemocytometer with an inverted-phase contrast microscope (Nikon Coporation, Model TMS, Tokyo, Japan).

3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The MTT assay was performed with a Celltier 96 non-radioactive kit. MTT is metabolically converted into formazan by mitochondrial dehydrogenases of healthy and live cells (Denizot & Lang, 1986). Briefly, cells (2×10^4) were seeded into each well of 96-well plate and in next day, treated with paraquat (0.05 - 1.0 mM) or co-treated with paraquat (0.05 - 1.0 mM) and 1 μM GBR12909 for 48 hr. After treatment, 15 μl of dye solution was added and continued to incubate at 37°C for 4 hr in a humid CO_2 incubator. After 4 hr, 100 μl of solubilization/stop solution was added to each well. Following incubation of one hour at 37°C, the absorbance was measured at 590 nm with a Fluostar Optima microplate reader (BMG labtech Inc., Durham, NC, USA).

Measurement of lactate dehydrogenase (LDH) release

The amount of LDH released into culture medium was measured as an indicator of cell membrane integrity (Moldeus et al., 1978). Briefly, cells grown in culture dishes (60 × 15 mm) were treated with paraquat (0.05 - 1.0 mM) or co-treated with paraquat (0.05 - 1.0 mM) and 1 μM GBR12909 for 48 hr. After treatment, the media containing detached cells were collected and centrifuged at 1000xg, 4°C for 10 min. The supernatants were added to 0.1 M potassium phosphate buffer (pH 7.4) containing 0.15 mM NADH, 0.2 M NaCl, and 0.09 mM sodium pyruvate in a final volume of 1 ml. The absorbance coupled with the reduction of pyruvate to L-lactate and the oxidation of NADH to NAD+ was measured at 340 nm for 5 min with a DU 640 spectrophotometer (Beckman Instrument Inc., Fullerton, CA, USA).

Measurement of reactive oxygen species (ROS)

The production of ROS was measured with a nonpolar compound 2',7'-dichlorofluorescein diacetate (DCF-DA) that readily enter the cells, where it is cleaved to form nonfluorescent 2',7'-dichlorofluorescein (DCFH) by endogenous esterases. DCFH reacts with reactive oxygen species to produce a fluorescent compound 2',7'-dichlorofluorescein, which is trapped inside the cells and indicates the intracellular ROS level (Oubrahim et al., 2001; Lee et al., 2003). To measure ROS, cells were treated with 0.5 mM paraquat for different time periods (0 - 48 hr) or 5 μM rotenone for 6 hr and then, cells were loaded with 50 μM for 30 min at 37^{o}C. After unloaded dye was removed out, cells washed with Hank`s balanced buffer and lysed in 0.1 M Tris (pH 7.5) containing 1% Triton X-100 (v/v). The cell lysates were centrifuged at 10,000xg, 4^{o}C for 10 min, and the supernatants were applied to 24 well plates to measure the DCF fluorescence at an excitation wavelength of 485 nm and an emission wavelength of 530 nm with a Fluostar Optima microplate reader. The fluorescence intensity obtained was normalized with on the protein concentration of individual extracts used.

Measurement of intracellular glutathione (GSH)

Monochlorobimane (MCB) is a fluorescent dye used to determine intracellular GSH levels. Within viable cells, GSH is specifically conjugated with MCB to form a fluorescent bimane-GSH adduct, which is catalyzed by glutathione S-transferases (Shrieve et al., 1988). To measure the intracellular levels of GSH, cells grown in culture dishes (60 × 15 mm) were first treated with 0.5 mM paraquat for different time periods

(0 - 48 hr) or 100 μM L-buthionine sulfoximine (BSO) for 24 hr, and then, cells were loaded with 40 μM MCB in serum-free medium at 37^{o}C for 30 min. After excess dye was washed off, cells were harvested and sonicated in Ca^{2+}/Mg^{2+} free PBS. The broken cells were centrifuged at 10,000xg, 10 min at 4^{o}C and the supernatants of 100 μl were applied to 24-well plates to measure fluorescence signals at an excitation wavelength of 380 nm and an emission wavelength of 485 nm with a Fluostar Optima microplate reader. The fluorescence signal obtained was normalized with protein concentration of individual extracts used.

Measurement of glutathione-related enzyme activity

For enzyme activity assays, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr). After treatment, cells were washed twice with PBS, harvested, and homogenized in cold lysis buffer of 100 μl containing 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 1 mM DTT. The lysates were centrifuged at 10,000xg, 4^{o}C for 20 min and the supernatants were assayed for enzyme activity.

Glutathione peroxidase (GPx)

GPx activity was measured with a cellular glutathione peroxidase assay kit according to the manufacturer`s protocol. Briefly, the lysates were mixed with 900 μl of reaction buffer containing 1 mM GSH, 0.2 mM NADPH, 0.22 mM *tert*-butyl hydroperoxide, and 0.4 units/ml glutathione reductase. The absorbance change coupled

with NADPH oixdation was monitored at 340 nm for 5 min. GPx activity was defined as nmol NADPH oxidized min/mg protein by use of the molar extinction coefficient of 6.22×10^3 M/cm.

Glutathione reductase (GR)

GR activity was measured with a glutathione reductase assay kit according to the manufacturer`s protocol. The lysates were first mixed with 50 µl of 2mM oxidized GSH and 900 µl reaction buffer of 100 mM potassium phosphate buffer (pH 7.5) including 1 mM EDTA, and then the reaction was initiated by adding 50 µl of 2 mM NADPH. The total volume of the mixture was 1 ml. The absorbance change was recorded by measuring the oxidation of NADPH at 340 nm for 5 min. GR activity was calculated as nmol NADPH oxidized min/mg protein using the molar extinction coefficient of 6.22×10^3 M/cm.

Glutathione S-transferase (GST)

GST activity was measured with a glutathione S-transferase assay kit according to the manufacturer`s protocol slightly modified. The reaction mixtures consisted of 100 µl cell lysates, 100 µl reduced GSH, 50 µl 1-chloro-2,4-dinitrobenzene (CDNB), and 750 µl assay buffer containing 100 mM potassium phosphate (pH 6.5), and 0.1% (v/v) Triton X-100. The absorbance change was recorded by measuring the formation of a dinitrophenyl thioether at 340 nm for 5 min. GST activity was calculated as nmol CDNB conjugate formed min/mg protein using a molar extinction coefficient of 9.6×10^3 M/cm.

Measurement of mitochondrial transmembrane potential (MTP)

Tetramethylrhodamine ethyl ester (TMRE) is a cationic dye, which is rapidly accumulated by mitochondria, due to their membrane potential and thus, damaged mitochondria cannot retain TMRE (Krohn et al., 1999). To measure MTP, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr) or 5 μM rotenone for 12 hr and then were incubated in serum-free media containing 200 nM TMRE at 37^{o}C for 15 min. After unloaded dye was removed out, cells were washed twice with PBS, harvested, and lysed in 100 μl of cold buffer containing 0.1 M Tris-Cl (pH 7.5) and 10% sodium dodecyl sulfate (w/v). The lysates were applied to 24-well plates to measure the TMRE signal at an excitation wavelength of 500 nm and an emission wavelength of 540 nm with a Synergy HT Multi-Detection microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The fluorescence signals obtained were normalized with protein concentration of each lysate used.

Lipid peroxidation

The lipid peroxidation end product malondialdehyde (MDA) was measured by the thiobarbituric acid (TBA) test as previously described (Wilber et al., 1949). Cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr). After treatment, cells were washed twice with PBS, harvested, and sonicated in 100 μl of cold lysis buffer consisting of 0.1 M Tris-Cl (pH 7.5), and 1 mM EDTA. Cell homogenates were mixed with 20% (v/v) trichloroacetic acid (TCA), vortexed, and then centrifuged at 12,000xg, 4^{o}C for 10 min. 0.67% (w/v) TBA

containing glacial acetic acid was added to the supernatants. The mixture was boiled at 100^{o}C for 10 min, then cooled on ice. MDA levels were determined in the samples at 530 nm with a DU 640 spectrophotometer (Beckman Instrument Inc., Fullerton, CA, USA). The absorbance values were normalized with the protein concentration of each supernatant used.

Protein oxidation

The protein oxidation end product carbonyl was measured as previously described (Jurma et al., 1997). Cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr). After treatment, cells were washed twice with PBS, harvested, and then sonicated in 100 µl of cold homogenizing buffer consisting of 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 5 µg/ml leupeptin, 5 µg/ml aprotinin, and 40 µg/ml phenylmethylsulfonyl fluoride (PMSF). The homogenates were then centrifuged at 10,000xg, 4^{o}C, for 10 min to remove cell debris. Supernatants were mixed with 10 mM of 2,4-dinitrophenyl hydrazine (DNPH) dissolved in 2 M HCL and incubated at room temperature for 60 min, with vortexing every 15 min. 20% (v/v) trichloroacetic acid (TCA) was added to supernatants, which were then centrifuged at 15,000xg, 4^{o}C, for 10 min. The precipitates were washed 3 times for 10 min each with ethanol /ethyl acetate (1:1, v/v) and then dissolved in 6 M guanidine HCL, pH 2.3, adjusted with TCA, by incubating at 37 ^{o}C for 15 min. Carbonyl levels were measured at 375 nm with a DU 640 spectrophotometer. The absorbance value was then normalized with protein concentrations of precipitates used.

DNA fragmentation

After treatment with 0.5 mM paraquat for up to 48 hr or 0.5 μM rotenone for 24 hr, respectively, cells in T-75 flasks were washed twice with PBS (pH 7.4), harvested, and centrifuged at 10,000xg for 5 min. Total genomic DNA was isolated from the same numbers of cells (5×10^6) with a Puregene DNA purification kit. Briefly, the collected cells were lysed with lysis solution, and treated with RNase A (40 μg/ml in a final concentration) at 37°C for 60 min. Protein precipitation solution was then added to the lysates, which were then vortexed vigorously and centrifuged at 16,000xg for 5 min. Supernatants were mixed with 100% isopropanol, inverted 20 times, and centrifuged at 15,000xg for 5 min to precipitate DNA. After being washed with cold 70% ethanol, DNA precipitates were air-dried in hood, dissolved in DNA hydration solution, and treated with proteinase K (0.4 mg/ml in a final concentration) at 55°C overnight. Finally, 20 μl of DNA were subject to electrophoresis on a 1.2% agarose gel, and DNA fragmentation was visualized under UV light after staining with ethidium bromide.

Measurement of proteasomal activity

Proteasomal activity was measured with a proteasome assay kit according to manufacturer`s protocol slightly modified. Briefly, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for various time periods (0 - 48 hr) or 0.5 μM rotenone for 12 hr. After treatment, cells were harvested, washed twice with PBS, and lysed for an hour in a cold buffer containing 50 mM HEPES (pH 7.6), 50 mM NaCl, 1 mM DTT. After centrifugation at 15,000xg for 30 min, 100 μl of supernatant was

aliquoted into 96-well plate. Then, 10 μl of 10 mM succinyl-leucine-leucine-valine-tyrosine-7-amino-4-methylcoumarin (Suc-LLVY-AMC) and 3% SDS were added to monitor the fluorescence signal released from free AMC at an excitation wavelength of 380 nm and an emission wavelength of 460 nm with a Fluostar Optima microplate reader. The specific 20S proteasomal activies were calculated as total activity minus remaining activity of lysates in the presence of 20 μM proteasome inhibitor MG132 (Höglinger et al., 2003).

Measurement of mitochondrial complex I and V activity

For enzyme activity assays, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr). After treatment, cells were harvested, washed twice with PBS, and suspended in 100 μl of cold isotonic buffer containing 250 mM sucrose, 1 mM EDTA, and 10 mM HEPES (pH 7.4). The cell suspension was subjected to three cycles of freezing and thawing in liquid nitrogen. The lysates were centrifuged at 1000xg, 4^{o}C for 10 min and the supernatants were assayed for enzyme activity by use of a DU 640 spectrophotometer.

Mitochondrial complex I (NADH-ubiquinone oxidoreductase)

Complex I activity was determined by measuring the rate of NADH oxidation as previously described (Clementi et al., 1998; Hölinger et al., 2003). The isolated mitochondrial fractions were added to 1 ml of reaction solution containing 10 mM potassium phosphate buffer (pH 8.0), 2.5 mg/ml bovine serum albumine (fraction V),

100 μM decylubiquinone, and 200 μM NADH. The absorbance change was monitored at 340 nm for 5 min in the presence and absence of 2 μM rotenone. Rotenone-sensitive absorbance was considered to represent a specific complex I activity.

Mitochondrial complex V (F_1F_0 ATPase)

Complex V activity was determined by measuring the rate of NADH oxidation as previously described (Barrientos et al., 1998; Theron et al., 2000). The isolated mitochondrial fractions were added to 1 ml of reaction solution containing 100 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 10 mM KCl, 25 mM ATP, 0.2 mM NADH, 4 units of pyruvate kinase and lactate dehydrogenase. The absorbance change was monitored at 340 nm for 5 min in the presence and absence of 3 μM oligomycin. Oligomycin-sensitive absorbance was considered to represent a specific complex V activity.

Measurement of intracellular ATP

Intracellular ATP levels were measured with an ATP assay kit, which utilizes luciferase to catalyze the formation of luminescence from ATP and luciferin. Briefly, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr) or 0.05 μM rotenone for 24 hr, respectively. After treatment, cells were harvested, washed twice with PBS and 10^4 cells were lyzed in 100 μl of nuclear releasing buffer provided by manufacturer. After centrifugation at 10,000xg, 4^{o}C for 10 min, the supernatants were subjected to 96-well plate. Then, 1 μl of ATP monitoring enzyme was added to measure the luminescence with a Fluostar Optima

microplate reader. The luminescence value was then normalized with protein concentration of each supernatant used.

Measurement of caspase 3 and 9 activity

Caspase 3 and 9 activity were measured with a caspase-3/-9 colorimetric assay kit according to manufacturer`s protocol. Briefly, cells grown in culture dishes (60 × 15 mm) were treated with 0.5 mM paraquat for different time periods (0 - 48 hr) or 5 μM rotenone for 24 hr. After treatment, cells were harvested, washed twice with PBS, and suspended in 100 μl of cold lysis buffer containing 5 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 0.5% Triton X-100 for 30 min. After centrifugation at 10,000xg for 10 min, 200 μg of supernatant and 50 μl of reaction buffer containing 10 mM DTT were aliquoted into a 96-well plate. Then, DEVD-*p*NA and LEHD-*p*NA were added as a substrate of caspase 3 or 9, respectively. The absorbance released from free *p*NA was monitored at 400 nm with a Fluostar Optima microplate reader for an hour.

Nuclear morphology

The morphological changes of nucli were observed with the nuclear dye Hoechst, which permeates nuclear membranes and yields blue chromatin (Gómez et al., 2001). Viable cells show normal nucleus size and chromatin density, whereas apoptotic cells display condensed chromatin with Hoechst staining. Briefly, cells grown in culture dish (60 × 15 mm) were first treated with 0.5 mM paraquat for various time periods (0 - 48 hr) or 0.1 μM rotenone for 24 hr. Then, cells were washed with warm PBS two times,

stained with 50 μM Hoechst and incubated at 37^{o}C incubator for 30 min. After excess dye was washed off, nuclear images were captured through 20X objective lens via Olympus IX 70 inverted fluorescence microscope (Olympus America Inc, Melville, NY, USA). Light emitted from the Hoechst dye was detected at an emission wavelength > 420 nm with an excitation 330- 385 nm.

Western blotting

After treatment of cells grown in culture dishes (60 × 15 mm) with 0.5 mM paraquat for up to 48 hr, 50 μM hemin for 12 hr, or 10 μM MG132 for 24 hr, respectively, cells were washed with PBS and harvested. Cells were lyzed with protein isolation buffer (0.1 M NaCl, 0.5 M Tris–HCl, pH 7.5, 10% SDS, 1% Triton X-100, 20 μ g/ml aprotinin, 20 μg/ml leupeptin) on ice for 40 min. After centrifugation at 15,000x*g* for 30 min at 4°C, supernatants were collected and protein concentration was determined by the Bradford assay. Isolated cytosolic proteins (40 - 50 μg) were run on a 12% sodium dodecyl sulfate polyacrylamide gel with 70 V for 3 hr. After electrophoresis, the separated proteins were transferred onto a PVDF membrane with 290 mA, 10 V for 65 min. The membrane was blocked with 5% (w/v) non-fat dry milk solution in Tris-buffered saline (TBS) for 2 hr and then incubated with primary antibodies (1:1000) at 4°C for 12 hr. After incubation, the membrane was washed three times with Tris-buffered saline–0.05% Tween 20 (TBS-T) at room temperature for 10 min and incubated with horseradish peroxidase-conjugated second antibodies (1:1000) for 4 hr at room temperature. The membrane was four times washed with TBS-T at room temperature for

10 min. After four final washing steps, protein bands were visualized on Kodak X-OMAT blue autoradiography film (Eastman Kodak Company, Rochester, NY, USA) with Western Lightning Chemiluminescence reagent. ImageJ version 1.33 (NIH, Maryland, MA, USA) was used to quantitate each protein band of western blot.

Statistical analysis

All experiments were performed 3 - 6 times and results are presented as mean ± SEM. Statistical significance was determined between untreated groups and treated groups at each time point, with the one-way analysis of variance (ANOVA) and the Dunnett`s test (GraphPad Software Inc., San Diego, CA, USA). A value of $p < 0.05$ was considered to be significant.

CHAPTER III

RESULTS

Effects of paraquat and GBR12909 on cell viability

To examine the adverse effects of paraquat, SY5Y cells were treated with various concentrations (0.05 - 1 mM) of paraquat for 48 hr and cell viability was assayed by trypan blue exclusion (Figure 10), MTT (Figure 11), and LDH (Figure 12). Paraquat decreased cell viability in a concentration-dependent manner. In particular, 0.5 and 1 mM paraquat significantly decreased viability to 56.2 ± 7.9 % or 42.8 ± 9.4 % of control, respectively. To investigate whether the adverse effects of paraquat were mediated by a dopamine transporter, SY5Y cells were co-incubated with a specific dopamine transporter inhibitor GBR12909 (1 μM) and various concentrations of paraquat (0.05 - 1 mM) for 48 hr and cell viability was checked. GBR12909 significantly protected SY5Y cells against the cytotoxicity of lower concentrations of paraquat (0.05 - 0.5 mM) and partially attenuated the toxicity of higher concentrations of paraquat (0.5 - 1.0 mM) (Figure 10-12). Based on the viability data, 0.5 mM paraquat was selected as a minimum effective concentration for other experiments. SY5Y cells were treated with 0.5 mM paraquat and cell morphology was examined in a time dependent manner (Figure 13). No significant changes were observed until 12 hr, whereas the shrinkage of neurites was seen at 24 hr and most cells were seriously damaged at 48 hr. Likewise, severe damage was observed in cells exposed to 0.1 μM rotenone for 24 hr.

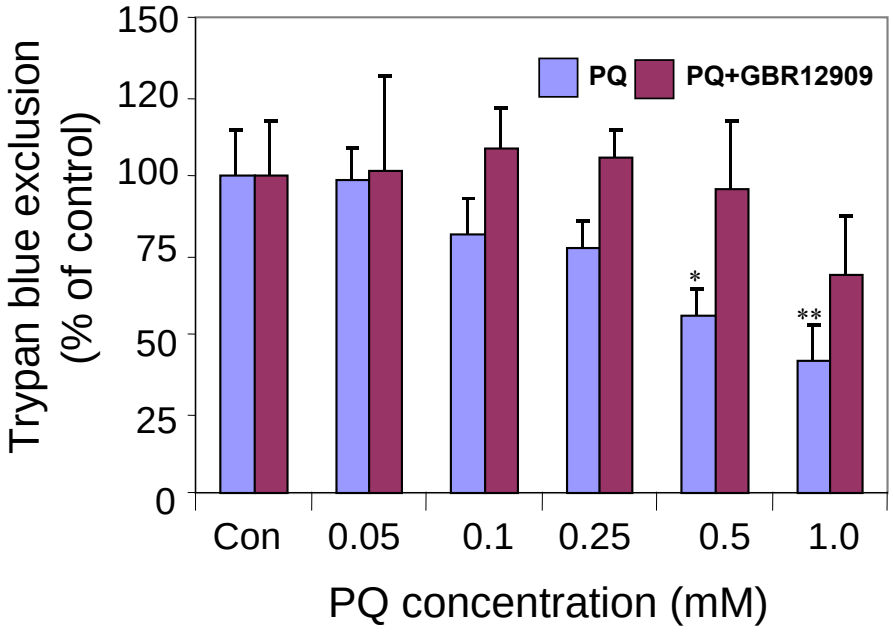

Figure 10. Effect of paraquat on trypan blue exclusion. Cells in culture dishes (60 × 15 mm) were exposed to various concentrations (0.1 - 1 mM) of paraquat or GBR12909 (1 μM) for 48 hr and viability was measured by trypan blue exclusion. Data are expressed as mean % of untreated control ± SEM for 4 independent experiments (n = 4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

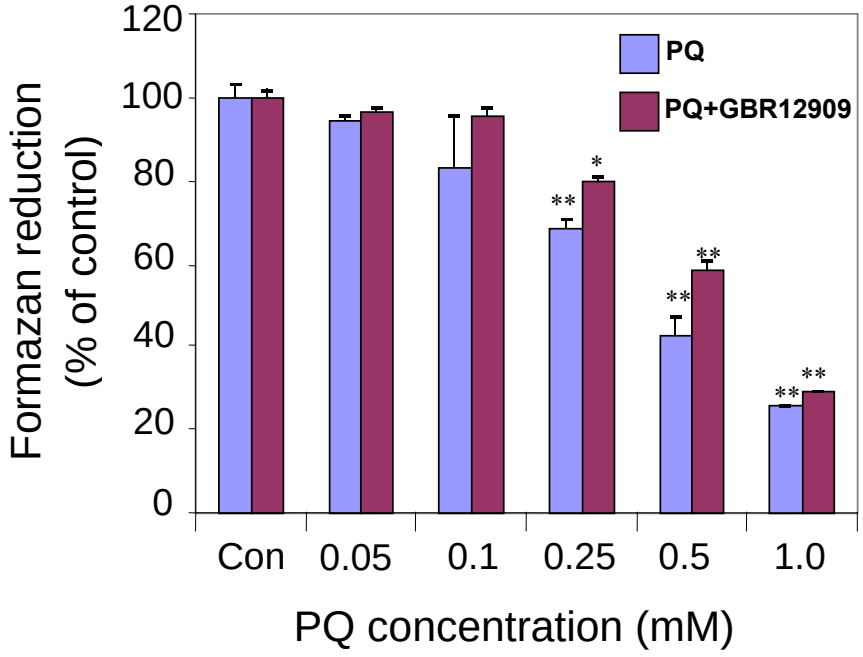

Figure 11. Effect of paraquat on formazan reduction. Cells in culture dishes (60 × 15 mm) were exposed to various concentrations (0.1 - 1 mM) of paraquat or GBR12909 (1 μM) for 48 hr and viability was determined by formazon reduction. Data are expressed as mean % of untreated control ± SEM for 4 independent experiments (n = 4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

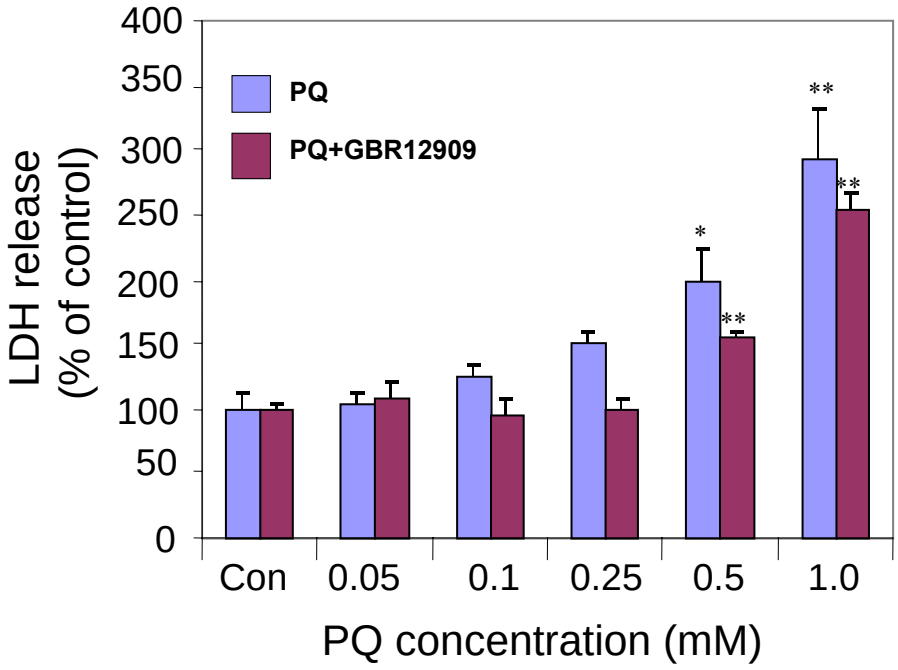

Figure 12. Effect of paraquat on LDH release. Cells in culture dishes (60 × 15 mm) were exposed to various concentrations (0.1 - 1 mM) of paraquat or GBR12909 (1 μM) for 48 hr and toxicity of paraquat was determined by the release of latactate dehydrogenase. Data are expressed as mean % of untreated control ± SEM for 4 independent experiments (n = 4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

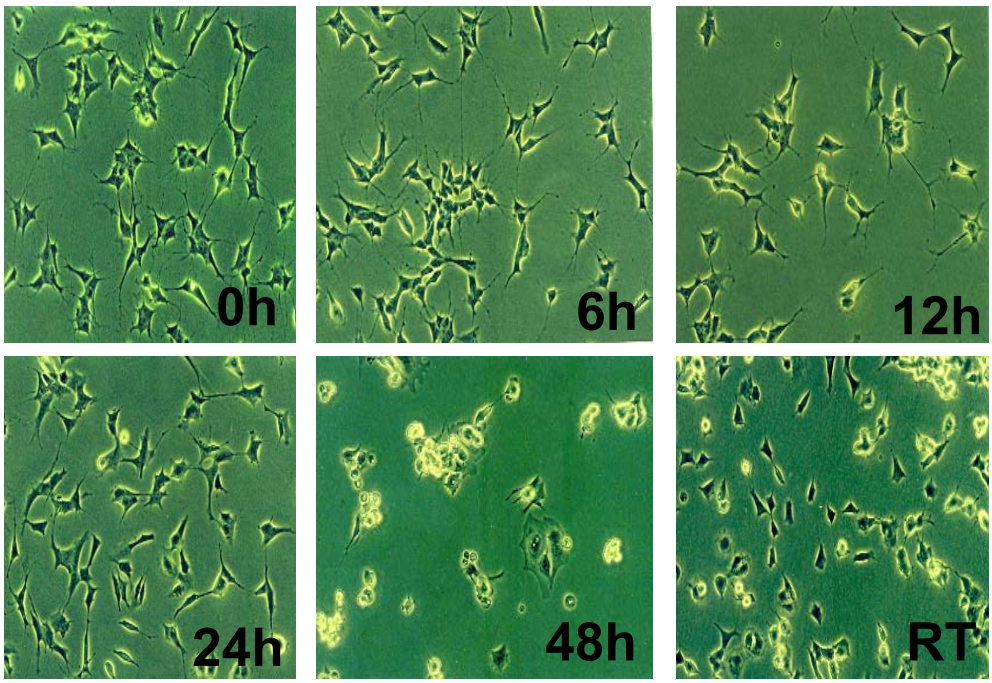

Figure 13. Effect of paraquat on the morphology of SY5Y cells. Cells in culture dishes (60 ×15 mm) were exposed to paraquat (0.5 mM) up to 48 hr or rotenone (0.1 μM) for 24 hr and after treatment, cell morphology was captured through 100X objective lens of phase-contrast light microscope. Each micrograph is representative of 6 fields.

Effect of paraquat on the levels of ROS and reduced GSH

A potent prooxidant, paraquat is known to generate reactive oxygen species (ROS) via the cytochrome P-450-mediated redox cycling reaction (Suntres, 2002). Thus, studies were carried out to determine whether ROS was generated from SY5Y cells treated with paraquat (Figure 14). Cells were treated with 0.5 mM paraquat for up to 48 hr or 5 μM rotenone for 6 hr and ROS levels were determined by measuring DCF fluorescence at each time point. Paraquat at 0.5 mM did not increase peroxide levels until 12 hr, whereas 5 μM rotenone significantly increased peroxide levels to 138 ± 7.62% of control within 6 hr. At 24 hr and 48 hr, 0.5 mM paraquat significantly increased peroxide levels to 129.8 ± 5.63 % and 151.1 ± 8.03% of control, respectively. Levels of intracellular GSH were measured because GSH is a major antioxidant for detoxifying ROS (Figure 15). SY5Y cells were treated with 0.5 mM paraquat for up to 48hr or with 100 μM L-buthionine sulfoximine (BSO) for 24 hr and GSH levels were determined by measuring MCB fluorescence at each time point. Paraquat at 0.5 mM did not significantly decrease GSH levels until 12 hr, but 100 μM BSO markedly decreased GSH levels to 47.3 ± 5.29% of control at 24 hr. At 24 hr and 48 hr, 0.5 mM paraquat reduced GSH levels to 59.4 ± 13.7 % or 38.5 ± 8.43% of control, respectively.

Effects of paraquat on the activities of GPx, GR, and GST

GPx, GR, and GST are antioxidant enzymes involved in scavenging of reactive oxygen species or conjugation of metabolites with GSH (Hayes & Strange, 1995; Griffith & Mulcahy, 1999). Thus, if these enxymes are inhibited, cells may suffer from

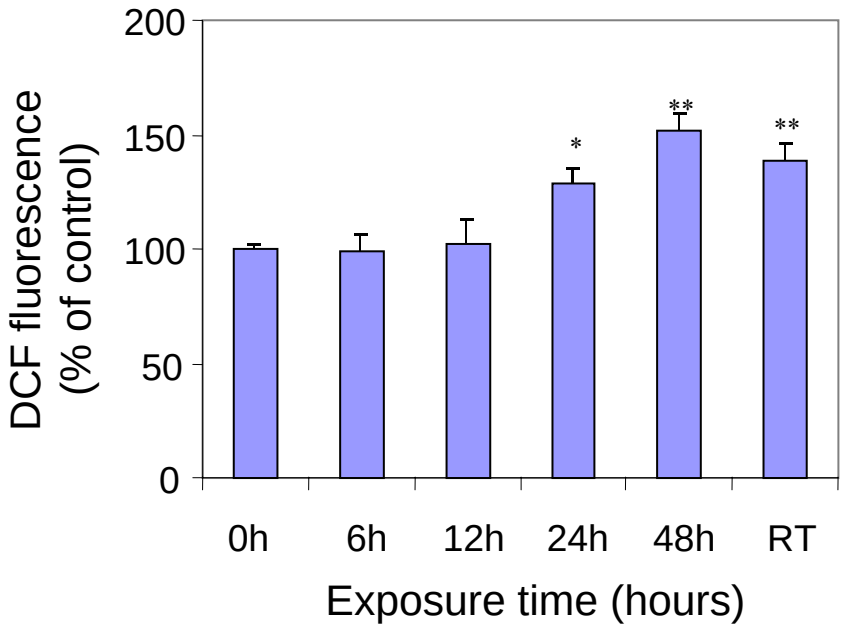

Figure 14. Effect of paraquat on ROS level. Cells in culture dishes (60 × 15 mm) were first exposed to paraquat (0.5 mM) for up to 48 hr or rotenone (5 μM) for 6 hr. After exposure, cells were loaded with DCF-DA (50 μM), and broken to isolate cytosol containing DCF. Levels of peroxides were determined by measuring the fluorescence of DCF cleaved from DCF-DA. Data are expressed as mean % ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

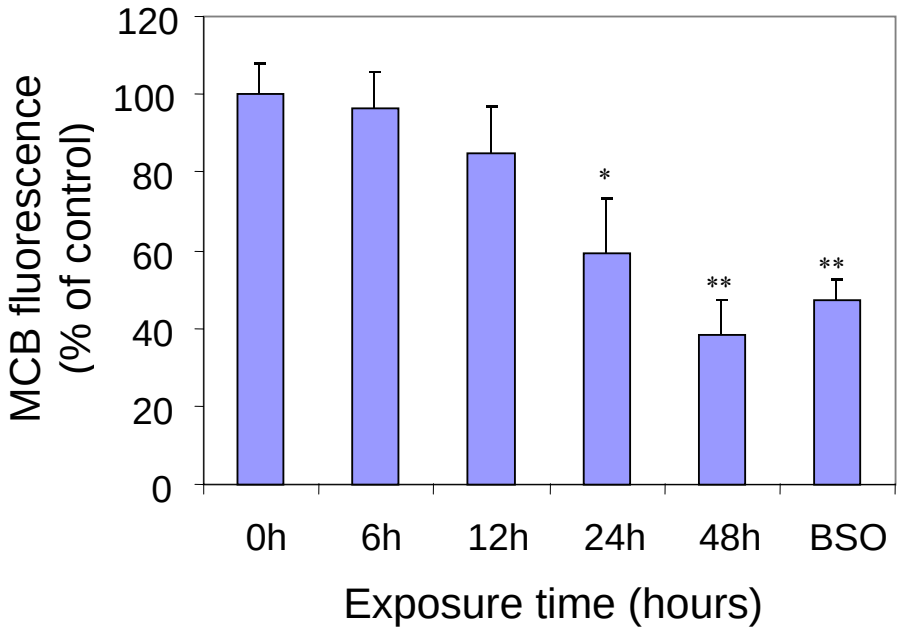

Figure 15. Effect of paraquat on intracellular GSH level. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr or BSO (L-buthionine sulfoximine, 100 μM) for 24 hr. After exposure, cells were loaded with MCB (40 μM), and broken to isolate cytosol containing MCB. GSH levels were determined by measuring GSH-MCB adducts.fluorescence with a microplate reader. Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

oxidative stress. The activities of these enzymes were therefore investigated in SY5Y cells treated with 0.5 mM paraquat for up to 48 hr (Figure 16). GR activity was not significantly affected by 0.5 mM paraquat. GST activity increased by 12 hr and reached its highest value (193.26 ± 14.21% of control) at 24 hr, before returning to the control value at 48 hr. On the other hand, GPx activity decreased in a time dependent manner: 50.9 ± 16.1% and 21.23 ± 6.18% of control at 24 hr or 48 hr, respectively.

Effect of paraquat on MTP

MTP is disrupted by pathological conditions such as oxidative stress, ATP depletion, Ca^{2+} overload, and high pH of the mitochondrial matrix (Škárka & Oštadál, 2002). Thus, given that paraquat increased reactive oxygen species (ROS) (Figure 14), but decreased GSH levels (Figure 15), it is conceivable that paraquat decreases MTP. SY5Y cells were treated with 0.5mM paraquat for up to 48 hr or 5 μM rotenone for 12 hr and MTP was determined by measuring TMRE fluorescence (Figure 17). Paraquat at 0.5 mM had no effect at 12 hr, though 5 μM rotenone greatly decreased MTP to 31.74 ± 1.71% of control. However, 0.5 mM paraquat significantly reduced MTP to 69.8 ± 2.98% and 54.3 ± 3.59% of control at 24 or 48 hr, respectively.

Oxidative damage of paraquat to intracellular macromolecules

Reactive oxygen species (ROS) are highly toxic and thus may harm cellular components including membrane lipids, cellular proteins, and DNA (Halliwell & Gutteridge, 1984). As shown in figure 14, paraquat markedly increased ROS levels.

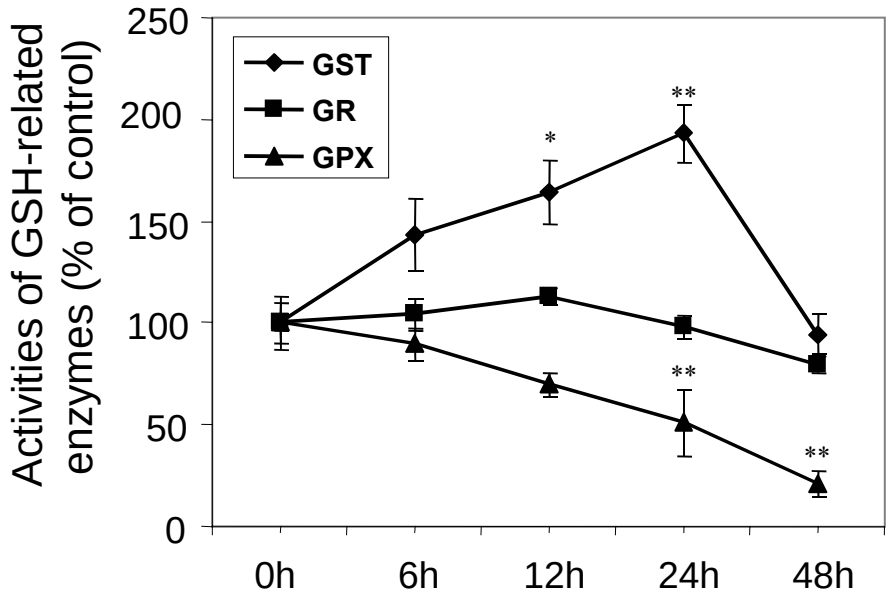

Figure 16. Effect of paraquat on the activities of GSH-related enzymes. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr and at each time point, enzyme activities were assayed in cell homogenates. GPx or GR activity was determined by measuring NADPH oxidized per min per mg protein, whereas GST activity was determined by measuring CDNB conjugate formed per min per mg protein. Data are expressed as mean % of untreated control ± SEM for 4 independent experiments done at each time point (n = 4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

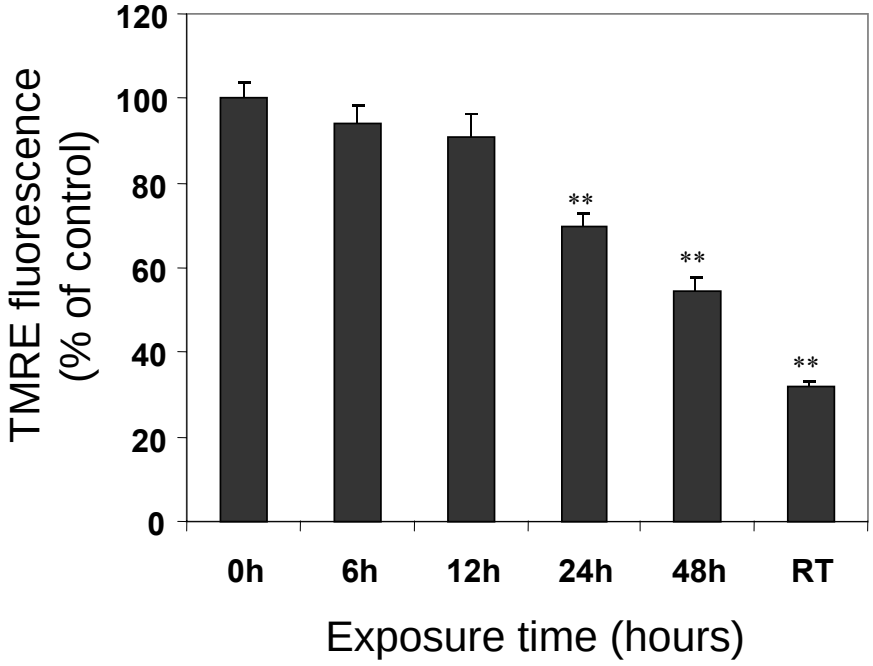

Figure 17. Effect of paraquat on MTP. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr or rotenone (2 μM) for 12 hr. After exposure, cells were loaded with TMRE (200 nM) and lysed to isolate cytosol containing TMRE. MTP was determined by measuring fluorescence of TMRE with a microplate reader. Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $P <$ 0.05 and ** $p < 0.01$ when compared to untreated control.

Thus, studies investigated whether paraquat would produce ROS-mediated damage to lipids, proteins, and DNA from SY5Y cells. 0. 5 mM paraquat significantly increased the concentration of malondialdehyde (MDA), a product of lipid peroxidation, in a time dependent manner (Figure 18). In particular, it elevated MDA levels to 194.2 ± 3.67% or 241.8 ± 4.7% of control at 24 hr and 48 hr, respectively. Likewise, cellular proteins sustained oxidative damage by 0.5 mM paraquat. Protein carbonyls significantly increased to 167.9 ± 9.5% and 188.1 ± 15.9% of control at 24 hr or 48 hr, respectively (Figure 19). Furthermore, 0.5 mM paraquat and 0.5 μM rotenone induced DNA fragmentation at 48 hr or 24 hr, respectively (Figure 20).

Effect of paraquat on the protein levels of TH and HO-1

TH is a specific biomarker of dopaminergic neurons (Kim et al., 2003) and heme oxygenase-1 (HO-1) is strongly induced in response to oxidative stress (Leon et al., 2003). Thus, it was tested whether the protein levels of TH, and HO-1 are affected by paraquat. SY5Y cells were treated with 0.5 mM paraquat or 50 μM hemin for up to 48 hr or for 12 hr, respectively, and the protein levels of TH and HO-1 were checked by western blotting. Paraquat did not significantly change the protein level of TH. However, paraquat significantly increased the protein level of HO-1 to 169.23 ± 8.3% or 193.98 ± 10.5% of 0 hr at 24 hr and 48 hr, respectively (Figure 22). Similarly, hemin greatly increased HO-1 protein level to 305.41 ± 13.4% of 0 hr at 12 hr.

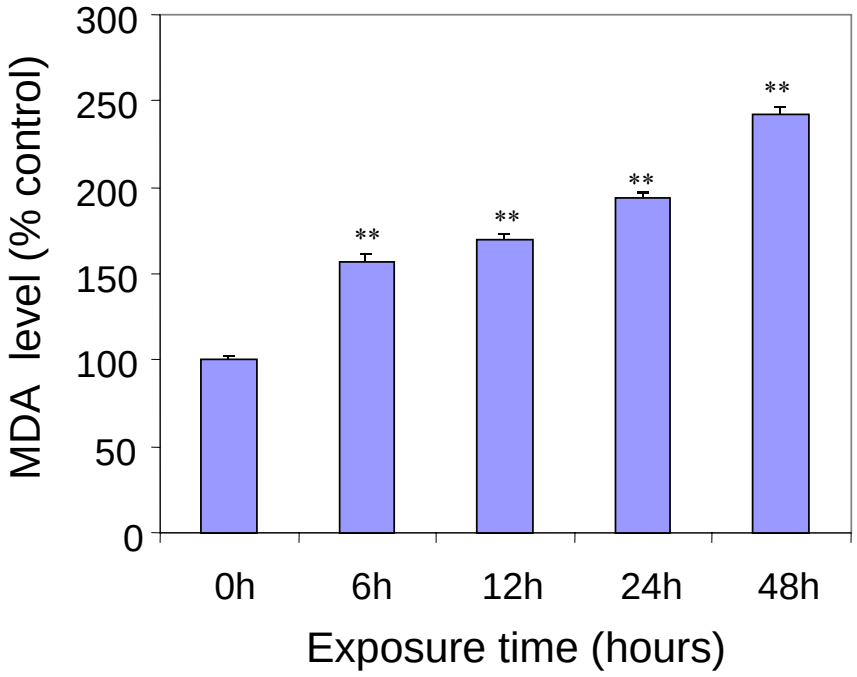

Figure 18. Effect of paraquat on malondialdehyde (MDA) level. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr and lysed to isolate cytosol containing oxidatively damaged lipids. The degree of lipid peroxidation was determined by measuring levels of MDA with a spectrophotometer. Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

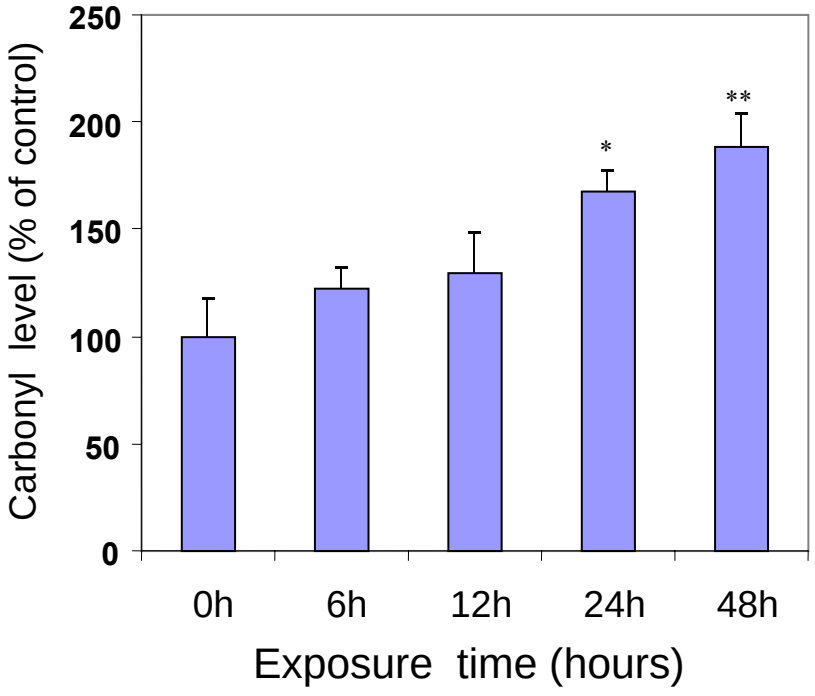

Figure 19. Effect of paraquat on carbonyl level. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr and lysed to isolate cytosol containing oxidatively damaged proteins. The degree of protein oxidation was determined by measuring levels of protein carbonyls with a spectrophotometer. Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

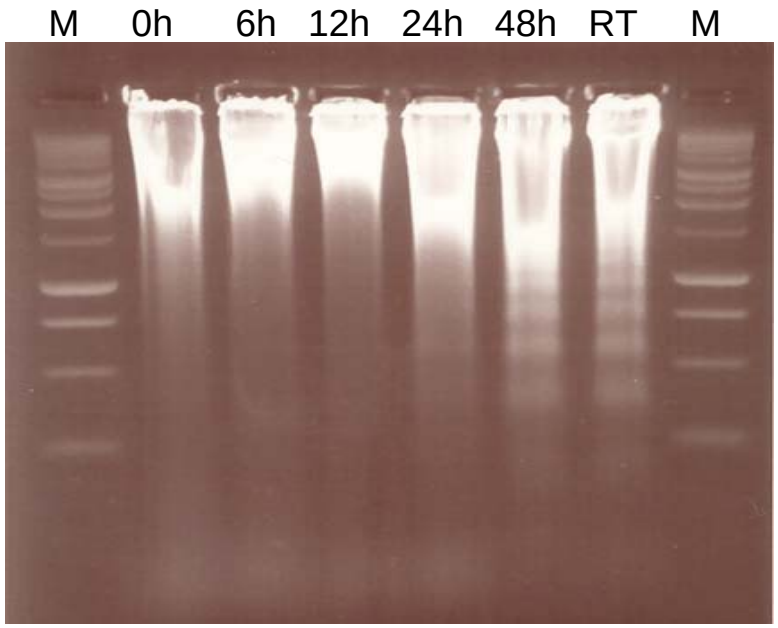

Figure 20. Paraquat-induced DNA fragmentation in SY5Y cells. Cells in T-75 flasks were exposed to paraquat (0.5 mM) for 6 hr (lane 3), 12 hr (lane 4), 24 hr (lane 5), and 48 hr (lane 6), respectively, or RT (0.5 μM rotenone, lane 7) for 12 hr. 0 hr group is shown in lane 2 and 1.0 Kb DNA size markers are shown in lane 1 and 8. DNA isolated from the same numbers of cells (5×10^6) was subjected to 1.2% agarose gels. After electrophoresis, gels were stained by ethidium bromide and the fragmented DNA was visualized under UV light. The result is representative from 4 independent experiments.

Effect of paraquat on proteasomal activity

Proteasomal activity is important in determining neuronal survival and death (Lopes et al., 1997; Pasquini et al., 2000). Some parkinsonian chemicals including maneb, 6-OHDA, dopamine, and neuromelanin have shown to decrease the activity of the proteasome (Keller et al., 2000; Elkon et al., 2004; Shamoto-Nagai et al., 2004; Zhou et al., 2004). Thus, study examined whether paraquat affects proteasomal activity. SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr or 0.5 μM rotenone for 12 hr and proteasomal activity was measured at each time point (Figure 23). Paraquat moderately decreased proteasomal activity by 12 hr, though 0.5 μM rotenone greatly decreased proteasomal proteasomal activity to 18.2 ± 8.1% of control at 12 hr. However, at 24 hr and 48 hr, paraquat significantly decreased proteasomal activity to 40.1 ± 12.9% or 22.8 ± 10.2% of control, respectively.

Effect of paraquat on the activities of mitochondrial complex I and complex V

ROS production and ATP synthesis are physiologically modulated by complex I or complex V of mitochondrial electron transport chains, respectively (McLennan et. al., 2000; Zheng & Ramirez et al., 2000). The inhibition of complex I have been suggested to cause proteasomal dysfunction (Höglinger et. al., 2003). Thus, in congunction with proteasomal dysfunction, experiments were carried out to test whether paraquat affects mitochondrial complex I and V function (Figure 24). Paraquat did not significantly affect the activity of complex I by 12 hr, whereas at 24 hr and 48 hr, it markedly decreased the activity of complex I to 52.2 ± 10.5% or to 25.9 ± 7.9% of control.

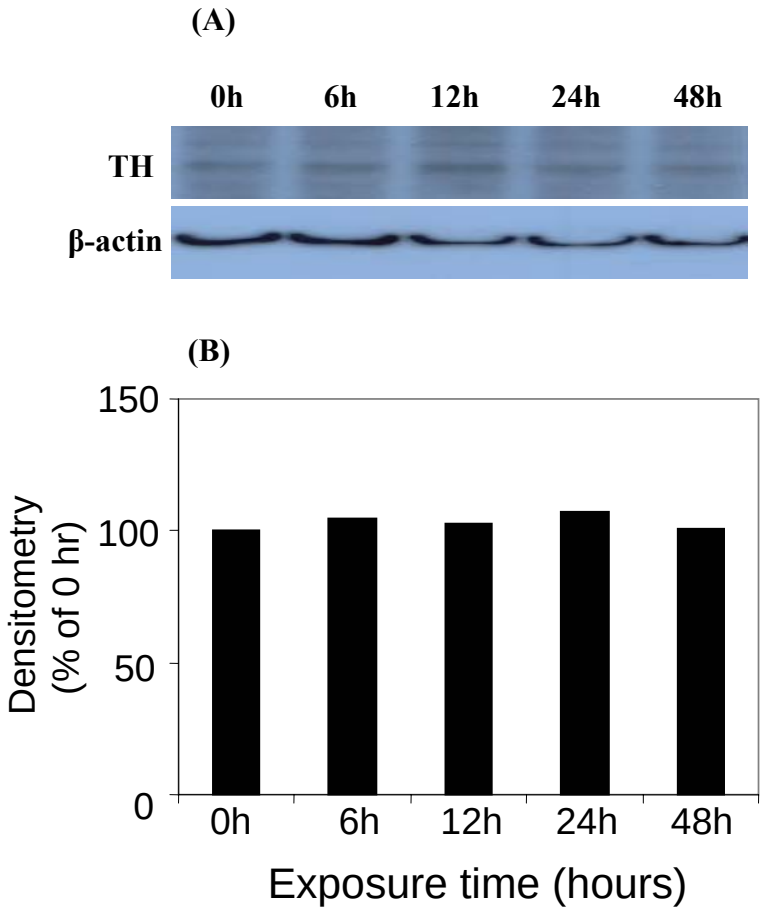

Figure 21. Effects of paraquat on the protein level of tyrosine hydroxylase (TH). Cells were exposed to paraquat (0.5 mM) for up to 48 hr. 50 μg of cytosolic proteins isolated at each time point were used to analyze TH by western blotting. (A) Representative immunoblot of TH from two separate experiment (B) TH level was quantified by densitometric analysis of each band of TH and normalized on the level of β-actin. The quantified TH level is expressed as % of 0 hr.

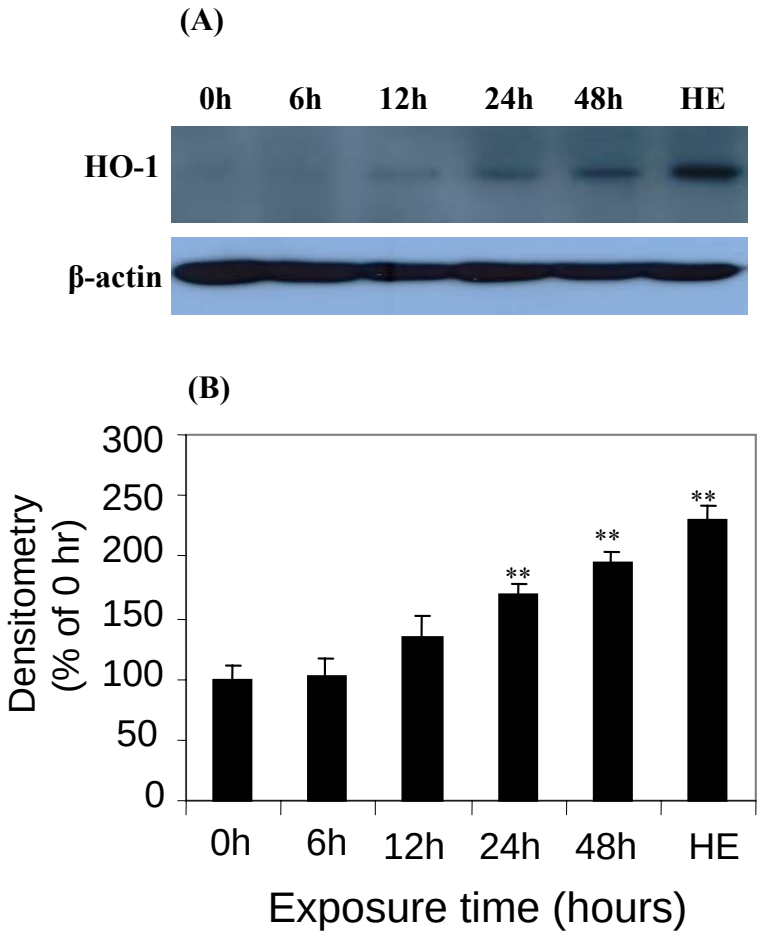

Figure 22. Effects of paraquat on the protein level of heme oxygenase-1 (HO-1). Cells were exposed to paraquat (0.5 mM) for up to 48 hr or hemin (HE) (50 μM) for 24 hr. 50 μg of cytosolic proteins isolated at each time point were used to analyze HO-1 by western blotting. (A) Representative immunoblot of HO-1 (B) HO-1 level was quantified by densitometric analysis of each band of HO-1 and normalized on the level of β-actin. The quantified HO-1 level is expressed as % of 0 hr. Values represent mean ± SEM for 4 independent experiments (n =4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; ** $p < 0.01$ when compared to 0 hr.

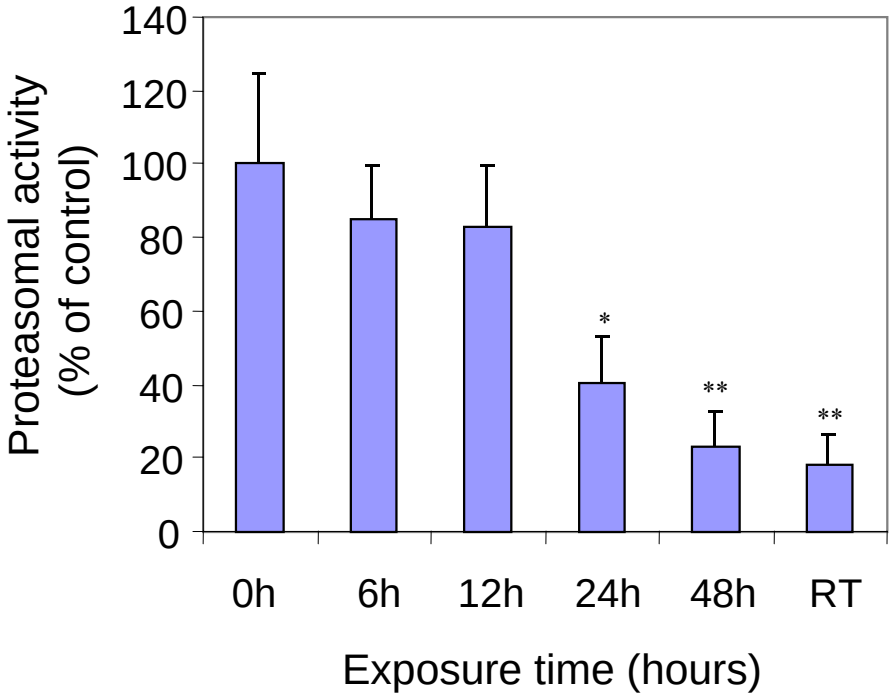

Figure 23. Effect of paraquat on proteasomal activity. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr or rotenone (5 μM) for 12 hr. At each time point, proteasomal activity was determined by measuring the fluorescence signal released from free AMC. The specific proteasomal activity was calculated as total activity minus remaining activity of lysates in the presence of a proteasome inhibitor MG 132 (20 μM). Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

proteasomal dysfunction, experiments were carried out to test whether paraquat affects mitochondrial complex I and V function (Figure 24). Paraquat did not significantly affect the activity of complex I by 12 hr, whereas at 24 hr and 48 hr, it markedly decreased the activity of complex I to 52.2 ± 10.5% or to 25.9 ± 7.9% of control, respectively. Likewise, paraquat did not affect the activity of complex V by 24 hr, but it significantly decreased the activity of complex V to 59.8 ± 13.9% of control at 48 hr.

Effect of paraquat on intracellular ATP level

ATP depletion is relevant to neuronal death and proteasomal dysfunction (Krieger & Duchen 2002; Hashimoto et al., 2003). ATP is required for the assembly of 20S proteasome and 19S PA 700 activator to form 26S proteasome complex (McNaught & Olanow, 2003). Thus, in connection with the proteasomal dysfunction, experiments were conducted to examine whether paraquat affects the level of ATP. The experiment was designed to correlate any change of ATP level with the decrease of proteasomal activity (Figure 23). SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr or 0.05 μM rotenone for 24 hr and ATP level was measured at each time point (Figure 25). Paraquat significantly decreased ATP levels to 76 ± 5.9% or 38.8 ± 4.8% of control, respectively at 24 hr and 48 hr. Similarly, 0.5 μM rotenone greatly decreased the ATP level to 20.9 ± 2.9% of control at 24 hr.

Effect of paraquat on the levels of proteasomal subunits

The 26S proteasome is a large protease complex, which consists of 20S catalytic

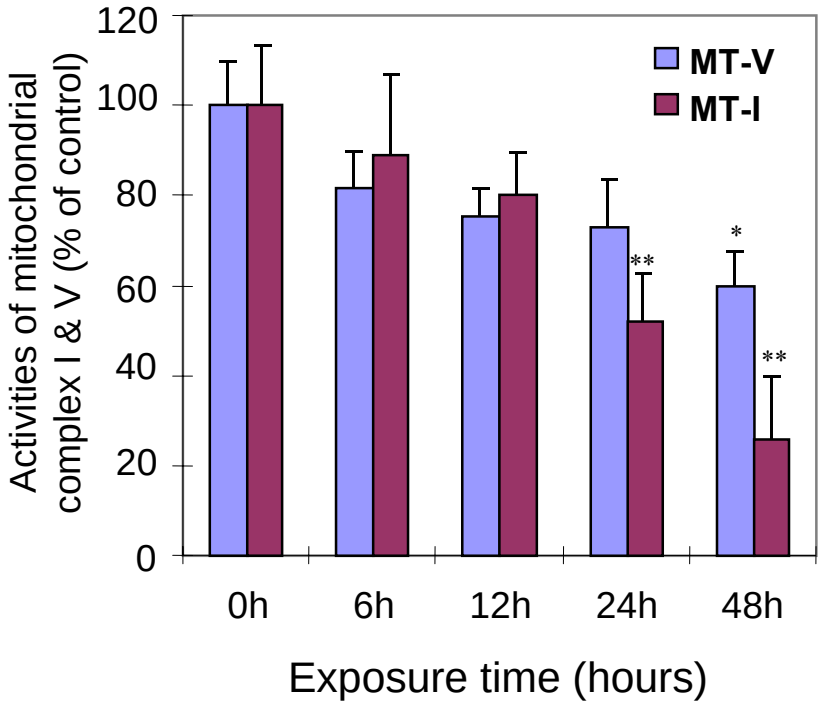

Figure 24. Effect of paraquat on the activities of mitochondrial complex I and V. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr. The specific activities of complex I and V were determined in the isolated mitochondrial fractions by measuring the absorbance change caused by NADH oxidation in the presence or absence of rotenone and oligomycin, respectively. Data are expressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $p < 0.05$ and ** $p < 0.01$ when compared to untreated control.

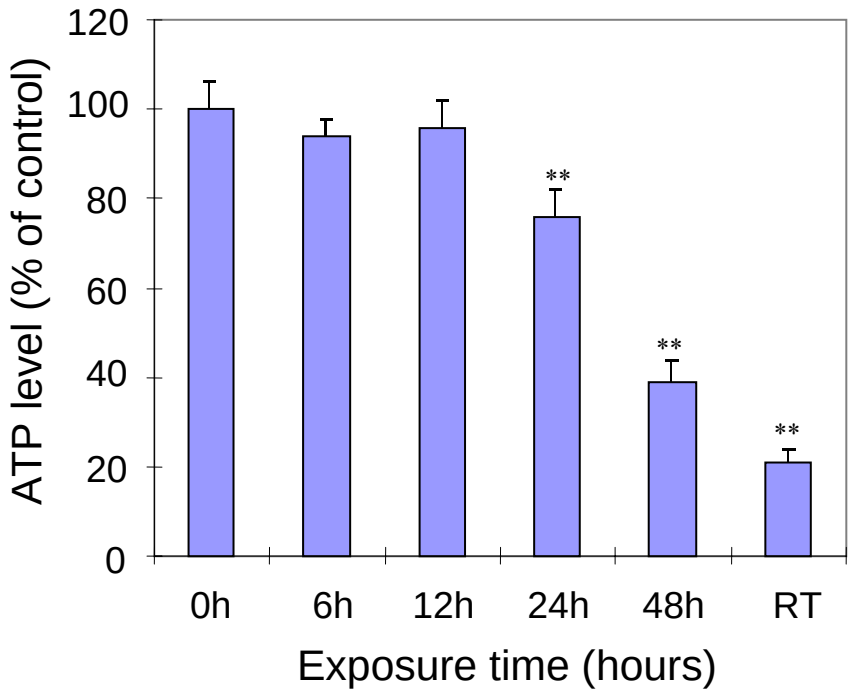

Figure 25. Effect of paraquat on the intracellular ATP level. Cells in culture dishes (60 × 15 mm) were exposed to paraquat (0.5 mM) for up to 48 hr or rotenone (0.5 μM) for 24 hr. After exposure, cells were broken and mixed with ATP monitoring enzyme. ATP levels were determined by measuring luminescence from ATP/luciferin binding. Data are aexpressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; ** $p < 0.01$ when compared to untreated control.

core subunits and 19S regulatory subunits called PA 700. The 20S core is composed of α and β subunit, and 19S component consist of ATPase and non-ATPase subunits (Roos-Mattjus & Sistonen, 2004). Structural defects in the proteasomal complex may cause proteasomal dysfunction (Carrad et al., 2002). In this study, we investigated whether paraquat affects the levels of proteasomal subunits. SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr and the levels of proteasomal subunits were checked at each time point by western blotting (Figure 26). Paraquat did not affect the protein levels of 20S α and β subunits, whereas paraquat significantly decreased the protein level of 19S subunits to 32.54 ± 12.21% of 0 hr at 48 hr.

Effect of paraquat on the levels of α-synuclein and ubiquitinated proteins

α-synuclein is linked both to normal synaptic function and neurodegeneration (Vekrellis et al., 2004), and ubiquitin plays a pivotal role in tagging unnecessary proteins for degradation by proteasome complex (Hyun et al., 2004). In a transgenic mouse model, neuronal expression of human α-synuclein results in progressive accumulation of α-synuclein and ubiquitin-immunoreactive inclusions in neurons of the neocortex, hippocampus, and substantia nigra which contained elevated levels of α-synuclein and ubiquitin (Masliah et al., 2000). In addition, proteasomal dysfunction has shown to form protein aggregates including α-synuclein and ubiquitin (Ross & Pickart, 2004). Thus, the current study examined whether paraquat affects the protein level of α-synuclein and causes the accumulation of ubiquitinated proteins. First, SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr or 10 μM MG132 for 24 hr, respectively

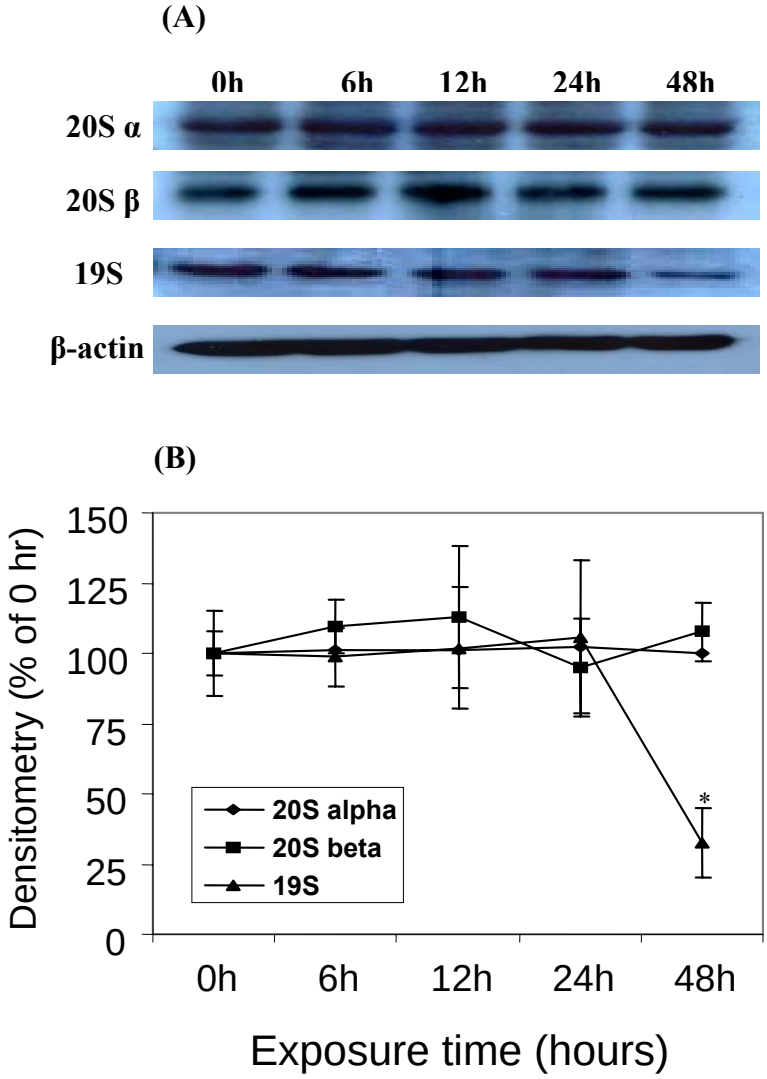

Figure 26. Effects of paraquat on the protein levels of proteasomal subunits. Cells were exposed to paraquat (0.5 mM) for up to 48 hr. 50 μg of cytosolic proteins isolated at each time point were used to analyze proteasomal subunits by western blotting. (A) Representative immunoblot of proteasomal subunit (B) The levels of proteasomal subunits were quantified by densitometric analysis of each band of proteasomal subuints and normalized on the level of β-actin. The quantified levels of proteasomal subunits are expressed as % of 0 hr. Values represent mean ± SEM for 4 independent experiments (n =4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $P < 0.05$ when compared to 0 hr.

and the levels of α-synuclein and ubiquitinated proteins were checked at each time point by western blotting (Figure 27). Paraquat significantly significantly increased the level of ubiquitinated proteins to 154.34 ± 17.81% and 166.81 ± 0.13% of 0 hr at 24 hr or 48 hr, respectively (Figure 27A). Likewise, paraquat increased the protein level of α-synuclein to 202.54 ± 27.93%, 235.78 ± 22.58%, or 305.21 ± 15.46% of 0 hr at 12hr, 24 hr, or 48hr, respectively (Figure 27B). Similarly, 10 μM MG132 highly elevated the levels of α-synuclein and ubiquitinated proteins to 635.29 ± 30.51 or 258.13 ± 18.92% of 0 hr at 24 hr, respectively.

Effect of paraquat on the protein levels of p53 and Bax

A tumor suppressor transcription factor p53 activates Bax to induce apoptosis and both 53 and Bax are degraded by proteasome-dependent pathway (Yang & Yu, 2003; Li & Dou, 2000). Thus, proteasomal dysfunction might cause the stabilization of p53 and Bax, resulting in the promotion of apoptosis. Based on the above finding that paraquat decreased proteasomal activity, we investigated whether paraquat affects the protein levels of p53 and Bax (Figure 28) through proteasomal dysfunction. SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr or 10 μM MG132 for 24 hr, respectively and the levels of p53 and Bax were checked at each time point by western blotting (Figure 28). Paraquat markedly increased the protein level of p53 to 350.21 ± 10.23%, 354.55 ± 22.81% or 375.76 ± 17.32% of 0 hr at 12 hr, 24 hr or 48 hr, respectively (Figure 28A). Correspondingly, paraquat significantly increased the protein level of Bax to 241.57 ± 19.71%, 268.54 ± 24.41% or 301.12 ± 16.42% of 0 hr at 12 hr, 24 hr, or 48

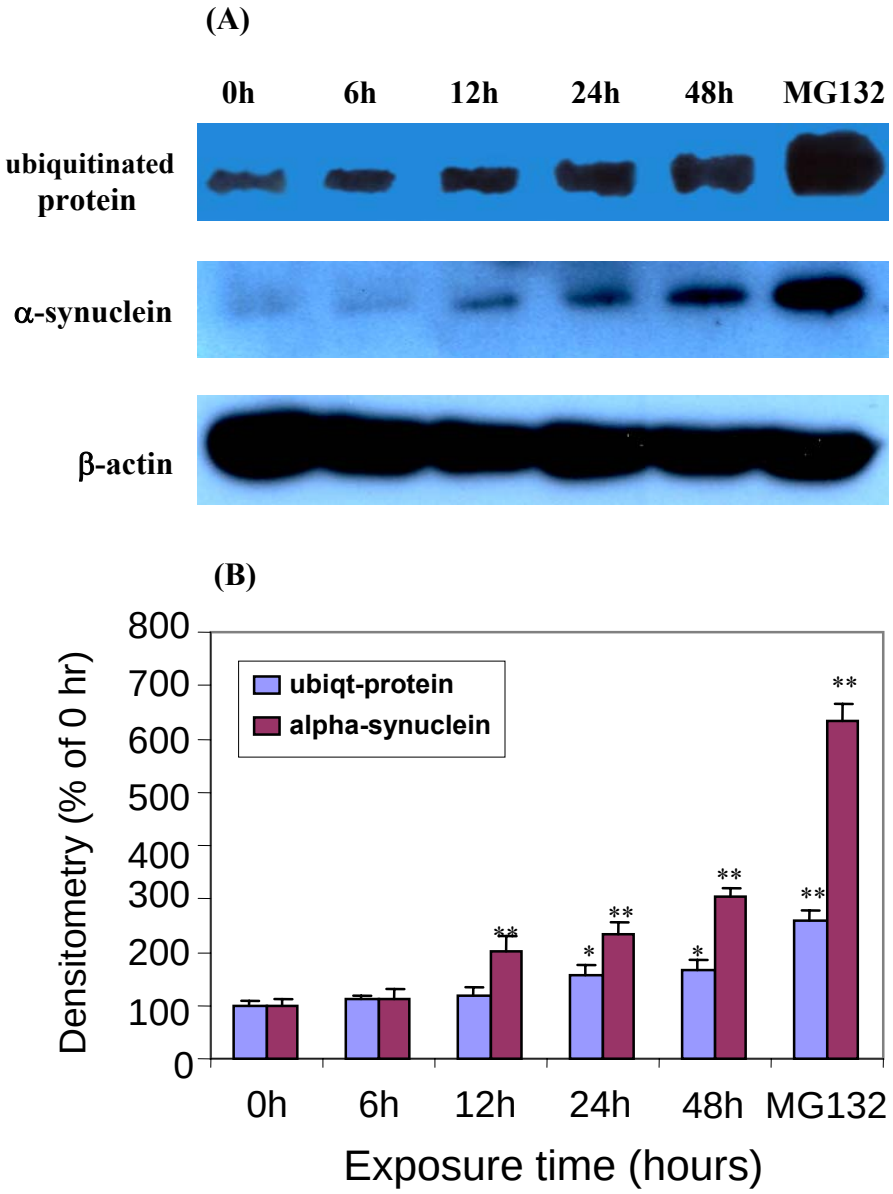

Figure 27. Effects of paraquat on the levels of ubiquitinated protein and α-synuclein. Cells were exposed to paraquat (0.5 mM) for up to 48 hr or MG132 (10 μM) for 24 hr. 50 μg of cytosolic proteins isolated at each time point were used to analyze ubiquitinated protein and α-synuclein by western blotting. (A) Representative immunoblot of ubiquitinated protein and α-synuclein (B) The levels of ubiquitinated protein and α-synuclein were quantified by densitometric analysis of each band of ubiquitinated protein and α-synuclein and normalized on the level of β-actin. The quantified levels of ubiquitinated protein and α-synuclein are expressed as % of 0 hr. Values represent mean ± SEM for 4 independent experiments (n =4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $P < 0.05$ and ** $p < 0.01$ when compared to 0 hr.

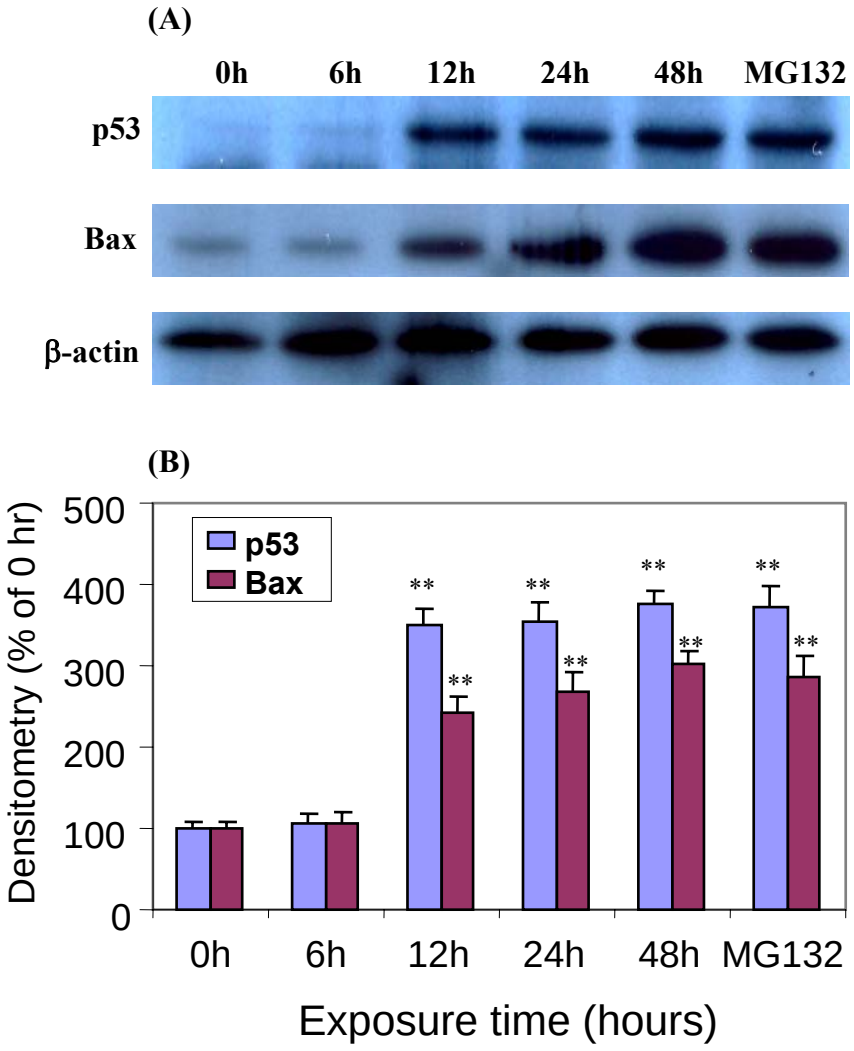

Figure 28. Effects of paraquat on the protein levels of p53 and Bax. Cells were exposed to paraquat (0.5 mM) for up to 48 hr or MG132 (10 μM) for 24 hr. 50 μg of cytosolic proteins isolated at each time point were used to analyze p53 and Bax by western blotting. (A) Representative immunoblot of p53 and Bax (B) The levels of p53 and Bax were quantified by densitometric analysis of each band of p53 and Bax and normalized on the level of β-actin. The quantified levels of p53 and Bax are expressed as % of 0 hr. Values represent mean ± SEM for 4 independent experiments (n =4). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; ** $p < 0.01$ when compared to 0 hr.

hr, respectively (Figure 28B).

Effect of paraquat on the activities of caspase 3 and 9

Mitochondrial dependent apoptosis requires the sequential activation of caspase 9 and 3, both of which are regulated by p53 and Bax (Moll & Zaika, 2001; Ohtsuka et al., 2004). A number of studies show that the activation of caspase 3 and 9 is associated with proteasomal inhibition (Almond et al., 2001; Lu et al., 2003; Jana et al., 2004). Thus, in connection with proteasomal dysfunction, studies examined whether paraquat affects the activities of caspase 3 and 9 (Figure 29). SY5Y cells were treated with 0.5 mM paraquat for up to 48 hr or 5 μM rotenone for 24 hr and the activities of caspase 3 and 9 were measured at each time point. Paraquat increased the activities of caspase 3 and 9 in a time dependent manner. In particular, paraquat significantly increased the activities of caspase 3 and 9 to 208.26 ± 21.45% or 181.35 ± 3.25% of control, respectively at 48 hr. A positive control rotenone markedly increased the activities of caspase 3 and 9 at 12 hr.

Nuclear condensation

Nuclear condensation appears at the late stage of apoptosis, which can be triggered by numerous factors including death receptor-mediated signals, oxidative stress, ionizing radiation, virus, and withdrawal of growth factors (Kannan & Jain, 2000). Proteasomal inhibition is implicated in nuclear condensation (Rideout et al., 2001, 2002; Sang et al., 2002). Thus, in connection with proteasomal dysfunction, the current study investigated whether paraquat causes nuclear condensation. SY5Y cells were treated

with 0.5 mM paraquat for up to 48 hr or 0.1 μM rotenone for 24 hr and nuclear morphology was examined at each time point (Figure 30). Paraquat did not change nuclear mophology by 24 hr but it significantly caused visible nuclear condensation at 48 hr. Likewise, rotenone clearly induced nuclear condensation at 24 hr.

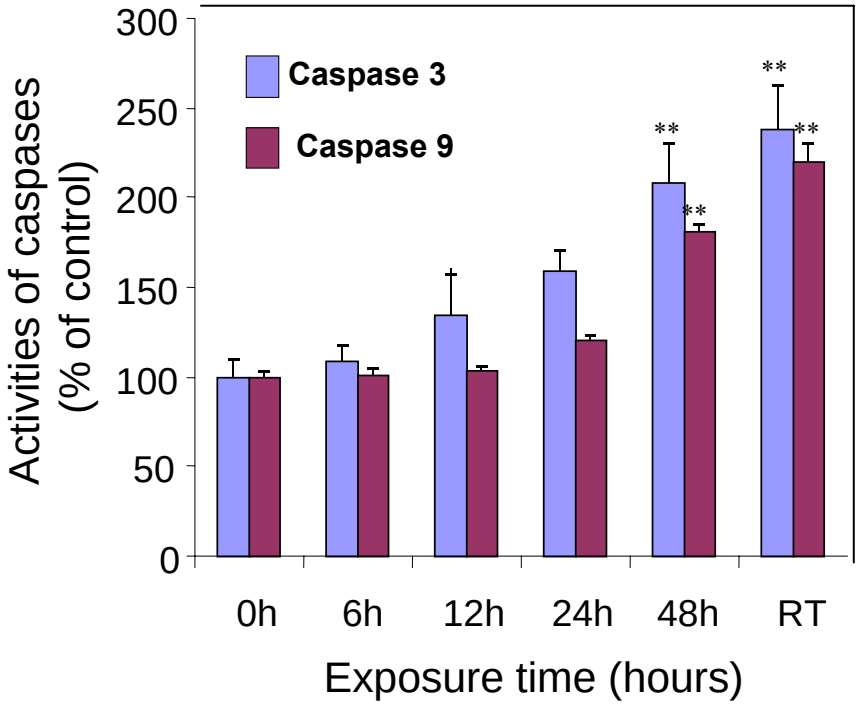

Figure 29. Effect of paraquat on the activities of caspase 3 and 9. Cells grown in culture dishes (60 × 15 mm) were treated with paraquat (0.5 mM) for up to 48 hr or rotenone (5 μM) for 24 hr. After treatment, cells were lysed and mixed with DEVD-*p*NA and LEHD-*p*NA as a substrate of caspase 3 or 9, respectively. The activities of caspase 3 and 9 were determined by measuring absorbance released from free *p*NA. Data are aexpressed as mean % of untreated control ± SEM for 6 independent experiments done at each time point (n = 6). Statistical significance was determined by the one-way analysis of variance (ANOVA) and the Dunnett`s test; * $P < 0.05$ and ** $p < 0.01$ when compared to untreated control.

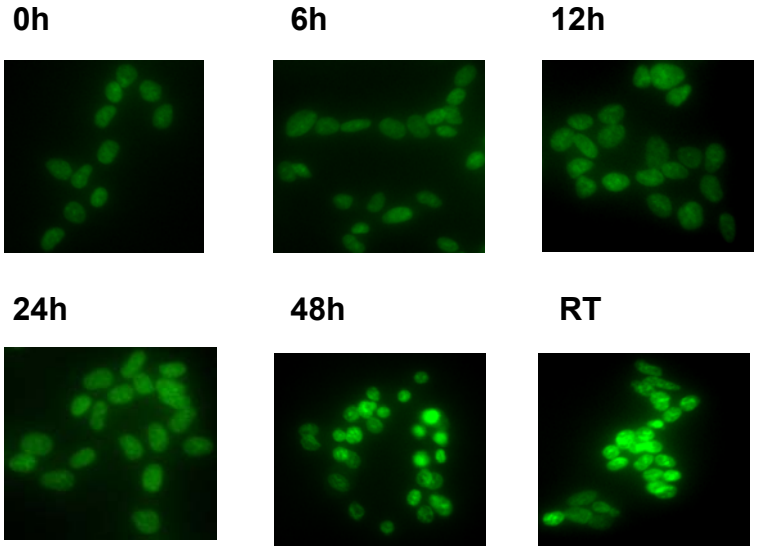

Figure 30. Paraquat-induced nuclear condensation in SY5Y cells. Cells were treated with paraquat (0.5 mM) for up to 48 hr or rotenone (0.5 μM) for 24 hr. After treatment, cells were stained with Hoechst and then, nuclear images were captured through 20X objective lens via an inverted fluorescence microscope. Each picture is representative of 6 fields.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Data from the current study demonstrated that paraquat produces oxidative stress and proteasomal dysfunction-mediated toxicity in dopaminergic SY5Y cells. There is extensive evidence that oxidative stress and proteasomal dysfunction are associated with neurodegenerative diseases such as PD, AD, HD, ALS, and Prion disease (Ciechanover & Brundin, 2003; Barnham et al., 2004). In particular, human postmortem studies of PD patient show that PD brains are in a state of oxidative stress (Olanow & Tatton, 1999). In addition, protein aggregates called Lewy bodies are observed in PD brains (Chung et al., 2001). Such oxidative stress and proteasomal dysfunction may be due to an abnormal endogenous process in dopamine metabolism (Olanow, 1993; Keller et al., 2000), or the action of exogenous and environmental neurotoxicants, notably, pesticides (Banerjee et al., 2001; Shamoto-Nagai et al., 2003; Abdollahi et al., 2004; Zhou et al., 2004). Thus, the hypotheses were tested that the bipyridyl herbicide paraquat produces oxidative stress-mediated toxicity and induces proteasomal dysfunction in dopaminergic SY5Y cells.

The trypan blue exclusion, MTT, and LDH assay were performed to examine the effect of paraquat or a specific dopamine transporter inhibitor GBR12909 on the viability of SY5Y cells. First, paraquat (0.5 mM - 1 mM) significantly reduced trypan blue exclusion and formazan reduction into less than 60 % of control (Figure 10, 11), and it significantly increased LDH release (Figure 12). Thus, 0.5 mM was selected as a minimum effective concentration to investigate the toxicity of paraquat in other in vitro

experiments and the toxic effect of paraquat was confirmed by the analysis of cell morphology (Figure 13). Second, GBR12909 (1 μM) significantly protected SY5Y cells against the toxicity of paraquat (0.05 - 0.25 mM) and partially attenuated the toxicity of paraquat (0.5 - 1 mM). Paraquat crosses the blood-brain barrier (BBB) through neutral amino acid transporters (McCormack & Di Monte, 2003) and then exerts its toxicity to dopaminergic neurons through a dopamine transporter (DAT) (Shimizu et al., 2003). Thus, this finding confirms the conclusion that the toxicity of paraquat is mediated by DAT and supports a previous report showing that a dopaminergic phenotype DAT is expressed in SY5Y cells (Manakova et al., 2004) (Figure 31).

The generation of ROS was measured in SY5Y cells exposed to paraquat (0.5 mM), because the redox cycling reaction of paraquat leads to the production of ROS including hydrogen peroxide, superoxide, and hydroxyl radicals, resulting in oxidative stress (Bus et al., 1974; Suntres, 2002). Current data confirmed that paraquat produced an increase of ROS in SY5Y cells in a time dependent manner (Figure 14). This finding is in agreement with the report that paraquat (0.01 - 1 mM) increases ROS in rat cortical neurons (Schmuck et al., 2002). Given that ROS-mediated oxidative damage is a prominent feature of dopaminergic pathogenesis (Cohen, 1986; Sinha et al., 1986; Chacon et al., 1987), current data suggest that paraquat may induce oxidative stress in dopaminergic neurons through the production of ROS. In this study, paraquat (0.5 mM) significantly decreased GSH levels in SY5Y cells at 24 and 48 hr (Figure 15). Similarly, Schmuck et al (2002) showed that paraquat (0.01 - 1 mM) markedly depletes GSH in rat cortical neurons even within 1 hr. In general, GSH functions as a cellular antioxidant and

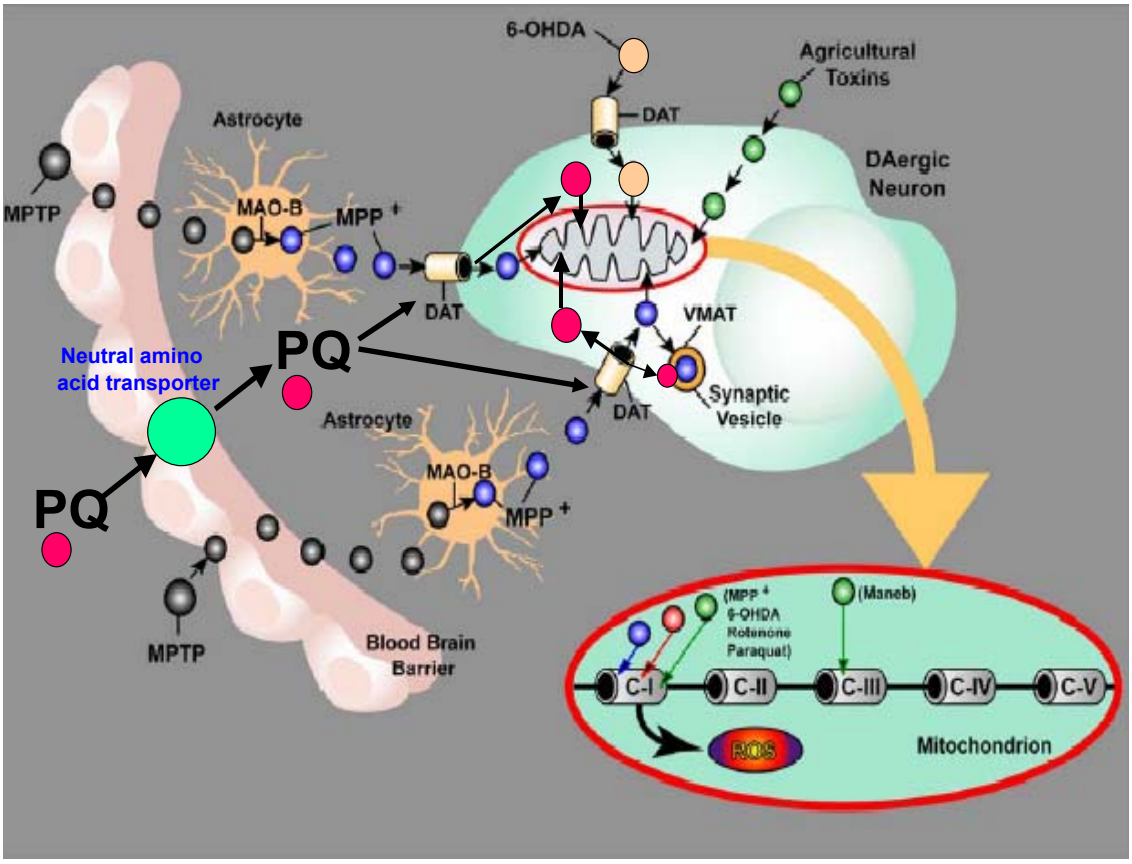

Figure 31. Proposed entry pathways and targets of paraquat in dopaminergic neruons (McCormack & Di Monte, 2003; Shimizu et al., 2003; Manakova et al., 2004). This figure is modified from the original. Paraquat first crosses blood-brain barrier (BBB) through neutral amino acid transporter and then enters dopaminergic neurons via dopamine transporter (DAT). Within dopaminergic neurons, paraquat can disrupt mitochondiral function, synaptic vesicles, and intracellular processes, ultimately leading to dopaminergic pathogenesis.

and a decrease of GSH levels may predispose cells to oxidative stress-mediated toxicity (Meister & Anderson, 1983). In fact, a decrease of GSH levels has been found in the remaining dopaminergic neurons of the PD brain, potentially rendering them more vulnerable to oxidative stress (Pearce et al., 1997). Thus, our finding indicates that depletion of GSH by paraquat may produce and aggravate oxidative stress in dopaminergic neurons.

The current study shows that paraquat (0.5 mM) decreased GPx activity in a time dependent manner, but did not significantly change GR activity. GST activity continuously increased for 24 hr, and then returned to basal level at 48 hr (Figure 16). A similar effect of paraquat (0.1 mM) on these enzymes was observed in pulmonary endothelial cells (Tsukamoto et al., 2002). No apparent change of activities in these enzymes was reported in PD brain (Marttila et al., 1988; Sian et al., 1994). However, these studies do not specifically reflect activities of these enzymes in dopaminergic neurons, because their results are from substantia nigra tissue, including both glial cells and dopaminergic neurons. Thus, our data can be used as a basis to determine activities of these enzymes in dopaminergic neurons of PD brain under oxidative stress. Furthermore, the current findings indicate that altered activities of GPx and GST by paraquat partially contributed to the increase of ROS (Figure 14) and depletion of GSH (Figure 15), because these two enzymes consume GSH to scavenge ROS or to detoxify xenobiotics and their metabolites (Dringen, 2000), respectively.

In the current study, paraquat (0.5 mM) significantly decreased MTP at 24 and 48 hr (Figure 17). This result is in agreement with the finding by Ding and Keller (2001)

that MTP is reduced in SY5Y cells exposed to paraquat (0.2 mM). In addition, paraquat (20 mM) dissipates complex I-dependent MTP in rat hepatocyte mitochondria (Vincente et al., 2001). MTP is decreased by oxidative stress, including the increase of ROS (Skulachev, 1996) and the depletion of GSH (Schulz et al., 2000). Indeed, the depletion of mitochondrial and cytoplasmic GSH depolarizes MTP in PC12 cells (Wüllner et al., 1999; Seyfried et al., 1999). Another type of free radical, reactive nitrogen species (RNS) also reduces MTP in SY5Y cells (Moriya et al., 2000) and rat cortical neurons (Solenski et al., 2003). Thus, our data indicate that MTP reduction represents mitochondrial damage by paraquat-induced increase of ROS (Figure 14) and decrease of GSH (Figure 15).

The current study demonstrates that paraquat (0.5 mM) produced a time dependent increase of malondialdehyde (MDA) (Figure 18) and protein carbonyls (Figure 19) and DNA fragmentation (Figure 20) in SY5Y cells. Similarly, others have shown that 0.2 and 0.5mM paraquat increases lipid peroxidation (Yang & Sun, 1998) and DNA fragmentation (Li & Sun, 1999) in PC12 cells. These indicators of oxidative damage are also found in dopaminergic neurons of PD brains (Dexter et al., 1989a; Yoritaka et al., 1996; Alam et al., 1997b; Floor & Wetzel, 1998; Zhang et al., 1999). Thus, current data indicate that paraquat may produce oxidative damage to dopaminergic neurons, and furthermore, extend our hypothesis to the possibility that paraquat may induce apoptotic cell death of dopaminergic neurons identified in PD brains (Mochizuki et al., 1996; Anglade et al., 1997; Tatton et al., 1998). The finding that increased MDA was the

earliest measurable response to paraquat of the endpoints measured suggests that paraquat also induced extracellular damage.

In this study, paraquat (0.5 mM) significantly increased the protein levels of heme oxygease-1 (HO-1) at 12 hr and its level continued to increase by 48 hr (Figure 21B). HO-1 is induced in response to oxidative stress (Leon et al., 2003) and the intense staining of HO-1 was observed in dopaminergic neurons of PD patients (Schipper et al., 1998). Thus, current finding indicates that SY5Y cells suffer from oxidative stress induced by paraquat. On the other hand, the protein levels of TH did not change in exposure to paraquat (Figure 21A). TH is a biomarker of dopaminergic neurons (Kim et al., 2003) and SY5Y cells are known to express it (Shaul et al., 2003). This finding confirms that a dopaminergic phenotype TH is expressed in SY5Y cells.

The current study shows that paraquat significantly decreased proteasomal activity (Figure 22). In general, the decrease of proteasomal activity might eventually lead to the formation of toxic protein aggregates. Both decreased proteasomal activity and protein aggregates called Lewy bodies are observed in the substantia nigra or dopaminergic neurons of PD brains, respectively (McNaught & Jenner, 2001). Significantly, parkinsogenic pesticides rotenone and maneb cause the decrease of proteasomal activity and the aggregation of α-synuclein in SY5Y cells or MES 23.5 cells, respectively (Shamoto-Nagai et al., 2003; Zhou et al., 2004). Consistently, rotenone was found to decrease proteasomal activity in SY5Y cells used in this study. Thus, current data suggest that paraquat might decrease proteasomal activity accompanied by protein aggregation in dopaminergic neurons.

In the current study, paraquat caused the decreases of mitochondrial complex I and V activity (Figure 23) and the depletion of ATP (Figure 24). Such a decrease of complex I activity is reported in the substantia nigra, platelets, muscles, fibroblasts, and lymphocytes of PD patients and mutation of mitochondrial genes is believed to contribute to the decrease of complex I (Swerdlow et al., 1996; Gu et al., 1998; Mizuno et al., 1998). The inhibition of complex I results in the increase of ROS and the decrease of ATP and this pathologic condition is known to cause oxidative stress (Tretter et al., 2004). Significantly, mitochondrial dysfunction accompanied by an impaired supply of ATP has been suggested to contribute to proteasomal dysfucntion. In support of this hypothesis, the parkinsogenic chemicals rotenone, maneb, dopamine, MPP+ inhibiting proteasomal activity (Keller et al., 2000; Shamoto-Nagai et al., 2003; Zhou et al., 2004), reduce complex I activity or ATP level in cell lines and animals (Höglinger et al., 2003; Zhang et al., 2003; Ben-Schachar et al., 2004). Thus, our data indicate that paraquat might decrease proteasomal activity through the aditional mechanism of mitochondrial dysfunction-induced ATP depletion. Given that mitochondria are major sources of ROS and the inhibition of complex I leads to the generation of large amount of ROS (Lenaz, 2001), it is also possible that abnormally generated ROS by mitochondrial dysfunction might inhibit proteasomal activity through oxidation of proteasomal subunits. Indeed, oxidative modifications of proteasomal subunits reduce proteasomal activity in cell lines and animals (Reinheckel et al., 1998; Bulteau et al., 2001).

The current study demonstrates that paraquat selectively decreased 19S subunits at 48 hr (Figure 25). In general, 19S subunits involve stabilization of 20S proteasome

complex and unfolding, recognition, transfer, and elimination of polyubiquitinated proteins (Vigouroux et al., 2004). Low levels of 19S subunits are reported in substantia nigra and this defect has been suggested to be relevant to the impaired proteasomal activity in PD patients (McNaught et al., 2003). In support of this hypothesis, a parkinsogenic neurotoxin neuromelanin inhibiting proteasomal activity reduces the protein levels of 19S sunbunits in SY5Y cells (Shamoto-Nagai et al., 2003). Thus, current data suggest that the reduction of 19S subunit levels by paraquat might cause the structural and functional instability of the 20S complex, contributing to the decrease of proteasomal activity (Figure 22). However, it remains to be determined whether such a decrease represents the downregulation or the enhanced turnover of 19S subunits by paraquat.

In this study, paraquat significantly increased the levels of α-synuclein and ubiquitinated proteins (Figure 26). The increases of those proteins may be relevant to some features of dopaminergic neuronal death. Protein aggregates called Lewy bodies are observed in dopaminergic neurons of PD patients and α-synuclein is a major component of those (Dev et al., 2003; Vekrellis et al., 2004). Proteasomal inhibition induces both the increase of α-synuclein and ubiquitin levels and the formation of intracellular protein inclusions in vitro and in vivo (Biasini et al., 2004; McNaught et al., 2002, 2004). Consistently, the parkinsogenic chemicals, rotenone, maneb, dopamine and 6-OHDA inhibit proteasomal activity (Keller et al., 2000; Shamoto-Nagai et al., 2003; Elkon et al., 2004; Zhou et al., 2004), cause the increase of α-synuclein levels, and induce the formation of protein aggregates in cell lines and animals (Elkon et al., 2001:

Gómez-Santos et al., 2003; Sherer et al., 2003; Zhou et al., 2004; Yoshimoto et al., 2005). In agreement with these results, our data indicate that paraquat might induce a proteasomal inhibition-mediated increase of α-synuclein and accumulation of ubiquitinated proteins in dopaminergic neurons. However, it can not be ruled out that the increase of α-synuclein might include an upregulation triggered by paraquat. Furthermore, the accumulation of ubiquitinated proteins might be caused by modification of amino acids in proteins. In fact, a lipid peroxidation product 4-hydroxynonenal (HNE) has shown to oxidize proteins, which, in turn, become aggregated. HNE-modified proteins are resistant to proteasomal degradation, resulting in the accumulation of protein inclusions in cells (Hyun et al., 2003; Grune & Davies, 2003). Furthermore, the accumulation of protein inclusions might be triggered secondarily by the inhibition of proteasomal activity, as protein aggregation inhibits proteasomal activity followed by accumulation of ubiquitin conjugates (Bence et al., 2001; Snyder et al., 2003; Lindersson et al., 2004).

The current study shows that paraquat significantly increased the protein levels of p53 and Bax at 12 hr and the level of Bax continued to increase by 48 hr (Figure 27B). A chief tumor suppressor, p53 and its downstream target gene Bax induce apoptosis, through which the formation of tumors is inhibited and the development of organs normally proceeds (Moll & Zaika, 2001; Fridman & Lowe, 2003; Chipuk & Green, 2004). The turnover of p53 and Bax is mediated by proteasome-dependent pathway, and thus, proteasomal dysfunction has been suggested to cause the accumulation of p53 followed by the increase of Bax, resulting in the acceleration of apoptosis (Wojcik,

1999). In support of this idea, proteasomal inhibition has shown to increase the protein levels of p53 and Bax in SY5Y cells and primary neurons (Kikuchi et al., 2003; Nakaso et al., 2004). In addition, the parkinsogenic drugs dopamine and 6-OHDA inhibiting proteasomal activity (Keller et al., 2000; Elkon et al., 2004), increase the protein levels of p53 and Bax in SY5Y cells or PC12 cells (Blum et al., 1997; Haque et al., 2003; Puttonen et al., 2003; Maňáková et al., 2004). These increases of p53 and Bax are reported in dopaminergic neurons of PD patients (de la Monte et al., 1998; Tatton, 2000; Horowitz et al., 2003). Thus, our data indicate that paraquat might induce proteasomal inhibition-mediated increases of p53 and Bax in dopaminergic neurons. However, it cannot be ruled out that their increases might also contribute to other paraquat-induced toxic events. For example, oxidative stress is known to increase the protein levels of p53 and Bax (Jang & Surh, 2003; Tamagno et al., 2003) and it has been previously demonstrated that paraquat causes oxidative stress in SY5Y cells.

In the current study, paraquat significantly increased the activities of caspase 3 and 9 at 24 and 48 hr (Figure 28). The activation of caspase 3 and 9 is reported in dopaminergic neurons of PD patients (Anderson, 2001). In general, caspase 9 is an intermediate of the mitochondrial dependent apoptotic pathway, and all apoptotic signals from the death receptor and mitochondria converge on caspase 3, which in turn activates other caspases to ultimately induce apoptosis (Bratton et al., 2000). Of interest, the parkinsogenic chemicals dopamine and rotenone inhibiting proteasomal activity (Keller et al., 2000; Shamoto-Nagai et al., 2003), increase the activity of caspase 9 or caspase 3 in SY5Y cells (Junn & Mouradian, 2001; Molina-Jimenez et al., 2003). In addition,

proteasomal inhibition has been shown to activate caspase 3 in Neuro2a cells and primary neurons (Qiu, et al., 2000; Lang-Rollin et al., 2003; Sawada et al., 2004). Thus, our data suggest that paraquat might cause proteasomal inhibition-mediated activation of caspase 3 and 9 in dopaminergic neurons. Furthermore, the current finding provides evidence that the activation of caspase 3 and 9 might be directly associated with paraquat-induced increases of p53 and Bax (Figure 27), because their activation is regulated by p53 and Bax (Moll & Zaika, 2001; Ohtsuka et al., 2004).

The current study demonstrates that paraquat apparently induced nuclear condensation at 48 hr (Figure 29). Nuclear condensation is a representative characteristic of apoptosis and is observed in dopaminergic neurons of PD patients (Anglade et al., 1997; Tatton et al., 1998). Proteasomal inhibition has been shown to induce caspase 3-mediated nuclear condensation in primary neurons (Qiu et al., 2000; Lang-Rollin et al., 2004). In agreement with these results, the parkinsogenic chemicals dopamine, 6-OHDA and rotenone inhibiting proteasomal activity (Keller et al., 2000; Shamoto-Nagai et al., 2003; Elkon et al., 2004), cause nuclear condensation in SY5Y cells (Haque et al., 2003; Gómez-Santos et al., 2003; Newhouse et al., 2004). Thus, our data suggest that paraquat might cause proteasomal inhibition-mediated nuclear condensation in dopaminergic neurons. In addition, this finding can be understood as an endpoint of a paraquat-activated cascade including proteasomal dysfunction, upregulation of p53/ Bax, activation of caspase 9/3, and apoptotic death.

Animal studies suggest a link between paraquat exposure and dopaminergic cell death in the substantia nigra. Dopaminergic pathogenesis has been reported in male

C57BL/6 adult mice that received 3 intraperitoneal injections of 10 mg/kg (equivalent to 15.8 μmol/kg) of paraquat dichloride for 3 consecutive weeks (Brooks et al., 1999; Manning-Bog et al., 2002; McCormack et al., 2002), as well as in male Wistar rats receiving intranigral injections of various concentrations of paraquat dichloride (2.5 - 10 nmole) for two weeks (Liou et al., 2001). The brain concentrations of paraquat in the brain and substantia nigra were not measured in these studies. Furthermore, brain concentrations of paraquat have not been determined in humans exposed to paraquat who developed parkinsonian symptoms, nor is it known what exposure periods and concentrations of paraquat might be relevant to PD incidence in human populations. Thus, the concentrations of paraquat that might enhance the risk for the development of overt dopaminergic pathogenesis or the clinical manifestation of PD under the certain exposure periods remain to be measured.

In conclusion, current findings suggest that paraquat might trigger dopaminergic pathogenesis through oxidative stress and proteasomal dysfunction (Figure 32). Furthermore, given that oxidative stress induces proteasomal dysfunction and conversely, proteasomal dysfunction causes oxidative stress, both mechanisms might interact to cause and exacerbate dopaminergic pathogenesis in response to paraquat. Thus, the characterization of novel mechanisms involving paraquat toxicity might contribute substantially to an understanding of how environmental neurotoxicants including pesticides might contribute to the incidence of PD in human populations. In addition, such an approach should provide a useful map to prevent and delay dopaminergic pathogenesis and eventually to develop therapeutics to treat PD patients.

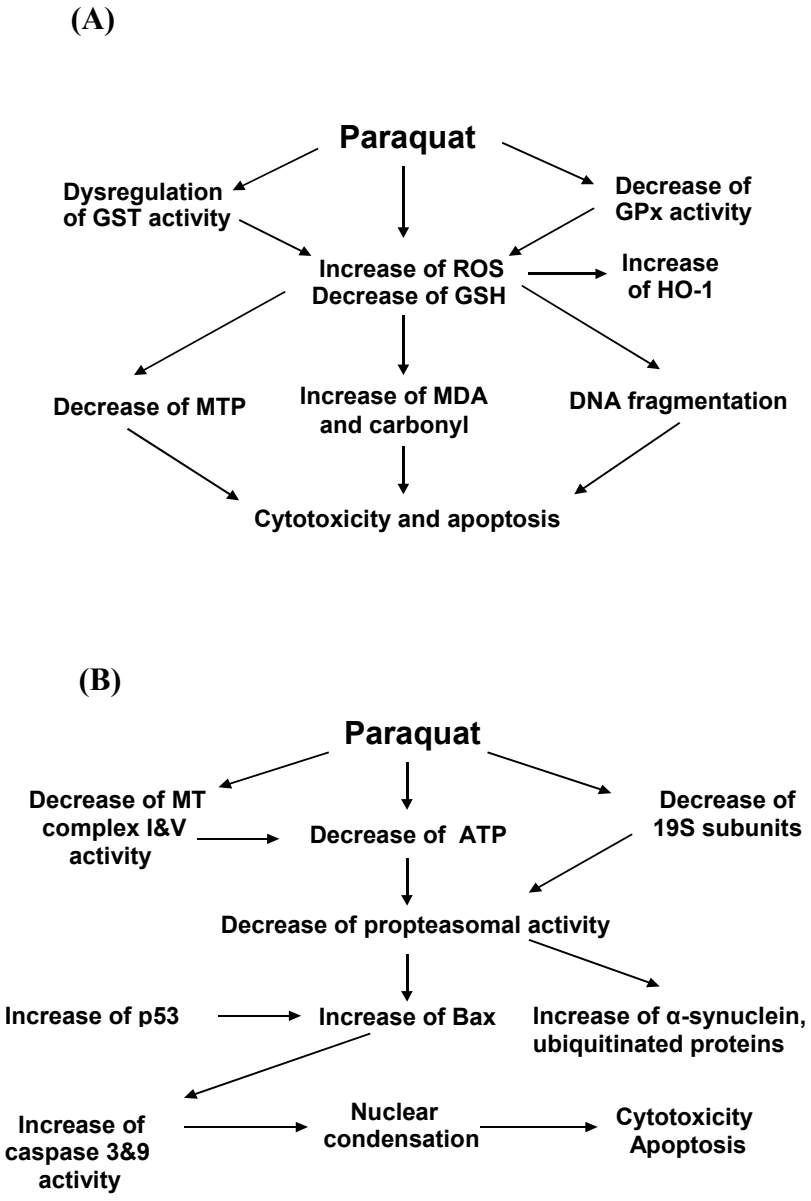

Figure 32. Proposed pathway of paraquat-induced toxicity in SY5Y cells. (A) Oxidative stress-mediated toxicity by paraquat. (B) Proteasomal dysfunction-mediated toxicity by paraquat. Both oxidative stress and proteasomal dysfunction can contribute to toxicity to SY5Y cells by paraquat.

Further studies

In this study, undifferentiated SY5Y cells with several dopaminergic properties were examined in order to explore a hypothetical link between paraquat-induced oxidative stress and proteasomal dysfunction and dopaminergic pathogenesis. These hypotheses were well supported by current findings. However, several avenues of research still need to be further investigated and confirmed by use of other dopaminergic cells, primary dopaminergic neurons, or midbrain slices of animals.

One promising area for future investigation is other characteristics of SY5Y cell line that might be involved in paraquat toxicity. SY5Y cell line expresses dopamine β-hydroxylase, which converts dopamine into norepinephrine (Thibault et al., 2000). Thus, it cannot be ruled that this adrenergic characteristic of SY5Y cells is involved in paraquat toxicity. In addition, differentiated SY5Y cells express more of TH, DAT, and D2R (Presgravesn et al., 2004) and show much stronger resistance against drug treatment than undifferentiated SY5Y cells used in this study. Considering that nearly all neurons including dopaminergic neurons are fully differentiated in adult humans, paraquat might be less toxic in differentiated SY5Y cells than in undifferentiated SY5Y cells. Furthermore, paraquat toxicity can be addressed in terms of function of transcription factors involved in dopaminergic neurogenesis, such as nuclear receptor-related factor 1 (Nurr 1), pituitary homeobox 3 (Pitx3), lim-homeodomain transcription factor 1B (Lmx b1), and engrailed 1 /2 (En1/2) (Wallen and Perlmann, 2003). In particular, Nurr 1 is expressed constitutively in dopaminergic neurons ranging from very early stages to the fully differentiated state, and this transcription factor is known to

participate significantly in both the fate and maintenance of normal function of dopaminergic neurons (Castillo et al., 1998; Saucedo-Cardenas et al., 1998). Thus, it can not be excluded that paraquat interferes with Nurr 1, resulting in dopaminergic pathogenesis.

Another promising avenue for future research is to address whether oxidative stress is triggered by metabolism of paraquat, dopamine or both. Given that metabolism of paraquat or dopamine is mediated by mitochondrial NADH oxidoreductase (or complex I), P450 reductases, MAO-A/B, and COMT, it is possible that paraquat affects those enzymes, in terms of their activities and expressions. Also, γ-glutamylcysteinyl synthetase and HO-1 have to be studied because the former produces a major antioxidant GSH used to scavenge ROS and the latter is exquisitively upregulated by oxidative stress. In fact, HO-1 was induced by paraquat in this study, but its function and mechanism of its upregulation remains to be characterized.

A third area for future research is the identification of signaling intermediates and transcription factors in death or survival pathways that are affected by paraquat-induced oxidative stress and proteasomal dysfunction. Oxidative stress activates death signaling pathways consisting of signaling intermediates p38, JNK and transcription factors AP-1, NF-κB, and p53, most of which are degraded by proteasome-dependent pathway. However, it is unclear that oxidative stress affects the survival pathway consisting of signaling intermediates PI3K, AKT (or PKB), Erk 1/2 and a transcription factor CREB.

Other areas that are attractive for future research are mechanisms of paraquat-induced proteasomal dysfunction and the possible involvement of lysosomal degradation. Proteasomal dysfunction might be induced by the oxidative modification of amino acids of proteasomal subunits, and 19S subunits are known to be more resistant to oxidative modification than 20S subunits. Thus, it should be addressed whether paraquat-induced ROS actually oxidizes proteasomal subunits and if so, which subunits is a preferentially modified by paraquat-induced ROS. In addition, proteins might also undergo a lysosomal pathway for degradation and thus, it remains to be explored whether paraquat might cause lysosomal dysfunction-mediated accumulation of α-synuclein and ubiquitinated proteins.

REFERENCES

Abdollahi, M., Ranjbar A., Shadnia, S., Nikfar, S., and Rezaiee, A. 2004. Pesticides and oxidative stress: Review. *Med. Sci. Moint*. 10:RA141-147.

Abou-Sleiman, P. M., Healy, D. G., Quinn, N., Lees, A. J., and Wood, N. W. 2003.The role of pathogenic DJ-1 mutations in Parkinson's disease. *Ann. Neurol.* 54:283-286.

Adem, A., Mattsson, M. E., Nordberg, A., and Pahlman, S. 1987. Muscarinic receptors in human SH-SY5Y neuroblastoma cell line: Regulation by phorbol ester and retinoic acid-induced differentiation. *Brain Res*. 430:235-242.

Ahmadi, F. A., Linseman, D. A., Grammatopoulos, T. N., Jones, S. M., Bouchard, R. J., Freed, C. R., Heidenreich, K. A., and Zawada, W. M. 2003. The pesticide rotenone induces caspase-3-mediated apoptosis in ventral mesencephalic dopaminergic neurons. *J. Neurochem*. 87:914-921.

Alam, M., and Schmidt, W. J. 2002. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav. Brain Res*. 136:317-324.

Alam, Z. I., Daniel, S. E., Lees, A. J., Marsden, D. C., Jenner, P., Halliwell, B. 1997a. A generalized increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. *J. Neurochem*. 69:1326-1329.

Alam, Z. I., Jenner, A., Daniel, S. E., Lees, A. J., Cairns, N., Marsden, C. D., Jenner, P., and Halliwell, B. 1997b. Oxidative DNA damage in the Parkinsonian brain: An apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J. Neurochem*. 69:1196-1203.

Albers, D. S., and Beal, M. F. 2000. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease. *J. Neural. Transm. Suppl*. 59:133-54.

Albores, R., Neafsey, E. J., Drucker, G., Fields, J. Z., and Collins, M. A., 1990. Mitochondrial respiratory inhibition by *N*-methylated beta-carboline derivatives structurally resembling *N*-methyl-4-phenylpyridine. *Proc. Natl. Acad. Sci. USA* 87:9368–9372.

Al-Chalabi, A., and Miller, C. C. 2003. Neurofilaments and neurological disease. *BioEssays* 25:346–355.

Alim, M. A., Hossain, M. S., Arima, K., Takeda, K., Izumiyama, Y., Nakamura, M., Kaji, H., Shinoda, T., Hisanaga, S., and Ueda, K. 2002. Tubulin seeds alpha-synuclein fibril formation. *J. Biol. Chem.* 277:2112-2117.

Almond, J. B., Snowden, R. T., Hunter, A., Dinsdale, D., Cain, K., and Cohen, G. M. 2001. Proteasome inhibitor-induced apoptosis of B-chronic lymphocytic leukaemia cells involves cytochrome c release and caspase activation, accompanied by formation of an approximately 700 kDa Apaf-1 containing apoptosome complex. *Leukemia* 15:1388-1397.

Andersen, J. K. 2001. Does neuronal loss in Parkinson's disease involve programmed cell death? *Bioessays* 23:640-646.

Andersen, J. K. 2003. Paraquat and iron exposure as possible synergistic environmental risk factors in Parkinson`s disease. *Neurotoxicol. Res*. 5:307-314.

Anglade, P., Vyas, S., Javoy-Agid, F., Herrero, M. T., Michel, P. P., Marquez, J., Mouatt-Prigent, A., Ruberg, M., Hirsch, E. C. and Agid, Y. 1997. Apoptosis and autophagy in nigral neurons of patients with Parkinson`s disease. *Histol. Histopathol*. 12:25-31.

Ardley, H. C., Scott, G. B., Rose, S. A., Tan, N. G., and Robinson, P. A. 2004. UCH-L1 aggresome formation in response to proteasome impairment indicates a role in inclusion formation in Parkinson's disease. *J. Neurochem*. 90:379-391.

Armstrong, M., Daly, A. K., Cholerton, S., Bateman, D. N., and Idle, J. R. 1992. Mutant debrisoquine hydroxylation genes in Parkinson's disease. *Lancet* 339:1017-1018.

Asada, H., Ip, N. Y., Pan, L., Razack, N., Parfitt, M. M., Plunkett, R. J. 1995. Time course of ciliary neurotrophic factor mRNA expression is coincident with the presence of protoplasmic astrocytes in traumatized rat striatum. *J. Neurosci. Res.* 40:22-30.

Asanuma, M., Miyazaki, I., and Ogawa, N. 2003. Dopamine- or L-DOPA-induced neurotoxicity: The role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease. *Neurotox. Res*. 5:165-176.

Aschner, M. 2000. Manganese: Brain transport and emerging research needs. *Environ. Health Perspect.*108:429-432.

Bandopadhyay, R., Kingsbury, A. E., Cookson, M. R., Reid, A. R., Evans, I. M., Hope, A. D., Pittman, A. M., Lashley, T., Canet-Aviles, R., Miller, D. W., McLendon, C., Strand, C., Leonard, A. J., Abou-Sleiman, P. M., Healy, D. G., Ariga, H., Wood, N. W., de Silva, R., Revesz, T., Hardy, J. A., and Lees, A. J. 2004. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* 127:420-430.

Banerjee, B. D., Seth, V., and Ahmed, R. S. 2001. Pesticide-induced oxidative stress: Perspectives and trends. *Rev. Environ. Health* 16:1-40.

Barnham, K. J., Masters, C. L., and Bush, A. I. 2004. Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discov.* 3:205-214.

Barone, S., Das, K. P., Lassiter, T. L., and White, L. D. 2000. Vulnerable processes of nervous system development: A review of markers and methods. *Neurotoxicology* 21:15-36.

Barrientos, A., Kenyon, L., Moraes, C. T. 1998. Human xenomitochondrial cybrids. Cellular models of mitochondrial complex I deficiency. *J. Biol. Chem.* 273:14210-14217.

Beal, M. F., 1992. Does impairment of energy metabolism results in excitotoxic neuronal death in neurodegenerative illness? *Ann. Neurol*. 31:119-130.

Bence, N. F., Sampat, R. M., and Kopito, R. R. 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292:1552-1555.

Ben-Shachar, D., Zuk, R., Gazawi, H., and Ljubuncic, P. 2004. Dopamine toxicity involves mitochondrial complex I inhibition: Implications to dopamine-related neuropsychiatric disorders. *Biochem. Pharmacol*. 67:1965-1974.

Biasini, E., Fioriti, L., Ceglia, I., Invernizzi, R., Bertoli, A., Chiesa, R., and Forloni, G. 2004. Proteasome inhibition and aggregation in Parkinson's disease: A comparative study in untransfected and transfected cells. *J. Neurochem*. 88:545-553.

Biedler, J. L., Roffler-Tarlov, S., Schachner, M., Freedman, L. S. 1978. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res*. 38:3751-3757.

Bismuth, C., Garnier, R., Baud, F. J., Muszynski, J., and Keyes, C. 1990. Paraquat poisoning. An overview of the current status. *Drug Safety* 5:243-251.

Blum, D., Wu, Y., Nissou, M. F., Arnaud, S., Alim-Louis, Benabid., and Verna, J. M. 1997. p53 and Bax activation in 6-hydroxydopamine-induced apoptosis in PC12 cells. *Brain Res*. 751:139-142.

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, AL., Sadoul, R., Verna, J. M. 2001. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Prog. Neurobiol*. 65:135-172.

Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A., and Heutink, P. 2003. Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. *Science* 299:256-259.

Bratton, S. B., MacFarlane, M., Cain, K., and Cohen, G. M. 2000. Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Exp. Cell Res*. 256:27-33.

Brooks, A. I., Chadwick, H. A., Gelbard, D. A., Cory-Slechta, D. A., and Federoff, H. J. 1999. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res*. 823:1-10.

Bulteau, A. L., Lundberg, K. C., Humphries, K. M., Sadek, H. A., Szweda, P. A., Friguet, B., and Szweda, L. I. 2001. Oxidative modification and inactivation of the proteasome during coronary occlusion/reperfusion. *J. Biol. Chem*. 276:30057-30063.

Bus, J. S., Aust, S. D., and Gibson, J. E. 1974. Superoxide- and singlet-oxygen-catalyzed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity. *Biochem. Biophys. Res. Commun.* 58:749-755.

Cao, Z., Hallur, S., Oiu, H. Z., Peng, X., and Li, Y. 2004. Induction of endogenous glutathione by the chemoprotective agent, 3H-1,2,-dithiole-3-thione, in human neuroblastoma SH-SY5Y cells affords protection against peroynitrite-induced cytotoxicity. *Biochem. Biophys. Res. Commun*. 316:1043-1049.

Carpenter, D. O. 2001. Effects of metals on the nervous system of humans and animals. *Int. J. Occup. Med. Environ. Health* 14:209-218.

Carrard, G., Bulteau, A. L., Petropoulos, I., and Friguet, B. 2002. Impairment of proteasome structure and function in aging. *Int. J. Biochem. Cell Biol*. 34:1461-1474.

Castillo, S. O., Baffi, J. S., Palkovits, M., Goldstein, D. S., Kopin, I. J., Witta, J., Magnuson, M. A., and Nikodem, V. M. 1998. Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. *Mol. Cell. Neurosci.* 11:36-46.

Chacon, J. N., Chedekel, M. R., Land, E. J., and Truscott, T. G. 1987. Chemically induced Parkinson`s disease: "Intermediates in the oxidation of 1-methyl-4-phenylpyridium ion". *Biochem. Biophys. Res. Commun.* 144:957-964.

Chadi, G., Cao, Y., Pettersson, R. F., Fuxe, K. 1994. Temporal and spatial increase of astroglial basic fibroblast growth factor synthesis after 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine neurons. *Neuroscience* 61:891-910.

Chan, B. S., Seale, J. P., andDuggin, G. G. 1997. The mechanism of excretion of paraquat in rats. *Toxicol. Lett*. 90:1-9.

Chan, D. K., Mellick, G. D., Buchanan, D. D., Hung, W. T., Ng, P. W., Woo, J., and Kay, R. 2002. Lack of association between CYP1A1 polymorphism and Parkinson's disease in a Chinese population. *J. Neural Transm*. 109:35-39.

Chan, P., Tanner, C. M., Jiang, X., and Langston, J. W. 1998. Failure to find the alpha-synuclein gene missense mutation (G209A) in 100 patients with younger onset Parkinson's disease. *Neurology* 50:513-514.

Checkoway, H., Franklin, G. M., Costa-Mallen, P., Smith-Weller, T., Dilley, J., Swanson, P. D., and Costa, L. G. 1998. A genetic polymorphism of MAO-B modifies the association of cigarette smoking and Parkinson's disease. *Neurology* 50:1458-1461.

Chen, G., Yuan, P. X., Jiang, Y. M., Huang, L. D., and Maniji, H. K. 1997. Lithium increases tyrosine hydroxylase levels both *in vivo* and *in vitro*. *J. Neurochem*. 70:1768-1771.

Chiba-Falek, O., and Nussbaum, R. L. 2001.Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. *Hum. Mol. Genet.* 10:3101-3109.

Chipuk, J. E., and Green, D. R. 2004. Cytoplasmic p53: bax and forward. *Cell Cycle* 3:429-431.

Chishti, M. A., Fisher, J. P., and Seegal, R. F. 1996. Aroclors 1254 and 1260 reduce dopamine concentrations in rat striatal slices. *Neurotoxicology* 17:653-660.

Choi, J., Levey, A. I., Weintraub, S. T., Rees, H. D., Gearing, M., Chin, L. S., and Li, L. 2004. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *J. Biol. Chem.* 279:13256–13264.

Choksi, N. Y., Kodavanti, P. R., Tilson, H. A., and Booth, R. G. 1997. Effects of polychlorinated biphenyls (PCBs) on brain tyrosine hydroxylase activity and dopamine synthesis in rats. *Fundam. Appl. Toxicol*. 39:76-80.

Cho, T. H. and Tiffany-Castiglioni, E. 2004. Neurofilament 200 as an indicator of differences between mipafox and paraoxon sensitivity in SY5Y neuroblastoma cells. *J. Toxicol. Environ. Health A* 67:987-1000.

Chung, K. K., Dawson, V. L., and Dawson, T. M. 2001. The role of the ubiquitin-proteasomal pathway in Parkinson's disease and other neurodegenerative disorders. *Trends Neurosci.Suppl*. 24:S7-14.

Ciechanover, A., and Brundin, P. 2003. The ubiquitin proteasome system in neurodegenerative diseases: Sometimes the chicken, sometimes the egg. *Neuron* 40:427-446.

Clementi, E., Brown, G. C., Feelisch, M., and Moncada, S. 1998. Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc. Natl. Acad. Sci. U S A*. 95:7631-7636.

Cohen, G. 1986. Monoamine oxidase, hydrogen peroxide and Parkinson`s disease. *Adv. Neurol.* 45: 119-125.

Collins, M. A., and Origitano, T. C. 1983. Catecholamine-derived tetrahydroisoquinolines: *O*-methylation patterns and regional brain distribution following intraventricular administration in rats. *J. Neurochem.* 41:1569-1575.

Conway, K. A., Harper, J. D., and Lansbury, P. T. 1998. Accelerated *in vitro* fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nat. Med.* 4:1318-1320.

Conway, K. A., Harper, J. D., and Lansbury, P. T. 2000. Fibrils formed *in vitro* from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* 39:2552-2563.

Corrigan, F. M., Murray, L., Wyatt, C. L., and Shore, R. F. 1998. Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. *Exp. Neurol*. 150:339-342.

Costa, P., Checkoway, H., Levy, D., Smith-Weller, T., Franklin, G. M., Swanson, P. D., and Costa, L. G. 1997. Association of a polymorphism in intron 13 of the monoamine oxidase B gene with Parkinson disease. *Am. J. Med. Genet.* 4:154-56.

Cremlyn, J. R. 1991. Agrochemicals: Preparation and Mode of Action. Chichester, England: Wiely

Dawson, V. L. and Dawson, T. M., 1996. Nitric oxide neurotoxicity. *J. Chem. Neuroanat.* 10:179–190.

Day, I. N., Hinks, L. J., and Thompson, R. J. 1990. The structure of the human gene encoding protein gene product 9.5 (PGP9.5), a neuron-specific ubiquitin C-terminal hydrolase. *Biochem. J.* 268:521-524.

de Jong, G. 1991. Long-term health effects of aldrin and dieldrin. A study of exposure, health effects and mortality of workers engaged in the manufacture and formulation of the insecticides aldrin and dieldrin. *Toxicol. Lett. Suppl*.1-206.

de la Monte, S. M., Sohn, Y. K., Ganju, N., and Wands, J. R. 1998. P53- and CD95-associated apoptosis in neurodegenerative diseases. *Lab Invest*. 78:401-411.

Dekker, M. C., Bonifati, V., and van Duijn, C. M. 2003. Parkinson's disease: Piecing together a genetic jigsaw. *Brain* 126:1722-1733.

Denizot, F., and Lang, R. 1986. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* 89:271-277.

De Martino, G. N., and Slaughter, C. A. 1999. The proteasome, a novel protease regulated by multiple mechanisms. *J. Biol. Chem*. 274:22123-22126.

Dennis, J., and Bennett, J. P. 2003. Interactions among nitric oxide and Bcl-family proteins after MPP+ exposure of SH-SY5Y neural cells I: MPP+ increases mitochondrial NO and Bax protein. *J. Neurosci. Res*. 72:76-88.

de Rijk MC, Breteler MM, Graveland GA, Ott A, Grobbee DE, van der Meche FG, Hofman A. 1995. Prevalence of Parkinson's disease in the elderly: The Rotterdam Study. *Neurology* 45:2143-2146.

Dev, K. K., Hofele, K., Barbieri, S., Buchman, V. L., and van der Putten, H. 2003. Part II: Alpha-synuclein and its molecular pathophysiological role in neurodegenerative disease. *Neuropharmacology* 45:14-44.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., and Marsden, C. D. 1989a. Basal lipid peroxidation in substantia nigra is increased in Parkinson`s disease. *J. Neurochem.* 52:381-389.

Dexter, D. T., Wells, F. R., Lees, A. J., Agid, F., Agid, Y., and Jenner, P. 1989b. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson’s disease. *J. Neurochem.* 52:1830-1836.

Dexter, D. T., Carayon, A., Vidailhet, M., Ruberg, M., Agid, F., and Agid, Y. 1990. Decreased ferritin levels in brain in Parkinson's disease. *J. Neurochem.* 55:6-20.

Dexter, D. T., Holley, A. E., Flitter, W. D., Slater, T. F., Wells, F. R., Daniel, S. E., Lees, A. J., Jenner, P., Marsden, C. D. 1994. Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: An HPLC and ESR study. *Mov. Disord*. 9:92-97.

Diaz-Corrales, F. J., Asanuma, M., Miyazaki, I., and Ogawa, N. 2004. Rotenone induces disassembly of the Golgi apparatus in the rat dopaminergic neuroblastoma B65 cell line. *Neurosci. Lett*. 354:59-63.

Difazio, M. C., Hollingsworth, Z., Young, A. B., and Penney Jr., J. B., 1992. Glutamate receptors in the substantia nigra of Parkinson's disease brains. *Neurology* 42:402-406.

Ding, Q., and Keller, J. N. 2001. Proteasome inhibition in oxidative stress neurotoxicity: Implications for heat shock proteins. *J. Neurochem*. 77:1010-1017.

Doong, R. A., Lee, C. Y., and Sun, Y. C. 1999. Dietary intake and residues of organochlorine pesticides in foods from Hsinchu, Taiwan. *J. AOAC. Int*. 82:677-682.

Doran, J. F., Jackson, P., Kynoch, P. A., and Thompson, R. J. 1983. Isolation of PGP 9.5, a new human neurone-specific protein detected by high-resolution two-dimensional electrophoresis. *J. Neurochem.* 40:1542-1547.

Dringen, R. 2000. Metabolism and functions of glutathione in brain. *Prog. Neurobiol*. 62:649-671.

Drucker, G., Raikoff, K., Neafsey, E. J., and Collins, M. A., 1990. Dopamine uptake inhibitory capacities of beta-carboline and 3,4-dihydro-beta-carboline analogs of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) oxidation products. *Brain Res.* 509:125–133.

Dunnett, S. B., and Bjorklund, A., 1999. Prospects for new restorative and neuroprotective treatments in Parkinson's disease. *Nature* 399:A32-39.

Duvoisin, R. C., and Yahr, M. D. 1965. Encephalitis and Parkinsonism. *Arch. Neurol*. 12:227-39.

Earle, K. M. 1968. Studies on Parkinson's disease including x-ray fluorescent spectroscopy of formalin fixed brain tissue. *J. Neuropathol. Exp. Neurol.* 27:1–14.

Ehrich, M., Intropido, L., and Costa, L. G. 1994. Interaction of organophosphorous compounds with muscarinic receptors in SH-SY5Y human neuroblastoma cells. *J. Toxicol. Environ. Health* 43:51-63.

Ehrich, M., and Correll, L. 1998. Inhibition of carboxylesterases in SH-SY5Y human and NB41A3 mouse neuroblastoma cells by organophosphorous esters. *J. Toxicol. Environ. Health* A 53:385-399.

Elkon, H., Melamed, E., and Offen, D. 2001. 6-Hydroxydopamine increases ubiquitin-conjugates and protein degradation: Implications for the pathogenesis of Parkinson's disease. *Cell Mol. Neurobiol*. 21:771-781.

Elkon, H., Melamed, E., and Offen, D. 2004. Oxidative stress, induced by 6-hydroxydopamine, reduces proteasome activities in PC12 cells: Implications for the pathogenesis of Parkinson's disease. *J. Mol. Neurosci*. 24:387-400.

Elsworth, J. D., and Roth, R. H. 1997. Dopamine synthesis, uptake, metabolism, and receptors: Relevance to gene therapy of Parkinson's disease. *Exp. Neurol*. 144:4-9.

Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., Margolis, R. L., Troncoso, J. C., Lanahan, A. A., Worley, P. F., Dawson, V. L., Dawson, T. M., and Ross, C. A. 1999. Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat. Genet.* 22:110-114.

Evert, B. O., Wullner, U., and Klockgether, T. 2000. Cell death in polyglutamine diseases. *Cell Tissue Res.* 301:189-204.

Fahn, S. 2003. Description of Parkinson`s disease as a clinical syndrome. *Ann. N. Y. Acad. Sci*. 991:1-14.

Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, k., Petrucelli, L., Hussey, J., Singleton, A., Tanner, C., Hardy, J., and Langston, J. W. 2001. Lewy bodies and Parkinsonism in families with parkin mutations. *Ann. Neurol.* 50:293-300.

Farrer, M., Maraganore, D. M., Lockhart, P., Singleton, A., Lesnick, T. G., de Andrade, M., West, A., de Silva, R., Hardy, J., and Hernandez, D. 2001. Alpha-Synuclein gene haplotypes are associated with Parkinson's disease. *Hum. Mol. Genet.* 10:1847-1851.

Farrer, M., Kachergus, J., Forno, L., Lincoln, S., Wang, D. S., Hulihan, M., Maraganore, D., Gwinn-Hardy, K., Wszolek, Z., Dickson, D., and Langston, J. W. 2004.

Comparisonof kindreds with Parkinsonism and alpha-synuclein genomic multiplications. *Ann. Neurol.* 55:174-179.

Farrington, J. A., Ebert, M., Land, E. J., and Fletcher, K. 1973. Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides. *Biochim. Biophys. Acta*. 314:372-381.

Ferrarese, C., Tremolizzo, L., Rigoldi, M., Sala, G., Begni, B., Brighina, L., Ricci, G., Albizzati, M. G., Piolti, R., Crosti, F., Dalpra, L., and Frattola, L. 2001. Decreased platelet glutamate uptake and genetic risk factors in patients with Parkinson's disease. *Neurol. Sci.* 22:65–66.

Ferraz, H. B., Bertolucci, P. H., Pereira, J. S., Lima, J. G., and Andrade, L. A. 1988. Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. *Neurology* 38:550-553.

Fishman, P. S., Gass, J. S., Swoveland, P. T., Lavi, E., Highkin, M. K., and Weiss, S. R. 1985. Infection of the basal ganglia by a murine coronavirus. *Science* 229:877-879.

Fiskum, G., Starkov, A., Polster, B. M., Chinopoulos, C. 2003. Mitochondrial mechanisms of neural cell death and neuroprotective interventions in Parkinson's disease. *Ann. N. Y. Acad. Sci*. 991:111-119.

Fleming, L., Mann, J. B., Bean, J., Briggle, T., and Sanchez-Ramos, J. R. 1994. Parkinson's disease and brain levels of organochlorine pesticides. *Ann. Neurol*. 36:100-103.

Floor, E., and Wetzel, M. G. 1998. Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *J. Neurochem*. 70:268-275.

Floyd, R. A., and Hensley, K. 2002. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging*. 23:795-807.

Forno, L. S., 1996. Neuropathology of Parkinson's disease. *J. Neuropathol. Exp. Neurol*. 55:259-272.

Fridman, J. S., and Lowe, S. W. 2003. Control of apoptosis by p53. *Oncogene* 22:9030-9040.

Gash, D. M., Zhang, Z., Cass, W. A., Ovadia, A., Simmerman, L., Martin, D., Russell, D., Collins, F., Hoffer, B. J., and Gerhardt, G. A. 1995. Morphological and

functional effects of intranigrally administered GDNF in normal rhesus monkeys. *J. Comp. Neurol*. 363:345-358.

Gearhart, D. A., Collins, M. A., Lee, J. M., and Neafsey, E. J. 2000. Increased beta-carboline 9*N*-methyltransferase activity in the frontal cortex of Parkinson's disease. *Neurobiol. Dis.* 7:201–211.

Gerber, G. B., Leonard, A., and Hantson, P. 2002. Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Crit. Rev. Oncol. Hematol*. 42:25-34.

Golbe, L. I. 1993. The genetics of Parkinson's disease. *Rev. Neurosci*. 4:1-16.

Golbe, L. I., Di Iorio, G., Bonavita, V., Miller, D. C., and Duvoisin, R. C. 1990. A large kindred with autosomal dominant Parkinson's disease. *Ann. Neurol.* 27:276-282.

Golbe, L. I., and Mouradian, M. M. 2004. Alpha-synuclein in Parkinson's disease: Light from two new angles. *Ann. Neurol.* 55:153-156.

Gómez, C., Reiriz, J., Pique, M., Gil, J., Ferrer, I., and Ambrosio, S. 2001. Low concentrations of 1-methyl-4-phenylpyridinium ion induce caspase-mediated apoptosis in human SH-SY5Y neuroblastoma cells. *J. Neurosci. Res*. 63:421-428.

Gómez-Santos, C, Ambrosio S, Ventura F, Ferrer I, Reiriz J. 2002. TGF-beta1 increases tyrosine hydroxylase expression by a mechanism blocked by BMP-2 in human neuroblastoma SH-SY5Y cells. *Brain Res*. 958:152-160.

Gómez-Santos, C., Ferrer, I., Santidrian, A. F., Barrachina, M., Gil, J., Ambrosio, S. 2003. Dopamine induces autophagic cell death and alpha-synuclein increase in human neuroblastoma SH-SY5Y cells. *J. Neurosci. Res*. 73:341-350.

Good, P., Olanow, C. W., and Perl, D. R. 1992. Neuromelanin-containing neurons of the substantia nigra accumulate iron and aluminum in Parkinson`s disease. *Brain Res*. 593:343-346.

Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L., Kortsha, G. X., Brown, G. G. 1997. Occupational exposures to metals as risk factors for Parkinson's disease. *Neurology* 48:650–658.

Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L., and Richardson, R. J. 1998. The risk of Parkinson`s disease with exposure to pesticides, farming, well water, and rural living. *Neurology* 50:1346-1350.

Goudreau, J. L., Maraganore, D. M., Farrer, M. J., Lesnick, T. G., Singleton, A. B., Bower, J. H., Hardy, J. A., and Rocca, W. A. 2002. Case-control study of dopamine transporter-1, monoamine oxidase-B, and catechol-*O*-methyl transferase polymorphisms in Parkinson's disease. *Mov. Disord.* 17:1305-1311.

Grant, H., Lantos, P. L., and Parkinson, C. 1980. Cerebral damage in paraquat poisoning. *Histopathology* 4:185-195.

Greenmyre, J. T., Eller, R. V., Zhang, Z., Ovadia, A., Kurlan, R. and Gash, D. M. 1994. AntiParkinsonian effects of remacemide hydrochloride, a glutamate antagonist, in rodent and primate models of Parkinson's disease. *Ann. Neurol.* 35:655–661.

Griffith, O. W., and Mulcahy, R. T. 1999. The enzymes of glutathione synthesis: Gamma-glutamylcysteine synthetase. *Adv. Enz. Relat. Areas Mol. Biol.* 73:209-267.

Grune, T., and Davies, K. J. 2003. The proteasomal system and HNE-modified proteins. *Mol. Aspects Med*. 24:195-204.

Gu, M., Cooper, J. M., Taanman, J. W., Schapira, A. H. 1998. Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Ann. Neurol*. 44:177-186.

Hagg, T., and Varon, S. 1993. Ciliary neurotrophic factor prevents degeneration of adult rat substantia nigra dopaminergic neurons *in vivo*. *Proc. Natl. Acad. Sci*. 90:6315-6319.

Halliwell, B., and Gutteridge, J. M. C. 1984. Oxygen toxicity, oxygen radicals, transition metals, and disease. *Biochem J*. 219:1-14.

Haque, M. E., Asanuma, M., Higashi, Y., Miyazaki, I., Tanaka, K., and Ogawa, N. 2003. Apoptosis-inducing neurotoxicity of dopamine and its metabolites via reactive quinone generation in neuroblastoma cells. *Biochim. Biophys. Acta*. 1619:39-52.

Harada, S., Fujii, C., Hayashi, A., and Ohkoshi, N. 2001. An association between idiopathic Parkinson's disease and polymorphisms of phase II detoxification enzymes: Glutathione *S*-transferase M1 and quinone oxidoreductase 1 and 2. *Biochem. Biophys. Res. Commun.* 288:887-892.

Hardy, J., Cookson, M. R., and Singleton, A. 2003. Genes and Parkinsonism. *Lancet Neurol.* 2:221-228.

Hasegawa, K., Funayama, M., Matsuura, N., Furusawa, H., Sakai, F., Kowa, H., and Obata, F. 2001. Analysis of alpha-synuclein, parkin, tau, and UCH-L1 in a Japanese family with autosomal dominant Parkinsonism. *Eur. Neurol.* 46:20–24.

Hasegawa, T., Matsuzaki, M., Takeda, A., Kikuchi, A., Furukawa, K., Shibahara S., and Itoyama, Y. 2003. Increased dopamine and its metabolites in SH-SY5Y neuroblastoma cell that express tyrosinase. *J. Neurochem*. 87:470-475.

Hashimoto, M., Rockenstein, E., Crews, L., and Masliah, E. 2003. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. *Neuromolecular Med*. 4:21-36.

Hayes, J. D., and Strange, R. C. 1995. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radical Res*. 22:193-207.

Healy, D. G., Abou-Sleiman, P. M., Ozawa, T., Lees, A. J., Bhatia, K., Ahmadi, K. R., Wullner, U., Berciano, J., Moller, J. C., Kamm, C., Burk, K., Barrone, P., Tolosa, E., Quinn, N., Goldstein, D. B., and Wood, N. W. 2004. A functional polymorphism regulating dopamine beta-hydroxylase influences against Parkinson's disease. *Ann. Neurol.* 55:443-446.

Heikkila, R. E., Nicklas, W. J., Vyas, I., and Duvoisin, R. C. 1985. Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: Implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. *Neurosci. Lett*. 62:389-394.

Heinz, G. H., Hill, E. F., and Contrera, J. F. 1980. Dopamine and norepinephrine depletion in ring doves fed DDE, dieldrin, and Aroclor 1254. *Toxicol. Appl. Pharmacol.* 53:75-82.

Hertzman, C., Wiens, M., Bowering, D., Snow, B., and Calne, D. 1990. Parkinson`s disease: A case-control study of occupational and environmental risk factors. *Am. J. Ind. Med.* 17, 349-355.

Höglinger, G. U., Carrard, G., Michel, P. P., Medja, F., Lombes, A., Ruberg, M., Friguet, B., and Hirsch, E. C. 2003. Dysfunction of mitochondrial complex I and the proteasome: Interactions between two biochemical deficits in a cellular model of Parkinson's disease. *J. Neurochem*. 86:1297-1307.

Holzmann, C., Kruger, R., Saecker, A. M., Schmitt, I., Schols, L., Berger, K., and Riess, O. 2003. Polymorphisms of the alpha-synuclein promoter: Expression analyses and association studies in Parkinson's disease. *J. Neural Transm.* 110:67-76.

Horowitz, J. M., Pastor, D. M., Goyal, A., Kar, S., Ramdeen, N., Hallas, B. H., and Torres G. 2003. BAX protein-immunoreactivity in midbrain neurons of Parkinson's disease patients. *Brain Res. Bull*. 62:55-61.

Howard, J. K. 1983. The myth of paraquat inhalation as a route for human poisoning. *J. Toxicol. Clin. Toxicol*. 20:191-193.

Howells, D.W., Porritt, M. J., Wong, J. Y., Batchelor, P. E., Kalnins, R., Hughes, A. J., and Donnan, G. A. 2000. Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol*. 166:127-135.

Hughes, J. T. 1988. Brain damage due to paraquat poisoning: A fatal case with neuropathological examination of the brain. *Neurotoxicology* 9:243-248.

Hunot, S., Bernard, V., Faucheux, B., Boissiere, F., and Leguern, E. 1996. Glial cell line-derived neurotrophic factor (GDNF) gene expression in the human brain: A post mortem in situ hybridization study with special reference to Parkinson's disease. *J. Neural Transm.* 103:1043-1052.

Hunot, S., Brugg, B., Ricard, D., Michel, P. P., Muriel, M. P., Ruberg, M., Faucheux, B. A., Agid, Y., and Hirsch, E. C. 1997. Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with Parkinson`s disease. *Proc. Natl. Acad. Sci*. USA 94:7531-7536.

Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P., and Lindsay, R. M. 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350:230-232.

Hyun, D. H., Lee, M. H., Halliwell, B., and Jenner, P. 2002. Proteasomal dysfunction induced by 4-hydroxy-2,3-trans-nonenal, an end-product of lipid peroxidation: A mechanism contributing to neurodegeneration? *J. Neurochem*. 83:360-370.

Hyun, D. H., Gray, D. A., Halliwell, B., and Jenner, P. 2004. Interference with ubiquitination causes oxidative damage and increased protein nitration: Implications for neurodegenerative diseases. *J. Neurochem*. 90:422-430.

Ichinose, H., Ohye, T., Suzuki, T., Sumi-Ichinose, C., Nomura, T., Hagino, Y., and Nagatsu, T. 1999. Molecular cloning of the human Nurr1 gene: Characterization of the human gene and cDNAs. *Gene* 230:233-239.

Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y., and Takahashi, R. 2001.An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* 105:891-902.

Irwin, I., Finnegan, K. T., Delanney, L. E., Di Monte, D., and Langston, J. W. 1992. The relationships between aging, monoamine oxidase, striatal dopamine and the effects of MPTP in C57BL/6 mice: A critical reassessment. *Brain Res*. 572:224-231.

Jana, N. R., Dikshit, P., Goswami, A., and Nukina, N. 2004. Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. *J. Biol. Chem*.279:11680-11685.

Jang, J. H., and Surh, Y. J. 2003. Protective effect of resveratrol on beta-amyloid induced oxidative PC12 cell death. *Free Radic. Biol. Med*. 34:1100-1110.

Jenner, P. 2003. Oxidative stress in Parkinson's disease. *Ann. Neurol.* 53:S26–S36.

Jensen, P. H., Hager, H., Nielsen, M. S., Hojrup, P., Gliemann, J., and Jakes, R. 1999. Alpha-synuclein binds to Tau and stimulates the protein kinase A-catalyzed tau phosphorylation of serine residues 262 and 356. *J. Biol. Chem.* 274:25481-25489.

Julien, J. P., and Mushynski, W. E. 1998. Neurofilaments in health and disease. *Prog. Nucleic Acid Res. Mol. Biol.* 61:1-23.

Junn, E., and Mouradian, M. M. 2001. Apoptotic signaling in dopamine-induced cell death: The role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases. *J. Neurochem*. 78:374-383.

Jurma, O. P., Hom, D. G., and Andersen, J. K. 1997. Decreased glutathione results in calcium-mediated cell death in PC12. *Free Radical Biol. Med*. 23:1055-1066.

Kalivendi, S. V., Cunningham, S., Kotamraju, S., Joseph, J., Hillard, C. J., and Kalyanaraman, B. 2003. Alpha-synuclein up-regulation and aggregation during MPP+-induced apoptosis in neuroblastoma cells: Intermediacy of transferrin receptor iron and hydrogen peroxide. *J. Biol. Chem*. 279:15240-15247.

Kannan, K., and Jain, S. K. 2000. Oxidative stress and apoptosis. *Pathophysiology* 7:153-163.

Kawai, H., Makino, Y., Hirobe, M., and Ohta, S. 1998. Novel endogenous 1,2,3,4-tetrahydroisoquinoline derivatives: Uptake by dopamine transporter and activity to induce parkinsonism. *J. Neurochem.* 70:745–751.

Kelada, S. N., Stapleton, P. L., Farin, F. M., Bammler, T. K., Eaton, D. L., Smith-Weller, T., Franklin, G. M., Swanson, P. D., Longstreth, W. T., and Checkoway, H. 2003. Glutathione S-transferase M1, T1, and P1 polymorphisms and Parkinson's disease. *Neurosci. Lett.* 337:5-8.

Keller, J. N., Huang, F. F., Dimayuga, E. R., and Maragos, W. F. 2000. Dopamine induces proteasome inhibition in neural PC12 cell line. *Free Radic. Biol. Med*. 29:1037-1042.

Kim, J. H., Park, K. C., Chung, S. S., Bang, O., and Chung, C. H. 2003. Deubiquitinating enzymes as cellular regulators. *J. Biochem.* 134:9-18.

Kim, T. D., Paik, S. R., Yang, C. H., and Kim, J. 2000. Structural changes in alpha-synuclein affect its chaperone-like activity *in vitro*. *Protein Sci.* 9:2489-2496.

Kim, T. E., Park, M. J., Choi, E. J., Lee, H. S., Lee, S. H., Yoon, S. H., Oh, C. K., Lee, B. J., Kim, S. U., Lee, Y. S., Lee, M. A. 2003. Cloning and cell type-specific regulation of the human tyrosine hydroxylase gene promoter. *Biochem. Biophys. Res. Commun*. 312:1123-1131.

Kim, W. G., Mohney, R. P., Wilson, B., Jeohn, G. H., Liu, B., and Hong, J. S. 2000. Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: Role of microglia. *J. Neurosci*. 20:6309-6316.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. 1998. Mutations in the Parkin Gene Cause Autosomal Recessive Juvenile Parkinsonism. *Nature* 392:605-608.

Klegeris, A., and McGeer, P. L. 2000. R-(-)-Deprenyl inhibits monocytic THP-1 cell neurotoxicity independently of monoamine oxidase inhibition. *Exp. Neurol*. 166:458-464.

Knott, C., Stern, G., and Wilkin, G. P. 2000. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Mol. Cell. Neurosci.* 16:724-739.

Kotake, Y., Tasaki, Y., Makino, Y., Ohta, S., and Hirobe, M. 1995. 1-Benzyl-1,2,3,4-tetrahydroisoquinoline as a parkinsonism-inducing agent: A novel endogenous amine in mouse brain and parkinsonian CSF. *J. Neurochem.* 65:2633-2638.

Krieger, C., and Duchen, M. R. 2002. Mitochondria, Ca2+ and neurodegenerative disease. *Eur. J. Pharmacol*. 447:177-188.

Krohn, A. J., Wahlbrink, T., and Prehn, J. H. 1999. Mitochondrial depolarization is not required for neuronal apoptosis. *J. Neurosci.*19: 7394-7404.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. 1998. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat. Genet.* 18:106-108.

Kruger, R., Fischer, C., Schulte, T., Strauss, K. M., Muller, T., Woitalla, D., Berg, D., Hungs, M., Gobbele, R., Berger, K., Epplen, J. T., Riess, O., and Schols, L. 2003. Mutation analysis of the neurofilament M gene in Parkinson's disease. *Neurosci. Lett.* 351:125-129.

Kusano, N., Aoyama, Y., Kawamura, A., and Kawashima, H. 1996. The diagnosis of Japanese encephalitis by means of fluorescent antibody technique in autopsy cases. *Neuropatol. Pol*. 4:449-56.

Kweon, G. R., Marks, J. D., Krencik, R., Leung, E. H., Schumacker, P. T., Hyland, K., and Kang, U. J. 2004. Distinct mechanisms of neurodegeneration induced by chronic complex I inhibition in dopaminergic and non-dopaminergic cells. *J. Biol. Chem*. 279:51783-51792.

Lai, B. C. L., Marion, S. A., Teschke, K., and Tsui, J. K. C. 2002. Occupational and environmental risk factors for Parkinson`s disease. *Parkinsonism Relat. Dis.* 8:297-309.

Lai, C. T., and Yu P. H. 1997. Dopamine- and L-β-3,4-dihydrophenylalanine hydrochloride (L-dopa)-induced cytotoxicity towards catecholaminergic neuroblastoma SH-SY5Ycells. *Biochem. Pharmacol*. 53:363-372.

Lang, A. E., and Lozano, A. M., 1998. Parkinson's disease. Second of two parts. *N. Engl. J. Med.* 339:1130-1143.

Lang-Rollin, I., Vekrellis, K., Wang, Q., Rideout, H. J., and Stefanis, L. 2004. Application of proteasomal inhibitors to mouse sympathetic neurons activates the intrinsic apoptotic pathway. *J. Neurochem*. 90:1511-1520.

Lapchak, P. A., Gash, D. M., Jiao, S., Miller, P. J., and Hilt, D. 1997. Glial cell line-derived neurotrophic factor: A novel therapeutic approach to treat motor dysfunction in Parkinson's disease. *Exp. Neurol*. 144:29-34.

Lavedan, C., Buchholtz, S., Nussbaum, R. L., Albin, R. L., and Polymeropoulos, M. H. 2002. A mutation in the human neurofilament M gene in Parkinson's disease that suggests a role for the cytoskeleton in neuronal degeneration. *Neurosci. Lett.* 322:57-61.

Law, S. W., Conneely, O. M., DeMayo, F. J., and O'Malley, B. W. 1992. Identification of a new brain-specific transcription factor, NURR1. *Mol. Endocrinol*. 6:2129-21235.

Le, W. D., Xu, P., Jankovic, J., Jiang, H., Appel, S. H., Smith, R. G., and Vassilatis, D. K. 2003. Mutations in NR4A2 associated with familial Parkinson disease. *Nat. Genet.* 33:85-89.

Le Couteur, D. G., Muller, M., Yang, M. C., Mellick, G. D., and McLean, A. J. 2002. Age-environment and gene-environment interactions in the pathogenesis of Parkinson's disease. *Rev. Environ. Health*.17:51-64.

Lee, C. S., Han, E. S., and Lee, W. B. 2003. Antioxidant effect of phenelzine on MPP+-induced cell viability loss in differentiated PC12 cells. *Neurochem. Res*. 28:1833-1841.

Lee, H. S., Park, C. W., and Kim, Y. S. 2000. MPP+ increases the vulnerability to oxidative stress rather than directly mediating oxidative damage in human neuroblastoma cells. *Exp. Neurol*. 165:164-171.

Legros, H., Dingeval, M. G., Janin, F., Costentin, J., Bonnet, J. J. 2004. Toxicity of a treatment asociating dopamine and disulfiram for catecholaminergic neuroblastoma SH-SY5Y cells: Relationships with 3,4-Dihydroxyphenylacetaldehyde formation. *Neurotoxicology* 25:365-375.

Lenaz, G. 2001. The mitochondrial production of reactive oxygen species: Mechanisms and implications in human pathology. *IUBMB Life*. 52:159-164.

Leon, A., Foll, L. I., Charriault-Marlangue, C., Leprince, J., Vaudry, H., Gabriel, C., and Duval, D. 2003. Level of haem oxygenase does not obligatorily reflect the sensitivity of PC12 cells to an oxidative shock induced by glutathione depletion. *J. Neurochem*. 84:459-470.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D., and Polymeropoulos, M. H. 1998. The ubiquitin pathway in Parkinson's disease. *Nature* 395: 451–452.

Levecque, C., Destee, A., Mouroux, V., Becquet, E., Defebvre, L., Amouyel, P., and Chartier-Harlin, M. C. 2001. No genetic association of the ubiquitin carboxy-terminal hydrolase-L1 gene S18Y polymorphism with familial Parkinson's disease. *J. Neural Transm.* 108:979-984.

Levy, B. S., and Nassetta, W. J. 2003. Neurologic effects of manganese in humans: Review. *Int. J. Occup. Environ. Health* 9:153-163.

Li, Y. J., Oliveira, S. A., Xu, P., Martin, E. R., Stenger, J. E., Scherzer, C. R., Hauser, M. A., Scott, W. K., Small, G. W., Nance, M. A., Watts, R. L., Hubble, J. P., Koller,

W. C., Pahwa, R., Stern, M. B., Hiner, B. C., Jankovic, J., Goetz, C. G., Mastaglia, F., Middleton, L. T., Roses, A. D., Saunders, A. M., Schmechel, D. E., Gullans, S. R., Haines, J. L., Gilbert, J. R., Vance, J. M., and Pericak-Vance, M. A. 2003. Glutathione *S*-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease. *Hum. Mol. Genet.* 12:3259-3267.

Li, B., and Dou, Q. P. 2000. Bax degradation by the ubiquitin/proteasome-dependent pathway: Involvement in tumor survival and progression. *Proc Natl Acad Sci U S A* 97:3850-3855.

Li, X., and Sun, A. Y. 1999. Paraquat induced activation of transcription factor AP-1 and apoptosis in PC12 cells. *J. Neural Transm*. 106:1-21.

Lin, L. F., Doherty, D. H., Lile, J. D., Bektesh, S., and Collins, F. 1993. GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 60:1130-1132.

Lincoln, S., Vaughan, J., Wood, N., Baker, M., Adamson, J., Gwinn-Hardy, K., Lynch, T., Hardy, J., and Farrer, M. 1999. Low frequency of pathogenic mutations in the ubiquitin carboxy-terminal hydrolase gene in familial Parkinson's disease. *NeuroReport* 10:427-429.

Lindersson, E., Beedholm, R., Hojrup, P., Moos, T., Gai, W., Hendil, K. B., and Jensen, P. H. 2004. Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J.Biol. Chem*. 279:12924-12934.

Liou, H. H., Chen, R. C., Tsai, Y. F., Chen, W. P., Chang, Y. C., and Tsai, M. C. 1996. Effects of paraquat on the substantia nigra of the Wistar rats: Neurochemical, histological, and behavioral studies. *Toxicol. Appl. Pharmacol*. 137:34-41.

Liou, H. H., Tsai, M. C., Chen, C. J., Jeng, J. S., Chang, Y. C., Chen, S. Y., and Chen, R. C. 1997. Environmental risk factors and Parkinson`s disease: A case-control study in Taiwan. *Neurology* 48:1583-1588.

Liou, H, H., Chen, R. C., Chen, H. H., Tsai, Y. F., and Tsai, M. M. 2001. Attenuation of paraquat-induced dopaminergic toxicity on the substantia nigra by (-)-deprenyl *in vivo*. *Toxicol. Appl. Pharmacol*. 172:37-43.

Liu, Y., Fallon, L., Lashuel, H. A., Liu, J., and Lansbury, P. T. 2002. The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. *Cell* 111:209–218

.

Lo, H. S., Chiang, H. C., Lin, A. M., Chiang, H. Y., Chu, Y. C., and Kao, L. S. 2004. Synergistic effects of dopamine and Zn2+ on the induction of PC12 cell death

and dopamine depletion in the striatum: Possible implication in the pathogenesis of Parkinson's disease. *Neurobiol. Dis.* 17:54-61.

Lopes, U. G., Erhardt, P., Yao, R., and Cooper, G. M. 1997. p53-dependent induction of apoptosis by proteasome inhibitors. *J. Biol. Chem*. 272:12893-12896.

Lu, M., Kitson, R. P., Xue, Y., and Goldfarb, R. H. 2003. Activation of multiple caspases and modification of cell surface fas (CD95) in proteasome inhibitor-induced apoptosis of rat natural killer cells. *J. Cell Biochem*. 88:482-492.

Lűcking, C. B., and Brice, A. 2000. Alpha-synuclein and Parkinson's disease. *Cell Mol. Life Sci*. 57:1894-908.

Machida, Y., Chiba, T., Takayanagi, A., Tanaka, Y., Asanuma, M., Ogawa, N., Koyama, A., Iwatsubo, T., Ito, S., Jansen, P. H., Shimizu, N., Tanaka, K., Mizuno, Y., and Hattori, N. 2005. Common anti-apoptotic roles of parkin and alpha-synuclein in human dopaminergic cells. *Biochem. Biophys. Res. Commun*. 332:233-240.

Majno, G., and Joris, I. 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am. J. Pathol*. 146:3-15.

Makino, Y., Ohta, S., Tachikawa, O., and Hirobe, M., 1988. Presence of tetrahydroisoquinoline and 1-methyl-tetrahydroisoquinoline in foods: Compounds related to Parkinson's disease. *Life Sci.* 43:373–378.

Maňáková, S., Puttonen, K., A., Raasmaja, A., Mannisto, P. T. 2004. The roles of dopamine transporter and Bcl-2 protein in the protection of CV1-P cells from 6-OHDA-induced toxicity. *Toxicol. Lett*. 154:117-123.

Mann, V. M., Cooper, J. M., Krige, D., Daniel, S. E., Schapira, A. H., Marsden, C. D. 1992. Brain, skeletal muscle, and platelet homogenate mitochondrial function in Parkinson's disease. *Brain* 115:333-342.

Maraganore, D. M., Farrer, M. J., Hardy, J. A., Lincoln, S. J., McDonnell, S. K., and Rocca, W. A. 1999. Case-control study of the ubiquitin carboxy-terminal hydrolase L1 gene in Parkinson's disease. *Neurology* 53:1858-1860.

Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A., and Mucke, L. 2000. Dopaminergic loss and inclusion body formation in alpha-synuclein mice: Implications for neurodegenerative disorders. *Science* 287:1265-1269.

Manning-Bog, A. B., McCormack, A. L., Li, J., Uversky, V. N., Fink, A. L., and Di Monte, D. A. 2002. The herbicide paraquat causes up-regulation and aggregation of α-synuclein in mice. *J. Biol. Chem.* 277:1641-1644.

Marttila, R. J., Lorentz, H., and Rinne, U. K. 1988. Oxygen toxicity protecting enzymes in Parkinson`s disease. *J. Neurol. Sci.* 86:321-331.

Maruyama, W., Nakahara, D., Ota, M., Takahashi, T., Takahashi, A., and Nagatsu, T. 1992. *N*-methylation of dopamine-derived 6,7-dihdroxy-1,2,3,4-tetrahydroisoquinoline, (*R*)-salsolinol, in rat brains: *In vivo* microdialysis study. *J. Neurochem.* 59:395-400.

Masayo, S. N., Wakako, M., Yoji, K., Isobe, K., Masashi, T., Makoto, N., and Toshihiko, O. 2003. An inhibitor of mitochondrial complex I, rotenone inactivates proteasome by oxidative modification and induces aggregation of oxidized proteins in SH-SY5Ycells. *J. Neurosci.Res*. 74:589-597.

Matsubara, K., Collins, M. A., Akane, A., Ikebuchi, J., Neafsey, E. J., and Kagawa, M. 1993. Potential bioactivated neurotoxicants, *N*-methylated beta-carbolinium ions, are present in human brain. *Brain Res.* 610:90-96.

Matsubara, K., Kobayashi, S., Kobayashi, Y., Yamashita, K., Koide, H., and Hatta, M. 1995. Beta-carbolinium cations endogenous MPP^+ analogs, in the lumbar cerebrospinal fluid of patients with Parkinson's disease. *Neurology* 45:2240-2245.

Matsubara, K., Gonda, T., Sawada, H., Uezono, T., Kobayashi, T., and Ohtaki, K. 1998. Endogenously occurring beta-carboline induces parkinsonism in nonprimate animals: A possible causative protoxin in idiopathic Parkinson's disease. *J. Neurochem.* 70:727-735.

Matsumine, H., Saito, M., Shimoda-Matsubayashi, S., Tanaka, H., Ishikawa, A., Nakagawa-Hattori, Y., Yokochi, M., Kobayashi, T., Igarashi, S., Takano, H., Sanpei, K., Koike, R., Mori, H., Kondo, T., Mizutani, Y., Schaffer, A. A., Yamamura, Y., Nakamura, S., Kuzuhara, S., Tsuji, S., and Mizuno, Y. 1997. Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2–27. *Am. J. Hum. Genet.* 60:588-596.

McCormack, A. L., Thiruchelvam, M., Manning-Bog, A. B., Thiffault, C., Langston, J. W., Cory-Slechta, D., and Di Monte, D. A. 2002. Environmental risk factors and Parkinson`s disease: Selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol. Dis*. 19:119-127.

McCormack, A. L., and Di Monte, D. 2003. Effects of L-dopa and other amino acids against paraquat-induced nigrostriatal degeneration. *J. Neurochem*. 85:82-86.

McEvoy, A. N., Murphy, E. A., Ponnio, T., Conneely, O. M., Bresnihan, B., FitzGerald, O., and Murphy, E. P. 2002. Activation of nuclear orphan receptor NURR1 transcription by NF-kappa B and cyclic adenosine 5′-monophosphate response element-binding protein in rheumatoid arthritis synovial tissue. *J. Immunol.* 168:2979-2987.

McGeer, P. L., Itagaki, S., and McGeer, E. G. 1988. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* 76:550-557.

McGrew, D. M., Irwin, I., and Langston, J. W. 2000. Ethylenebisdithiocarbamate enhances MPTP-induced striatal dopamine depletion in mice. *Neurotoxicology* 21:309-312.

McLennan, H. R., and Degli Esposti, M. 2000. The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species. *J. Bioenerg. Biomembr*. 32:153-162.

McNaught, K. S., and Jenner, P. 2001. Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci. Lett*. 297:191-194.

McNaught, K. S., Olanow, C. W., Halliwell, B., Isacson, O., and Jenner, P. 2001. Failure of the ubiquitin-proteasome system in Parkinson's disease. *Nat. Rev. Neurosci*. 2:589-594.

McNaught, K. S., Belizaire, R., Jenner, P., Olanow, C. W., and Isacson, O. 2002. Selective loss of 20S proteasome alpha-subunits in the substantia nigra pars compacta in Parkinson's disease. *Neurosci. Lett*. 326:155-158.

McNaught, K. S., Belizaire, R., Isacson, O., Jenner, P., and Olanow, C. W. 2003. Altered proteasomal function in sporadic Parkinson's disease. *Exp. Neurol*. 179:38-46.

McNaught, K. S., Perl, D. P., Brownell, A. L., and Olanow, C. W. 2004. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann. Neurol*. 56:149-162.

Meco, G., Bonifati, V., Vanacore, N., and Fabrizio, E. 1994. Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). *Scand. J. Work Environ. Health* 20:301-305.

Mecocci, P., MacGarvey, U., Kaufman, A. E., Koontz, D., Shoffner, J. M., Wallace, D. C., and Beal, M. F. 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol*. 34:609-616.

Meister, A., and Anderson, M. E. 1983. Glutathione. *Ann. Rev. Biochem.* 52:711-760.

Melchiorri, D., Del Duca, C., Piccirilli, S., Trombetta, G., Bagetta, G., and Nistico, G. 1998. Intrahippocampal injection of paraquat produces apoptotic cell death which is prevented by the lazaroid U74389G, in rats. *Life Sci*. 62:1927-1932.

Mellick, G. D., Buchanan, D. D., Silburn, P. A., Chan, D. K., Le Couteur, D. G., Law, L. K., Woo, J., and Pang, C. P. 2000. The monoamine oxidase B gene GT repeat polymorphism and Parkinson's disease in a Chinese population. *J. Neurol.* 247:52-55.

Menegon, A., Board, P. G., Blackburn, A. C., Mellick, G. D., and Le Couteur, D. G. 1998. Parkinson's disease, pesticides, and glutathione transferase polymorphisms. *Lancet* 352:1344-1346.

Miller, G. W., Kirby, M. L., Levey, A. I., and Bloomquist, J. R. 1999. Heptachlor alters expression and function of dopamine transporters. *Neurotoxicology* 20:631-637.

Mitsumoto, A., and Nakagawa, Y. 2001. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radic. Res.* 35:885-893.

Mizuno, Y., Yoshino, H., Ikebe, S., Hattori, N., Kobayashi, T., Shimoda-Matsubayashi, S., Matsumine, H., and Kondo, T. 1998. Mitochondrial dysfunction in Parkinson disease. *Ann. Neurol*. 44:S99-109.

Mochizuki, H., Goto, K., Mori, H., and Mizuno, Y. 1996. Histochemical detection of apoptosis in Parkinson`s disease. *J. Neurol. Sci*. 137:120-123.

Mochizuki, H., Mori, H., and Mizuno, Y. 1997. Apoptosis in neurodegenerative disorders. *J. Neural. Transm. Suppl*. 50:125-40.

Mody, I. and MacDonald, J. F. 1995. NMDA receptor-dependent excitotoxicity: The role of intracellular Ca2+ release. *Trends Pharmacol. Sci*. 6:356-359.

Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., and Nagatsu, T. 1999. Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neurosci. Lett*. 270:45-48.

Moldeus, P., Hogberg, J., and Orrenius, S. 1978. Isolation and use of liver cells. *Methods Enzymol*. 52:60-71.

Molina-Jimenez, M. F., Sanchez-Reus, M. I., and Benedi, J. 2003. Effects of fraxetin and myricetin on rotenone-induced cytotoxicity in SH-SY5Ycells: Comparison with N-acetylcysteine. *Eur. J. Pharmcol*. 472:81-87.

Moll, U. M., and Zaika, A. 2001. Nuclear and mitochondrial apoptotic pathways of p53. *FEBS Lett*. 493:65-69.

Momose, Y., Murata, M., Kobayashi, K., Tachikawa, M., Nakabayashi, Y., Kanazawa, I. and Toda, T. 2002. Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. *Ann. Neurol.* 51:133-136.

Morato, G. S., Lemos, T., and Takahashi, R. N. 1989. Acute exposure to maneb alters some behavioral functions in the mouse. *Neurotoxicol. Teratol*. 11:421-425.

Moriya, R., Uehara, T., and Nomura, Y. 2000. Mechanism of nitrite oxide-induced apoptosis in human neuroblastoma SH-SY5Y cells. *FEBS Lett*. 484:253-260.

Morris, C. M., O'Brien, K. K., Gibson, A. M., Hardy, J. A., and Singleton, A. B. 2003. Polymorphism in the human DJ-1 gene is not associated with sporadic dementia with Lewy bodies or Parkinson's disease. *Neurosci. Lett.* 352:151-153.

Nagakubo, D., Taira, T., Kitaura, H., Ikeda, M., Tamai, K., Iguchi-Ariga, S. M., and Ariga, H. 1997. DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. *Biochem. Biophys. Res. Commun.* 231:509-513.

Nagatsu, T., and Yoshida, M. 1988. An endogenous substance of the brain, tetrahydroisoquinoline, produces Parkinsonism in primates with decreased dopamine, tyrosine hydroxylase and biopterin in the nigrostriatal regions. *Neurosci. Lett.* 87:178-182.

Nagatsu, T., Mogi, M., Ichinose, H., and Togari, A. 2000. Cytokines in Parkinson's disease. *J. Neural. Transm. Suppl*. 58:143-151.

Nakamura, K., Bindokas, V. P., Marks, J. D., Wright, D. A., Frim, D. M., Miller, R. J., and Kang, U. J. 2000. The selective toxicity of 1-methyl-4-phenylpyridinium to dopaminergic neurons: The role of mitochondrial complex I and reactive oxygen species (ROS) revisited. *Mol.Pharmacol*. 58:271-278.

Nakaso, K., Yano, H., Fukuhara, Y., Takeshima, T., Wada-Isoe, K., and Nakashima, K. 2003. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. *FEBS Lett*. 546:181-184.

Nakaso, K., Yoshimoto, Y., Yano, H., Takeshima, T., and Nakashima, K. 2004. p53-mediated mitochondrial dysfunction by proteasome inhibition in dopaminergic SH-SY5Y cells. *Neurosci. Lett*. 354:213-216.

Newhouse, K., Hsuan, S. L., Chang, S. H., Cai, B., Wang, and Y., Xia, Z. 2004. Rotenone-induced apoptosis is mediated by p38 and JNK MAP kinases in human dopaminergic SH-SY5Y cells. *Toxicol. Sci*. 79:137-146.

Nicholls, D. G., 2002. Mitochondrial function and dysfunction in the cell: Its relevance to aging and aging-related disease. *Int. J. Biochem. Cell. Biol*. 34:1372-1381.

Nicholls, D. G. and Budd, S. L. 1998. Neuronal excitotoxicity: The role of mitochondria. *Biofactors* 8:287–299.

Niwa, T., Takeda, N., Kaneda, N., Hashizume, Y., and Nagatsu, T., 1987. Presence of tetrahydroisoquinoline and 2-methyl-tetrahydroquinoline in parkinsonian and normal human brains. *Biochem. Biophys. Res. Commun.* 144:1084-1089.

Ohtsuka, T., Ryu, H., Minamishima, Y. A., Macip, S., Sagara, J., Nakayama, K. I., Aaronson, S. A., and Lee, S. W. 2004. ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. *Nat. Cell Biol*. 6:121-128.

Olanow, C. W. 1993. A radical hypothesis for neurodegeneration. *Trends Neurosci*. 16:439-444

Olanow, C. W., and Tatton, W. G. 1999. Etiology and pathogenesis of Parkinson`s disease. *Annu. Rev. Neurosci.* 22:123-144.

Oliveira, S. A., Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Koller, W. C., Lyons, K. E., Pahwa, R., Stern, M. B., Hiner, B. C., Jankovic, J., Ondo, W. G., Allen, F. H., Scott, B. L., Goetz, C. G., Small, G. W., Mastaglia, F. W., Stajich, J.M., Zhang, F., Booze, M. W., Reaves, J. A., Middleton, L. T., Haines, J. L., Pericak-Vance, M. A., Vance, J. M., and Martin, E. R. 2003. Association study of Parkin gene polymorphisms with idiopathic Parkinson disease. *Arch. Neurol.* 60:975-980.

Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S., and Stadtman, E. R. 1987. Age-related changes in oxidized proteins. *J. Biol. Chem*. 262:5488-5491.

Oliver, K. R., Brennan, P., and Fazakerley, J. K. 1997. Specific infection and destruction of dopaminergic neurons in the substantia nigra by Theiler's virus. *J. Virol*. 71:6179-6182.

Olney, J. W., Collins, R. C., Sloviter, R. S. 1986. Excitotoxic mechanisms of epileptic brain damage. *Adv. Neurol*. 44:857-77.

Onyon, L. J., and Volans, G. N. 1987. The epidemiology and prevention of paraquat poisoning. *Human Toxicol*. 6:19-26.

Ostrerova, N., Petrucelli, L., Farrer, M., Mehta, N., Choi, P., Hardy, J., and Wolozin, B. 1999. Alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. *J. Neurosci.* 19:5782-5791.

Oubrahim, H., Stadtman, E. R., and Chock, P. B. 2001. Mitochondria play no roles in Mn(II)-induced apoptosis in Hela cells. *Proc. Natl. Acad. Sci. USA* 98:9505-9510.

Pahlman, S., Odelstad, L., Larsson, E., Grotte, G., and Nilsson, K. 1981. Phenotypic changes of human neuroblastoma cells in culture induced by 12-O-tetradecanoyl-phorbol-13-acetate. *Int. J. Cancer* 28:583-589.

Pal, P. K., Samii, A., and Calne, D. B., 1999. Manganese neurotoxicity: A review of clinical features. *Neurotoxicology* 20:227–238.

Pasquini, L. A., Marta, C. B., Adamo, A. M., Pasquini, J. M., and Soto, E. F. 2000. Relationship between the ubiquitin-dependent pathway and apoptosis in different cells of the central nervous system: Effect of thyroid hormones. *Neurochem. Res*. 25:627-635.

Pastor, P., Munoz, E., Ezquerra, M., Obach, V., Marti, M. J., Valldeoriola, F., Tolosa, E., and Oliva, R. 2001. Analysis of the coding and the 5′ flanking regions of the alpha-synuclein gene in patients with Parkinson's disease. *Mov. Disord.* 16:1115-1119.

Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., and Taylor, J. B. 1994. Human glutathione *S*-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem. J.* 300:271-276.

Perez, R.G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., and Zigmond, M. J. 2002. A role or alpha-synuclein in the regulation of dopamine biosynthesis. *J. Neurosci.* 22:3090-3099.

Perez-Polo, J. R., Werbach-Perez, K., and Tiffany-Castiglioni, E. 1979. A human clonal cell line model of differentiating neurons. *Dev. Biol*. 71:341-355.

Perrin, R. J., Woods, W. S., Clayton, D. F., and George, J. M. 2000. Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids: Structural analysis using site-directed mutagenesis. *J. Biol. Chem.* 275:34393-34398.

Pearce, R. K. B., Owen, A., Daniel, S., Jenner, P., and Marsden, C. D. 1997. Alterations in the distribution of glutathione in the substantia nigra in Parkinson`s disease. *J. Neural Transm*. 104:661-677.

Plante-Bordeneuve, V., Taussig, D., Thomas, F., Ziegler, M., and Said, G. 1995. A clinical and genetic study of familial cases of Parkinson's disease. *J. Neurol. Sci.*133:164-172.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045–2047.

Presgraves, S. P., Ahmed, T., Borwege, S., and Joyce, J. N. 2004. Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. *Neurotox. Res*. 5:579-598.

Priyadarshi, A., Khuder, S. A., Schaub, E. A., and Shrivastava, S. 2000. A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology* 21:435-40.

Priyadarshi, A., Khuder, S., Schaub, E. A., and Priyadarshi, S. S. 2001. Environmental risk factors and Parkinson`s disease: A metaanalysis. *Environ. Res.* 86:122-127.

Puttonen, K. A., Manakova, S., Raasmaja, A., and Mannisto, P. T. 2003. Increased p53 levels without caspase-3 activity and change of cell viability in 6-hydroxydopamine-treated CV1-P cells. Cell Biol. Toxicol. 19:177-187.

Qiu, J. H., Asai, A., Chi, S., Saito, N., Hamada, H., and Kirino, T. 2000. Proteasome inhibitors induce cytochrome c-caspase-3-like protease-mediated apoptosis in cultured cortical neurons. *J. Neurosci*. 20:259-265.

Rahbar, A., Kempkes, M., Muller, T., Reich, S., Welter, F. L., Meves, S., Przuntek, H., Bolt, H. M., and Kuhn, W. 2000. Glutathione *S*-transferase polymorphism in Parkinson's disease. *J. Neural Transm.* 107;331-334.

Reinheckel, T., Sitte, N., Ullrich, O., Kuckelkorn, U., Davies, K. J., and Grune, T. 1998. Comparative resistance of the 20S and 26S proteasome to oxidative stress. *Biochem. J*. 335:637-642.

Rideout, H. J., Larsen, K. E., Sulzer, D., and Stefanis, L. 2001. Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. *J. Neurochem*. 78:899-908.

Rideout, H.J., and Stefanis, L. 2002. Proteasomal inhibition-induced inclusion formation and death in cortical neurons require transcription and ubiquitination. *Mol. Cell Neurosci*. 21:223-238.

Ridge, J., Terle, D. A., Dragunsky, E., and Levenbook, I. 1996. Effects of gamma-IFN and NGF on subpopulations in a human neuroblastoma cell line: Flow cytometric and morphological analysis. In Vitro Cell Dev. Biol. Anim. 32:238-248.

Riedl, A. G., Watts, P. M., Jenner, P., and Marsden, C. D. 1998. P450 enzymes and Parkinson's disease: The story so far. *Mov. Disord*. 13:212-220.

Ritz, B., and Yu, F. 2000. Parkinson's disease mortality and pesticide exposure in California 1984-1994. *Int. J. Epidemiol*. 29:323-329.

Rizzu, P., Hinkle, D. A., Zhukareva, V., Bonifati, V., Severijnen, L. A., Martinez, D., Ravid, R., Kamphorst, W., Eberwine, J. H., Lee, V. M., Trojanowski, J. Q., and Heutink, P. 2004. DJ-1 colocalizes with tau inclusions: A link between Parkinsonism and dementia. *Ann. Neurol.* 55:113-118.

Roberts, T. R., Dyson, J. S., and Lane, M. C. 2002. Deactivation of the biological activity of paraquat in the soil environment: A review of long-term environmental fate. *J. Agric. Food Chem*. 50:3623-3631.

Rodriguez, M. C., Obeso, J. A., Olanow, C. W. 1998. Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: A target for neuroprotection. *Ann. Neurol*. 44:S175-S188.

Roos-Mattjus, P., and Sistonen, L. 2004. The ubiquitin-proteasome pathway. *Ann. Med*. 36:285-295.

Rose, M. S., Smith, L. L., and Wyatt, I. 1974. Evidence for energy-dependent accumulation of paraquat into rat lung. *Nature* 252:314-315.

Rose, M. S., Lock, E. A., Smith, L. L., and Wyatt, I. 1976. Paraquat accumulation: Tissue and species specificity. *Biochem. Pharmacol*. 25:419-423.

Ross, C. A., and Pickart, C. M. 2004. The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases. *Trends Cell Biol*. 14:703-711.

Ross, R. A., Spengler, B. A., and Biedler, J. L. 1983. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J. Natl. Cancer Inst*. 71:741-747.

Rothman, S. M. and Olney, J. W. 1986. Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann. Neurol*. 19:105-111.

Sanchez-Ramos, J., Facca, A., Basit, A., and Song, S. 1998. Toxicity of dieldrin for dopaminergic neurons in mesencephalic cultures. *Exp. Neurol*. 150:263-271.

Sanchez-Ramos, J. R., Hefti, F., Weiner W. J. 1987. Paraquat and Parkinson`s disease. *Neurology* 37:728.

Sang, C., Kobayashi, Y., Du, J., Katsumo, M., Adachi, H., Doyu, M., and Sobue, G. 2002. c-Jun N-terminal kinase pathway mediates Lactacystin-induced cell death in a neuronal differentiated Neuro2a cell line. *Brain Res. Mol. Brain Res*. 108:7-17.

Sardar, A. M., Czudek, C., and Reynolds, G. P. 1996. Dopamine deficits in the brain: The neurochemical basis of parkinsonian symptoms in AIDS. *Neuroreport* 7:910-912.

Satoh, J., and Kuroda, Y. 2001. A polymorphic variation of serine to tyrosine at codon 18 in the ubiquitin C-terminal hydrolase-L1 gene is associated with a reduced risk of sporadic Parkinson's disease in a Japanese population. *J. Neurol. Sci.* 189:113-117.

Saucedo-Cardenas, O., Quintana- Hau, J. D., Le, W. D., Smidt, M. P., Cox, J. J., De Mayo, F., Burbach, J. P. H., and Conneely, O. M. 1998. Nurr1 is essential for the induction of the dopaminergic phenotype and survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc. Natl Acad. Sci. USA*, 95: 4013-4018.

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. 2004. Proteasome mediates dopaminergic neuronal degeneration, and its inhibition causes alpha-synuclein inclusions. *J. Biol. Chem*. 279:10710-10719.

Sayre, L. M., Wang, F. J., Arora, P. K., Riachi, N. J., Harik, S. I., and Hoppel, C. L. 1991. Dopaminergic neurotoxicity *in vivo* and inhibition of mitochondrial

respiration *in vitro* by possible endogenous pyridinium-like substances. *J. Neurochem.* 57:2106-2115.

Schapira, A. H. 1994. Evidence for mitochondrial dysfunction in Parkinson's disease--a critical appraisal. *Mov. Disord*. 9:125-138.

Schapira, A. H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., Marsden, C. D. 1990. Mitochondrial complex I deficiency in Parkinson's disease. *J. Neurochem*. 54:823-827.

Schinder, A. F., Olson, E.C., Spitzer, N.C., and Montal, M. 1996. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. *J. Neurosci.* 16:6125-6133.

Schipper, H. M., Liberman, A., and Stopa, E. G. 1998. Neural heme oxygenase-1 expression in idiopathic Parkinson`s disease. *Exp. Neurol*. 150:60-68.

Schlossmacher, M. J., Frosch, M. P., Gai, W. P., Medina, M., Sharma, N., Forno, L., Ochiishi, T., Shimura, H., Sharon, R., Hattori, N., Langston, J. W., Mizuno, Y., Hyman, B. T., Selkoe, D. J., and Kosik, K. S. 2002. Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. *Am. J. Pathol.* 160:1655-1667.

Schmuck, G., Röhrdanz, E., Tran-thi, G. H., Kahl, R., and Schlüter, G. 2002. Oxidative stress in rat cortical neurons and astrocytes induced by paraquat *in vitro*. *Neurotoxicol. Res.* 4:1-13.

Schulz, J. B., Lindenau, J., Seyfried, J., and Dichgans, J. 2000. Glutathione, oxidative stress, and neurodegeneration. *Eur. J. Biochem.* 267:4904-4911.

Seegal, R. F., Bush, B., and Brosch, K. O. 1985. Polychlorinated biphenyls induce regional changes in brain norepinephrine concentrations in adult rats. *Neurotoxicology* 6:13-23.

Seegal, R. F., Brosch, K., Bush, B., Ritz, M., and Shain, W. 1989. Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. *Neurotoxicology*. 10:757-764.

Seidegard, J., and Pero, R. W. 1988. The genetic variation and the expression of human glutathione transferase mu. *Klin. Wochenschr.* 66:125-126.

Seidler, A., Hellenbrand, W., Robra, B. P., Viergge, P., Nischan, P., and Jooerg, J. 1996. Possible environmental, occupational, and other etiologic factors for Parkinson’s disease: A case-control study in Germany. *Neurology* 46:1275–1284.

Semchuck, K. M., Love, E. J., and Lee, R. G. 1993. Parkinson's disease: A test of the multifactorial etiologic hypothesis. *Neurology* 43: 1173-1180.

Senthilkumar, K., Kannan, K., Subramanian, A., and Tanabe, S. 2001. Accumulation of organochlorine pesticides and polychlorinated biphenyls in sediments, aquatic organisms, birds, bird eggs and bat collected from south India. *Environ. Sci. Pollut. Res. Int*. 8:35-47.

Seyfried, J., Soldner, F., Schulz, J. B., Klockgether, T., Kovar, K. A. and Wüllner, U. 1999. Differential effects of L-buthionine sulfoximine and ethacrynic acid on glutathione levels and mitochondrial function in PC12 cells. *Neurosci. Lett*. 264:1-4.

Shamoto-Nagai, M., Maruyama, W., Kato, Y., Isobe, K., Tanaka, M., Naoi, M., and Osawa, T. 2003. An inhibitor of mitochondrial complex I, rotenone, inactivates proteasome by oxidative modification and induces aggregation of oxidized proteins in SH-SY5Y cells. J. Neurosci. Res. 74:589-597.

Sharma, R. P., Winn, D. S., and Low, J. B. 1976. Toxic, neurochemical and behavioral effects of dieldrin exposure in mallard ducks. *Arch. Environ. Contam. Toxicol*. 5:43-53.

Shaul, U., Ben-Shachar, D., Karry, R., Klein, E. 2003. Modulation of frequency and duration of repetitive magnetic stimulation affects catecholamine levels and tyrosine hydroxylase activity in human neuroblastoma cells: Implication for the antidepressant effect of rTMS. *Int. J. neuropsychopharmacol*. 6:233-241.

Shavali, S., Carlson, E. C., Swinscoe, J. C., and Ebadi, M. 2004. 1-benzyl-1,2,3,4,-tetrahydroisoquinoline, a Parkinsonism-inducing endogenous toxin, increases α-synuclein expression and causes nuclear damage in human dopaminergic cells. *J. Neurosci. Res*. 76:563-572.

Sherer, T. B., Kim, J. H., Betarbet, R., and Greenamyre, J. T. 2003. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp. Neurol*. 179:9-16.

Shimizu, K., Ohtaki, K., Matsubara, K., Aoyama, K., Uezono, T., Saito, O., Suno, M., Ogawa, K., Hayase, N., Kimura, K., and Shiono, H. 2001. Carrier-mediated processes in blood--brain barrier penetration and neural uptake of paraquat. *Brain Res*. 906:135-142.

Shimizu, K., Matsubara, K., Ohtaki, K., and Shiono, H. 2003. Paraquat leads to dopaminergic neural vulnerability in organotypic midbrain culture. *Neurosci.Res*. 46:523-532.

Shi, Q., and Tao, E. 2003. An Ile93Met substitution in the UCH-L1 gene is not a disease-causing mutation for idiopathic Parkinson's disease. *Chin. Med. J.* 116:312–313.

Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S., and Selkoe, D. J. 2001.Ubiquitination of a new form of alpha-synuclein by parkin from human brain: Implications for Parkinson's disease. *Science* 293:263-269.

Shrieve, D. C., Bump, E. A., and Rice, G. C. 1988. Heterogeneity of cellular glutathione among cells derived from a murine fibrosarcoma or a human renal cell carcinoma detected by flow cytometric analysis. *J. Biol. Chem.* 263:14107-14114.

Sian, J., Dexter, D. T., Lees, A. J., Daniel, S., Jenner, P., and Mardsen, C. D. 1994. Glutathione-related enzymes in brain in Parkinson`s disease. *Ann. Neurol.* 36:356-361.

Simas, J. P., and Fazakerley, J. K. 1996. The course of disease and persistence of virus in the central nervous system varies between individual CBA mice infected with the BeAn strain of Theiler's murine encephalomyelitis virus. *J. Gen. Virol*. 77:2701-2711.

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., and Gwinn-Hardy, K. 2003. {alpha}-Synuclein Locus triplication causes Parkinson's disease. *Science* 302:841.

Sinha, B. K., Singh, Y. and Krishna, G. 1986. Formation of superoxide and hydroxyl radicals from 1-methyl-4 phenylpyridium ion (MPP+): Reductive activation by NADPH cytochrome P450 reductase. *Biochem. Biophys. Res. Commun.* 135:83-88.

Škárka, L., and Oštadál, B. 2002. Mitochondrial membrane potential in cardiac myocytes. *Physiol Res.* 51:425-434.

Skulachev, V. P. 1996. Why are mitochondria involved in apoptosis? *FEBS Lett.* 397:7-10.

Smith, P., and Heath, D. 1975. The pathology of the lung in paraquat poisoning. *J. Clin. Pathol. Suppl*. 9:81-93.

Snyder, H., Mensah, K., Theisler, C., Lee, J., Matouschek, A., and Wolozin, B. 2003. Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *J. Biol. Chem*. 278:11753-11759.

Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G. P., and Hebenstreit, G. 1988. Increased iron(III) and total iron content in post mortem substantia nigra of parkinsonian brain. *J. Neural. Transm.* 74:199-205.

Solenski, N. J., Kostecki, V. K., Docey, S., Periasamy, A. 2003. Nitric-oxide-induced depolarization of neuronal mitochondria: Implications for neuronal cell death. *Mol. Cell Neurosci*. 24:1151-1169.

Soleo, L., Defazio, G., Scarselli, R., Zefferino, R., Livrea, P., and Foa, V. 1996. Toxicity of fungicides containing ethylene-bis-dithiocarbamate in serumless dissociated mesencephalic-striatal primary coculture. *Arch. Toxicol*. 70:678-682.

Son, J. H., Chun, H. S., Joh, T. H., Cho, S., Conti, B., and Lee, J. W. 1999. Neuroprotection and neuronal differentiation studies using substantia nigra dopaminergic cells derived from transgenic mouse embryos. *J. Neurosci.* 19:10-20.

Spiewak, R. 2001. Pesticides as a cause of occupational skin diseases in farmers. *Ann. Agric. Environ. Med.* 8:1-5.

Spillantini, M. G., Crowther, R. A., Jakes, R., Cairns, N. J., Lantos, P. L., and Goedert, M. 1998.Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neurosci. Lett.* 251:205-208.

Steventon, G. B., Sturman, S., Waring, R. H., and Williams, A. C. 2001. A review of xenobiotic metabolism enzymes in Parkinson's disease and motor neuron disease. *Drug Metabol. Drug Interact*. 18:79-98.

Stokes, A. H., Hastings, T. G., Vrana, K. E. 1999. Cytotoxic and genotoxic potential of dopamine. *J. Neurosci. Res*. 55:659-665.

Storch, A., Kaftan, A., Burkhardt, K., and Schwarz, J. 2000. 6-Hydroxydopamine toxicity towards human SH-SY5Y dopaminergic neuroblastoma cells: Independent of mitochondrial energy metabolism. *J. Neural Transm*. 107:281-293.

Suntres, Z. E. 2002. Role of antioxidants in paraquat toxicity. *Toxicology* 180:65-77.

Suwalsky, M., Benites, M., Villena, F., Aguilar, F., and Sotomayor, C. P. 1997. Interaction of the organochlorine pesticide dieldrin with phospholipid bilayers. *Z. Naturforsch*. 52:450-458.

Suzuki, K., Mizuno, Y., and Yoshida, M. 1990. Inhibition of mitochondrial respiration by 1,2,3,4-tetrahydroisoquinoline-like endogenous alkaloids in mouse brain. *Neurochem. Res.* 15:705-710.

Swerdlow, R. H., Parks, J. K., Miller, S. W., Tuttle, J. B., Trimmer, P. A., Sheehan, J. P., Bennett, J. P., Davis, R. E., and Parker, W. D. 1996. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann. Neurol*. 40:663-671.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K., and Ariga, H. 2004. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep*. 5:213-218.

Takahashi, K., Taira, T., Niki, T., Seino, C., Iguchi-Ariga, S. M., and Ariga, H. 2001. DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. *J. Biol. Chem.* 276:37556-37563.

Takahashi, M., and Yamada, T. 2001. A possible role of influenza A virus infection for Parkinson`s disease. *Adv. Neurol.* 86:91-104.

Takahashi, R. N., Rogerio, R., Zanin, M. 1989. Maneb enhances MPTP neurotoxicity in mice. *Res. Commun. Chem. Pathol. Pharmacol*. 66:167-170.

Takahashi, T., Deng, Y., Maruyama, W., Dostert, P., Kawai, M., and Naoi, M. 1994. Uptake of neurotoxin-candidate, (R)-1,2-dimethyl –6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline into human dopaminergic neuroblastoma SH-SY5Y cells by dopamine transport system. *J. Neural. Transm*. 98:107-118.

Takeda, A. 2003. Manganese action in brain function. *Brain Res. Brain Res. Rev.* 41:79-87.

Tamagno, E., Parola, M., Guglielmotto, M., Santoro, G., Bardini, P., Marra, L., Tabaton, M., and Danni, O. 2003. Multiple signaling events in amyloid beta-induced, oxidative stress-dependent neuronal apoptosis. *Free Radic. Biol*. *Med*. 35:45-58.

Tan, E. K., Chung, H., Zhao, Y., Shen, H., Chandran, V. R., Tan, C., Teoh, M. L., Yih, Y., Pavanni, R., and Wong, M. C. 2003. Genetic analysis of Nurr1 haplotypes in Parkinson's disease. *Neurosci. Lett.* 347:139-142.

Tang, Y., Donnelly, K. C., Tiffany-Castiglioni, E., and Mumtaz, M. M. 2003. Neurotoxicity of polycyclic aromatic hydrocarbons and simple chemical mixtures. *J. Toxicol. Environ. Health* A 66:919-940.

Tanner, C. M. 2003. Is the cause of Parkinson's disease environmental or hereditary? Evidence from twin studies. *Adv Neurol*. 91:133-42.

Tanner, C. M., Ottoman, R., Goldman, S. M., Elenberg, J., Chan, P., Mayeux, R., and Langston, J. W. 1999. Parkinson disease in twins. *J. Am. Med. Assoc.* 281:341-346.

Tanner, C. M., and Yoav, B. S. 1999. Epidemiology of Parkinson`s disease. *Adv. Neurol*. 80:153-9.

Tatton, W. G., Chalmers-Redman, R., Brown, D., and Tatton, N. 2003. Apoptosis in Parkinson's disease: Signals for neuronal degradation. *Ann. Neurol*. *Suppl* 3:S61-70.

Tatton, N. A., Maclean-Fraser, A., Tatton, W. G., Perl, D. P., and Olanow, C. W. 1998. A fluorescent double-labelling method to detect and confirm apoptotic nuclei in Parkinson`s disease. *Ann. Neurol*. 44:S142-S148.

Tatton, N. A. 2000. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp. Neurol*. 166:29-43.

Tawara, T., Fukushima, T., Hojo, N., Isobe, A., Shiwaku, K., Setogawa, T., and Yamane Y. 1996. Effects of paraquat on mitochondrial electron transport system and catecholamine contents in rat brain. *Arch. Toxicol*. 70:585-589.

Teismann, P., Tieu, K., Choi, D. K., Wu, D. C., Naini, A., Hunot, S., Vila, M., Jackson-Lewis, V., and Przedborski, S. 2003. Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc. Natl. Acad. Sci*. USA 100:5473-5478.

Teismann, P., and Schulz, J. B. 2004. Cellular pathology of Parkinson's disease: Astrocytes, microglia and inflammation. *Cell Tissue Res*. 318:149-161.

Theron, M., Guerrero, F., and Sebert, P. 2000. Improvement in the efficiency of oxidative phosphorylation in the freshwater eel acclimated to 10.1 MPa hydrostatic pressure. *J. Exp. Biol*. 203:3019-3023.

Thibault, C., Lai, C., Wilke, N., Duong, B., Olive, M. F., Rahman, S., Dong, H., Hodge, C. W., Lockhart, D. J., and Miles, M. F. 2000. Expression profiling of neural

cells reveals specific patterns of ethanol-responsive gene expression. *Mol. Pharmacol*. 58:1593-1600.

Thiffault, C., Langston, J. W., and Di Monte, D. A. 2000. Increased striatal dopamine turnover following acute administration of rotenone to mice. *Brain Res*. 885:283-288.

Thiruchelvam, M., Richfield, E. K., Goodman, B. M., Baggs, R. B., and Cory-Slechta, D. A. 2002. Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype. *Neurotoxicology* 23:621-633.

Tilson, H. A., and Kodavanti, P. R. 1997. The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology* 19:517-525.

Tirmenstein, M. A., Hu, C. X., Scicchitano, M. S., Narayanan, P. K., McFarland, D. C., Thomas, H. C., and Schwartz, L. W. 2005. Effects of 6-hydroxydopamine on mitochondrial function and glutathione status in SH-SY5Y human neuroblastoma cells. Toxicol. In Vitro 19:471-479.

Toda, T., Momose, Y., Murata, M., Tamiya, G., Yamamoto, M., Hattori, N., and Inoko, H. 2003. Toward identification of susceptibility genes for sporadic Parkinson's disease. *J. Neurol. Suppl.* 250:40-43.

Tomac, A., Widenfalk, J., Lin, L. F., Kohno, T., Ebendal, T., Hoffer, B. J., and Olson, L. 1995. Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc. Natl. Acad. Sci.* USA 92:8274-8278.

Tooyama, I., McGeer, E. G., Kawamata, T., Kimura, H., McGeer, P. L. 1994. Retention of basic fibroblast growth factor immunoreactivity in dopaminergic neurons of the substantia nigra during normal aging in humans contrasts with loss in Parkinson's disease. *Brain Res*. 656:165-1688.

Tretter, L., Sipos, I., and Adam-Vizi, V. 2004. Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease. 2004. *Neurochem. Res*. 29:569-577.

Tsang, F., and Soong, T. W. 2003. Interactions between environmental and genetic factors in the pathophysiology of Parkinson's disease. *IUBMB Life*. 55:323-327.

Tsukamoto, M., Tampo, Y., Sawada, M., and Yonaha, M. 2002. Paraquat-induced oxidative stress and dysfunction of the glutathione redox cycle in pulmonary microvascular endothelial cells. *Toxicol. Appl. Pharmacol*. 178:82-92.

Turski, L., Bressler, K., Rettig, K. J., Loschmann, P. A., and Wachtel, H. 1991. Protection of substantia nigra from MPP^+ neurotoxicity by *N*-methyl-aspartate antagonists. *Nature* 349: 414–418.

Uversky, V. N., Li, J., and Fink, A. L. 2001. Pesticides directly accelerate the rate of alpha-synuclein fibril formation: A possible factor in Parkinson's disease. *FEBS Lett*. 500:105-108.

Uversky VN, Li J, Bower K, Fink AL. 2002. Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: Implications for Parkinson's disease. *Neurotoxicology* 23:527-536.

Vale, J. A., Meredith, T. J., and Buckley, B. M. 1987. Paraquat poisoning: Clinical features and immediate general management. *Hum. Toxicol*. 6:41-47.

Van Duijn, C. M., Dekker, M. C., Bonifati, V., Galjaard, R. J., Houwing-Duistermaat, J. J., Snijders, P. J., Testers, L., Breedveld, G. J., Horstink, M., Sandkuijl, L. A., van Swieten, J. C., Oostra, B. A., and Heutink, P. 2001. Park7, a novel locus for autosomal recessive early-onset Parkinsonism, on chromosome 1p36. *Am. J. Hum. Genet.* 69:629-634.

Vekrellis, K., Rideout, H. J., and Stefanis, L. 2004. Neurobiology of alpha-synuclein. *Mol. Neurobiol*. 30:1-21.

Vigouroux, S., Briand, M., and Briand, Y. 2004. Linkage between the proteasome pathway and neurodegenerative diseases and aging. *Mol. Neurobiol*. 30:201-221.

Vincente, J. A. F., Peixoto, F., Lopes, M. L., and Madeira, V. M. 2001. Differential sensitivities of plant and animal mitochondria to the herbicide paraquat. *J. Biochem. Mol. Toxicol.*15:322-330.

Wallen, A., and Perlmann, T. 2003. Transcriptional control of dopamine neuron development. *Ann. N. Y. Acad. Sci*. 991:48-60.

Wang, J., Tung, Y. C., Wang, Y., Li, X. T., Iqbal, K., and Grundke-Iqbal, I. 2001. Hyperphosphorylation and accumulation of neurofilament proteins in Alzheimer disease brain and in okadaic acid-treated SY5Y cells. *FEBS Lett.* 507:81-87.

Wang, X., Qin, Z. H., Leng, Y., Wang, Y., Jin, X., Chase, T. N., and Bennett, M. C. 2002. Prostaglandin A1 inhibits rotenone-induced apoptosis in SH-SY5Ycells. *J. Neurochem*. 83:1094-1102.

Wang, X. J., and Xu, J. X. 2005. Possible involvement of Ca2+ signaling in rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells. *Neurosci. Lett*. 376:127-132.

Wanpen, S., Govitrapong, P., Shavali, S., Sangchot, P., and Ebadi, M. 2004. Salsolinol, a dopamine-derived tetrahydroisoquinoline, induces cell death by causing oxidative stress in dopaminergic SH-SY5Y cells, and the said effect is attenuated by metallothionein. *Brain Res*. 1005:67-76.

Warner, T. T., and Schapira, A. H. 1998. The role of the alpha-synuclein gene mutation in patients with sporadic Parkinson's disease in the United Kingdom. *J. Neurol. Neurosurg. Psychiatry* 65:378–379.

Watabe, M., and Nakaki, T. 2004. Rotenone induces apoptosis via activation of bad in human dopaminergic SH-SY5Y cells. *J. Pharmacol. Exp. Ther*. 311:948-953.

Weinshilboum, R. M. 1983. Biochemical genetics of catecholamines in humans. *Mayo Clin. Proc.* 58:319-330.

Wilber, K. M., Bernheim, F., and Shapiro, O. W. 1949. The thiobarbituric acid test for the oxidation of unsaturated fatty acids by various agents. *Arch. Biochem. Biophys*. 24:305-313.

Wilkinson, K. D., Lee, K. M., Deshpande, S., Duerksen-Hughes, P., Boss, J. M., and Pohl, J. 1989. The neuron-specific protein PGP 9.5 is an ubiquitin carboxyl-terminal hydrolase. *Science* 246:670–673.

Wojcik, C. 1999. Proteasomes in apoptosis: Villains or guardians? *Cell Mol. Life Sci.* 56:908-917.

Wu, R. M., Cheng, C. W., Chen, K. H., Lu, S. L., Shan, D. E., Ho, Y. F., and Chern, H. D. 2001. The COMT L allele modifies the association between MAOB polymorphism and PD in Taiwanese. *Neurology* 56:375-382.

Wűllner, U., Seyfried, J., Groscurth, P., Beinroth, S., Winter, S., Gleichmann, M., Heneka, M., Loschmann, P. A., Schulz, J. B., Weller, M., and Klockgether, T. 1999.Glutathione depletion and neuronal cell death: The role of reactive oxygen intermediates and mitochondrial function. *Brain Res*. 826:53-62.

Wyllie, A. H., Kerr, J. F., and Currie, A. R. 1980. Cell death: The significance of apoptosis. *Int. Rev. Cytol*. 68:251-306.

Xia, Y., Rohan de Silva, H. A., Rosi, B. L., Yamaoka, L. H., Rimmler, J. B., Pericak-Vance, M. A., Roses, A. D., Chen, X., Masliah, E., DeTeresa, R., Iwai, A.,

Sundsmo, M., Thomas, R. G., Hofstetter, C. R., Gregory, E., Hansen, L. A., Katzman, R., Thal, L. J., and Saitoh, T. 1996. Genetic studies in Alzheimer's disease with a NACP/alpha-synuclein polymorphism. *Ann. Neurol.* 40:207-215.

Yang, M. C., McLean, A. J., Rivory, L. P., and Le Couteur, D. G. 2000. Hepatic disposition of neurotoxins and pesticides. *Pharmacol. Toxicol*. 87:286-291.

Yang, W. L., and Sun, A. Y. 1998. Paraquat-induced cell death in PC12 cells. *Neurochem. Res*. 23:1387-1394.

Yang, Y., and Yu, X. 2003. Regulation of apoptosis: The ubiquitous way. *FASEB J.* 17:790-799.

Yoritaka, A., Hatorri, N., Uchida, K., Tanaka, M., Stadtman, E. R., and Mizuno, Y. 1996. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson`s disease. *Proc. Natl. Acad. Sci. USA* 93:2696-2701.

Yoritaka, A., Hattori, N., Yoshino, H., and Mizuno, Y. 1997. Catechol-*O*-methyltransferase genotype and susceptibility to Parkinson's disease in Japan. Short communication. *J. Neural Transm.* 104:1313-1317.

Yoshimoto, Y., Nakaso, K., and Nakashima, K. 2005. L-dopa and dopamine enhance the formation of aggregates under proteasome inhibition in PC12 cells. *FEBS Lett*. 579:1197-1202.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Gomez Tortosa, E., del Ser, T., Munoz, D. G., and de Yebenes, J. G. 2004. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann. Neurol.* 55:164-173.

Zecca, L., Pietra, R., Goj, C., Mecacci, C., Radice, D., and Sabbioni, E., 1994. Iron and other metals in neuromelanin, substantia nigra, and putamen of human brain. *J. Neurochem.* 62:1097–1101.

Zetterstrom, R. H., Solomin, L., Jansson, l., Hoffer, B. J., Olson, L., and Perlmann, T. 1997. Dopamine neuron agenesis in Nurr1-deficient mice. *Science* 276:248-250.

Zhang, J., Fitsanakis, V. A., Gu, G., Jing, D., Ao, M., Amarnath, V., and Montine, T. J. 2003. Manganese ethylene-bis-dithiocarbamate and selective dopaminergic neurodegeneration in rat: A link through mitochondrial dysfunction. *J. Neurochem*.84:336-346.

Zhang, J., Perry, G., Smith, M. A., Robertson, D., Olson, S. J., Graham, D. G., and Montine, T. J. 1999. Parkinson`s disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am. J. Pathol.* 154:1423-1429.

Zhang, Y., Gao, J., Chung, K. K., Huang, H., Dawson, V. L., and Dawson, T. M. 2000. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc. Natl. Acad. Sci. U S A* 97:13354-13359.

Zheng, J., and Ramirez, V. D. 2000. Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. *Br. J. Pharmacol*. 130:1115-1123.

Zheng, K., Heydari, B., and Simon, D. K. 2003. A common NURR1 polymorphism associated with Parkinson disease and diffuse Lewy body disease. *Arch. Neurol.* 60:722-725.

Zhou, W., and Freed, C. R. 2004. Tyrosine-to-cysteine modification of human alpha-synuclein enhances protein aggregation and cellular toxicity. *J. Biol. Chem*. 279:10128-10135.

Zhou, Y., Shie, F. S., Piccardo, P., Montine, T. J., and Zhang, J. 2004. Proteasomal inhibition induced by manganese ethylene-bis-dithiocarbamate: Relevance to Parkinson's disease. *Neuroscience* 128:281-291.

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