Oxidative Stress, α-Synuclein, and Apoptosis in Parkinson's Disease

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Summary

Parkinson's Disease is a neurodegenerative disease that is pathologically characterized by abnormal α-synuclein protein aggregation, dopamine depletion in nigrostriatal neurons, and the subsequent death of these neurons. Oxidative stress has been widely implicated as a cause for neurodegenerative diseases, especially Parkinson's Disease (PD). The mechanisms by which oxidative stress occurs in PD neurons and causes cell death remain obscure. Recent research has revealed several mechanisms by which α-synuclein and oxidative stress may induce cell death in PD, providing potential targets for therapies. The roles of α-synuclein, PD-inducing drugs, and DJ-1 as well as their involvement in oxidative stress production and apoptosis are reviewed.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that afflicts one million people in the U.S., and four million people worldwide. It is characterized by resting tremors, postural instability, and bradykinesia (Olanow and Tatton, 1999)—all symptoms which result from the death of dopamine producing neurons in the substantia nigra pars compacta. Although five percent of PD cases result from genetic mutations in one of several key genes, ninety-five percent of PD cases are sporadic. This review explores oxidative stress as a contributor to sporadic PD.

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and the hydroxyl radical, are reactive molecules that cause cellular stress. Mitochondria can generate ROS within the cell during normal metabolic reactions, and these free radicals can significantly damage other cellular structures, including lipid membranes, proteins in important cellular machinery, and DNA. In PD patients, increased lipid peroxidation (Dexter et al., 1986), protein carbonylation (Alam et al., 1997a), and oxidation of DNA (Alam et al., 1997b) have all been detected in the substantia nigra, implying the occurrence of oxidative stress within these neurons. The inhibition of complex I of the mitochondria has been shown to generate ROS, which can damage the electron-transport chain and lead to further production of ROS (Dauer and Przedborski, 2003). Several studies have confirmed the deficiency of complex I in the brains of PD patients (Schapira et al., 1989; Mizuno et al., 1989; Parker et al., 1989), and the death of dopaminergic neurons has been shown to increase when complex I of the mitchondria is deficient (Kweon et al., 2004). These markers of oxidative stress have led to research aiming to discover the mechanisms behind oxidative stress and cell death in PD.

Modeling PD with drugs

Several molecular species and drugs are thought to lead to the onset of PD and have been used to successfully produce Parkinsonism in model organisms. Dopamine, itself, is thought to be the single most important species that contributes to oxidative stress conditions in the brains of PD patients. While this neurotransmitter is the molecular species that enables neuronal activity, its oxidized metabolites can act as endogenous neurotoxins within these neurons (Hastings and Zigmond, 1997). Dopamine can undergo auto-oxidation and form reactive dopamine quinones or 6-hydroxydopamine (6-OHDA), which can generate the deleterious hydroxyl radical and hydrogen peroxide species (Heikkila and Cohen, 1973; Slivka and Cohen, 1985). 6-OHDA induces nirgostriatal neuronal death in rats (Mendez and Finn, 1975; Javoy et al., 1976) and human neuroblastoma (Simantov et al., 1996). Because dopamine depletion is a common pathology of PD, therapies, such as L-dopa, have aimed to replace dopamine within surviving neurons in order to restore their function, thus reducing the symptoms of PD. Although L-dopa, which was first used in the 1960s, is effective in restoring nigrostriatal function, its long term effects are now under investigation because L-dopa is converted to dopamine in the patient, and which can then undergo auto-oxidation and form toxic species.

The connection between oxidative stress and PD has also been strengthened by the effects of several drugs on nigrostriatal dopaminergic neurons. The drug MPTP has been shown to cause extensive dopaminergic neuronal death in the substantia nigra, leading to Parkinson's Disease. This drug has been used to model PD in animals and has revealed findings such as parkinsonism in non-human primates (Burns et al., 1983) as well as the loss of movement in mice (Sundstrom et al., 1990), rats (Sahgal et al., 1984), and zebrafish (Bretaud et al., 2004). When this drug is oxidized to its metabolite, MPP+, it inhibits mitochondrial respiration by blocking the flow of electrons in complex I (Ramsay et al., 1991), leading to increased levels of ROS (Singer et al., 1987). Because MPTP causes oxidative stress and PD, examining its effects in model organisms may elucidate mechanisms that show oxidative stress is a precursor or result of PD.

Rotenone is another complex I inhibitor that has been used to model PD in rats (Betarbet et al., 2000) and *Drosophila* (Coulom et al., 2004). It has been shown to induce apoptosis at low concentrations in neuronal PC-12 cells (Hartley et al., 1994) and to increase oxidative stress conditions, increase α-synuclein accumulation, and induce apoptosis in human neuroblastoma (Sherer et al., 2002). Another molecule, paraquat, is an oxidative herbicide that has also been used to model PD. It was first shown to cause neurodegeneration in rats (Corasaniti et al., 1992) and has since been shown to cause apoptosis in PC-12 cells (Li and Sun, 1999) and dopaminergic cell loss in mice (Brooks et al., 1999). The mechanisms by which

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6-OHDA, MPTP, rotenone, and paraquat cause cell death are only partially understood. Both MPP+ and rotenone bind to either the same or adjacent sites in complex I of the mitochondria to inhibit mitochondrial respiration (Ramsay et al., 1999; Krueger et al., 1990), indicating that they induce neuronal death via similar mechanisms. In addition, MPP+ is transported into cells via the dopamine transporter (DAT), accounting for its selectivity in targeting dopaminergic neurons (Kitayama et al., 1992; Kitayama et al., 1993). On the other hand, one study has shown that, while MPP+ causes cell death via inhibition of mitochondrial complex I, 6-OHDA apoptosis occurs independent of mitochondrial impairment (Wu et al., 1996).

Most recent studies have shown a relationship between 6-OHDA neurotoxicity and cyclooxygenase (COX) activity (Carrasco et al., 2005). COX is an enzymatic mediator of inflammation (McDaniel et al., 1996) that has been found at increased levels in the dopaminergic neurons of PD patients (Knott et al., 2000). Regular use of non-steroidal anti-inflammatory drugs, such as ibuprofen, has been shown to reduce the risk of PD (Casper et al., 2000; Chen et al., 2005). A recent study has shown that ibuprofen, a non-selective inhibitor of COX activity, attenuated the death of embryonic rat mesencephalic neurons by 6-OHDA, but not MPP+ (Carrasco et al., 2005). Furthermore, a specific inhibitor of COX-2 was shown to decrease 6-OHDA toxicity, while no correlation was shown for COX-1. This study further supports that, in addition to the ability to generate ROS, 6-OHDA could be involved in a toxic mechanism that is seemingly independent of oxidative stress conditions.

While rotenone is a well-studied complex I inhibitor, it has recently been shown to be involved in an apoptotic pathway. Bcl-2 is a protein that prevents apoptosis induced by the fas cell death receptor (Jaattela et al., 1995), and BAD is a protein that blocks the activity of Bcl-2, thereby causing cell death (Yang et al., 1995). Rotenone has been shown to dephosphorylate the BAD protein in human dopaminergic SH-SY5Y cells, an event that allows BAD to block Bcl-2 (Watabe and Nakaki, 2004). Both rotenone's involvement in the Bcl-2 pathway and 6-OHDA's involvement in COX activity present evidence that these PD-inducing agents may cause neuronal toxicity via apoptotic pathways not only by contributing to oxidative stress conditions. This suggests that these species may have dual roles in causing PD. Further studies may someday link these toxic species' involvement in apoptosis to oxidative stress, as mitochondria are primary producers of ROS and are involved in the initiation of apoptotic pathways. Fortunately, cells are equipped with machinery to combat the ROS that can lead to untimely cell death.

Oxidative stress enzymes and PD

Over the course of evolution, cells have constructed enzymatic machinery to combat oxidative stress. For example, superoxide dismutase (SOD) is an enzyme that catalyzes the reaction of the superoxide radical ($O_2^{\bullet -}$) to hydrogen peroxide and molecular oxygen (O_2). This enzyme is a protein complex that can have various types of metal ion content (mangansese, copper-zinc, and iron). Manganese superoxide dismutase (SOD2) is found in the matrix of mitochondria (Slot et al., 1986), which is the sight of oxidative phosphorylation. Theoretically, an antioxidant defense system would be required in this area of the cell since the normal process of oxidative phosphorylation can give rise to superoxide radicals. Copper-zinc superoxide dismutase (SOD1) is located in the cytosol and comprises a rather large fraction of a cell's total protein (10μM in yeast) (Rae et al., 1999), while iron superoxide dismutase (SOD3) is extracellular. Catalase and glutathione are two other antioxidant enzymes that catalyze the reaction of hydrogen peroxide to molecular oxygen and water, thereby reducing a major source of oxidative stress. All of these enzymes have been shown to confer protection of neurons against oxidative stress.

SODs, specifically, have been examined in many studies of neurodegeneration. Perhaps the best known example is the role of SOD1 in amyotrophic lateral sclerosis (ALS), also known as Lou Gherig's disease. Twenty percent of familial ALS cases are caused by mutations in the SOD1 gene, which causes abnormal SOD1 protein aggregation (Rosen et al., 1993). SOD1 has also been shown to alleviate Aβ toxicity by scavenging free radicals generated by the abberant Alzheimer's protein (Bowling and Beal, 1995). Moreover, both SOD1 and SOD2 are upregulated in neuronal regions containing neurofibrillary tangles and plaques in AD patients (Delacourte et al., 1988).

Many studies have also examined the roles of SODs in the protection against oxidative stress in PD. Because SOD2 is the major free radical scavenger in mitochondria, the SOD2 gene has been closely examined in many studies in an attempt to find a possible link between the gene and genetic PD cases; however, no such correlation has been identified (Jones et al., 1998; Farin et al., 2001). But because SOD2 is an essential enzyme against mitochondrial oxidative stress, SOD2 knockouts have been used as a model of endogenous oxidative stress (Hinerfeld et al., 2004). Moreover, MPTP-treated mice are more susceptible to striatal lesions and dopamine depletion when SOD2 is partially deficient (Andreassen et al., 2001). Interestingly, SOD2 was also identified as a gene that protected against α-synuclein toxicity to yeast cells expressing the α-synuclein protein (Willingham et al., 2003). By delineating the activity of oxidative stress enzymes in protecting model organisms from cell death under PD-inducing conditions, the role of oxidative stress in PD is strengthened. In a genetic cause of PD, this relationship between cell death and oxidative stress becomes more apparent.

Genetic Evidence of Oxidative Stress in PD

A familial mutation and the deletion of the DJ-1 gene have been shown to induce autosomal, recessive, early-onset PD (Bonifati et al., 2003). DJ-1 is an antioxidant protein that eliminates hydrogen peroxide by undergoing auto-oxidation (Taira et al., 2004). Neuroblastoma cells with DJ-1 knockdown by siRNA are susceptible to death by 6-OHDA, hydrogen peroxide, and MPP+, all Parkinson's inducing agents (Taira et al., 2004). Moreover, DJ-1 overexpression protects cultured primary dopamine neurons and rat dopaminergic neurons from hydrogen peroxide and 6-OHDA induced death by the upregulation of glutathione levels (Zhou and Freed, 2005). Another study has shown that the knockdown of DJ-1 or the expression of mutant DJ-1 in a mouse cell line resulted in the downregulation of extracellular iron-SOD expression (Nishinaga et al., 2005), suggesting that normal DJ-1 can regulate gene expression to protect against cell death. The discovery of this gene's connection to PD

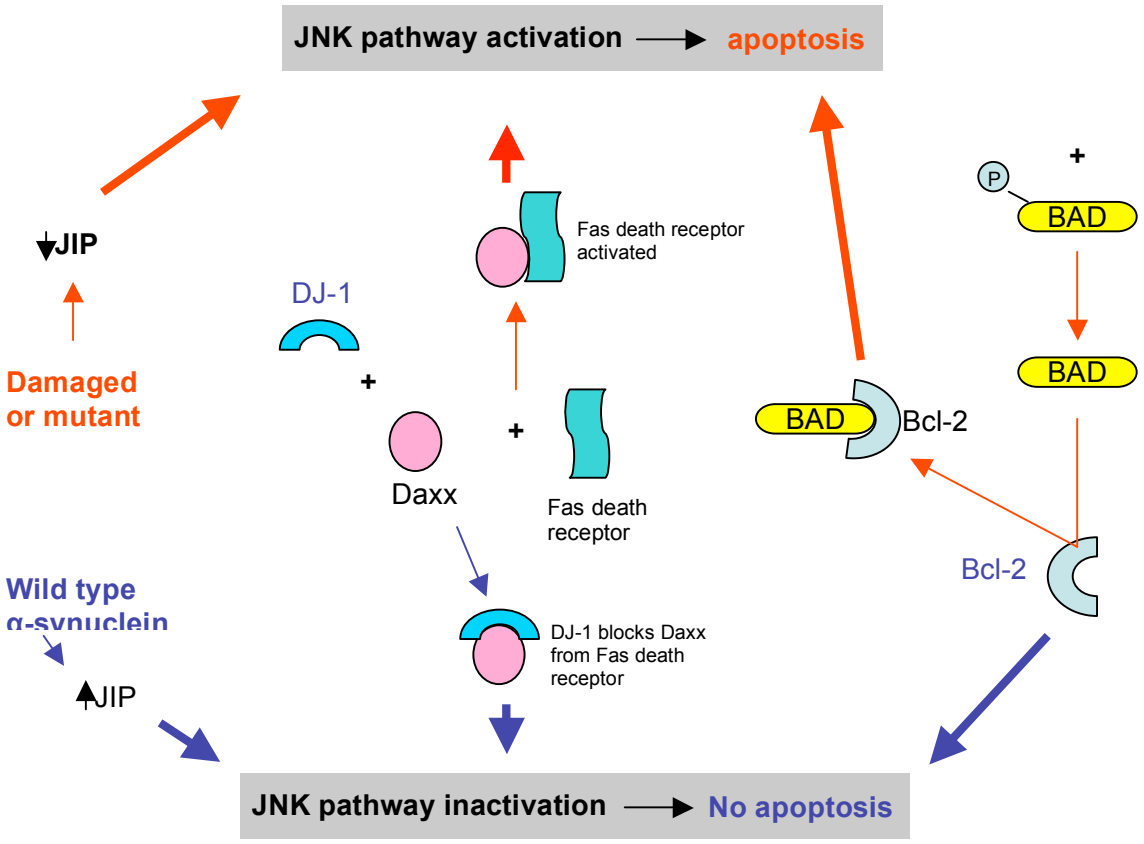

Figure 1: α-synuclein, oxidative stress, and DJ-1 involvement in the JNK pathway may explain cell death in PD.
Several studies support the involvement of the JNK apoptotic pathway in the cell death observed in PD. α-Synuclein is can bind to the JNK-interacting protein (JIP) to inactivate JNK and prevent apoptosis. DJ-1 can bind to Daxx and prevent its association with the fas death receptor, inactivating the JNK pathway and also preventing apoptosis. Rotenone can dephosphorylate BAD, which blocks Bcl-2 from inactivating the JNK pathway, thereby causing apoptosis.

presents significant genetic evidence that implicates oxidative stress as a cause of PD.

DJ-1 has many other functions in addition to its ability to quench ROS. A recent study has revealed that DJ-1 binds to the Daxx protein (Junn et al., 2005), a protein that binds to a fas death receptor (Yang et al., 1997) and activates the JNK (c-Jun N-terminal kinase) apoptotic pathway. Junn and colleagues discovered that when DJ-1 binds to Daxx, it sequesters the protein into the nucleus and prevents it from gaining access to the cytoplasm and from inducing apoptosis at the fas death receptor. Interestingly, Daxx upregulation occurs in cells that are treated with the oxidant hydrogen peroxide (Kim et al., 2005), linking oxidative stress to the activation of this apoptotic pathway. These studies together show that DJ-1 is involved in inactivating the apoptotic JNK pathway that is activated by oxidative stress, indicating its indirect role in guarding against oxidative stress-induced cell death (Figure 1).

α-Synuclein and Oxidative Stress

The common pathological hallmark among most cases of PD is the accumulation of misfolded α-synuclein protein into aggregates called Lewy Bodies (Spillantini et al., 1997). A connection between a-synuclein misfolding and oxidative stress has yet to be fully established. α-Synuclein has been shown to bind phospholipids in several *in vitro* and *in vivo* studies (Davidson et al., 1998; Sharon et al., 2001; Eliezer et al., 2001; Outeiro and Lindquist, 2003), and lipid peroxidation has been observed in dopaminergic neurons within the brains of PD patients. If lipid content is damaged within dopaminergic neurons, α-synuclein may be unable to bind to the membrane and will accumulate within the cytoplasm (Figure 2), accounting for the formation of Lewy bodies.

An emerging theory of α-synuclein toxicity conversely proposes that toxic events occur with *increased* binding of α-synuclein protofibrils to the lipid membrane (Volles et al., 2001). This study shows that in contrast to monomeric and oligomeric forms of α-synuclein, protofibrillar forms bind more tightly to vesicles, permeating and destroying them. This provides evidence that those protofibrillar forms of α-synuclein may be the toxic species in neurons in that they destroy lipid membranes. Therefore, it is also a possibility that oxidative damage that increases α-synuclein binding to lipid membranes can lead to toxic events in neurons (Figure 2).

α-Synuclein has been more concretely linked to oxidative stress with its involvement in the JNK pathway previously discussed. A study by Hashimoto and colleagues revealed that hydrogen peroxide

treatment resulted in the activation of the JNK pathway and death in a neuronal cell line. α-Synuclein

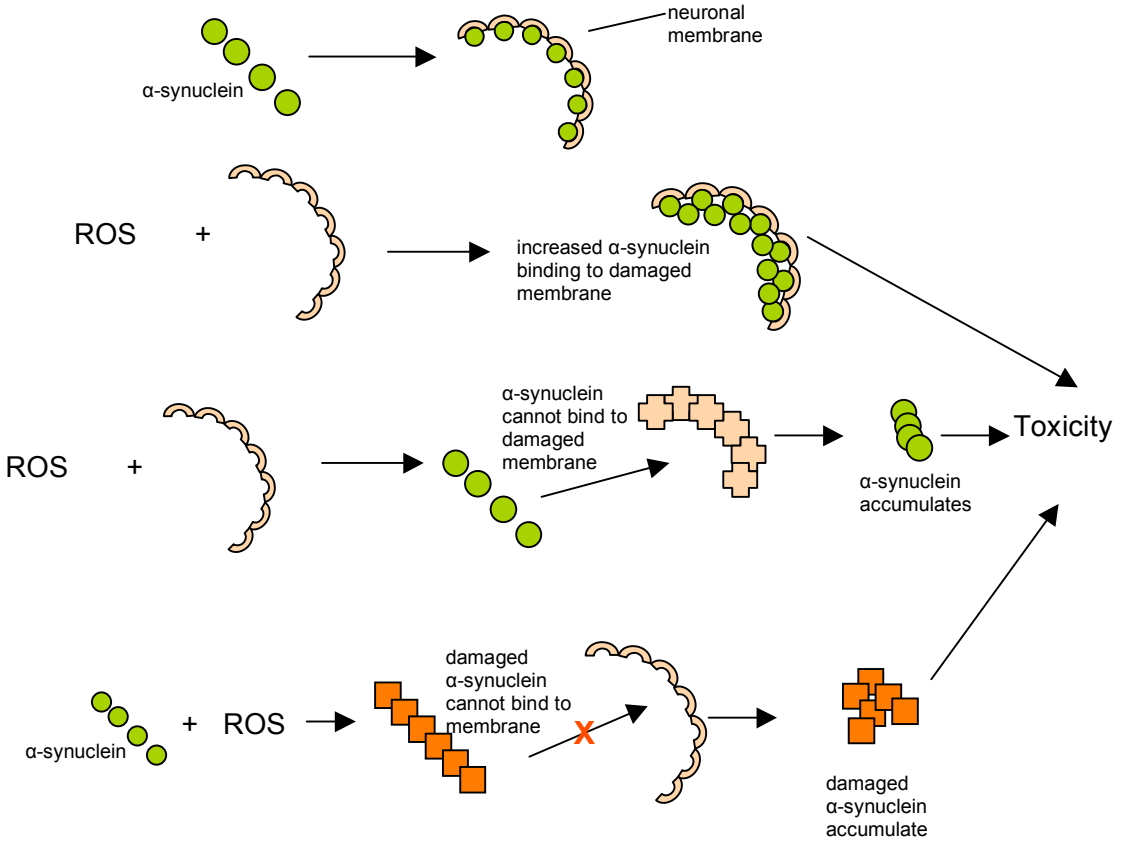

Figure 2: Oxidative stress can contribute to α-synuclein dysfunction and cell death in PD.
ROS can damage α-synuclein's lipid binding ability and contribute to the formation of α-synuclein aggregates. ROS can also damage lipid membranes and prevent or promote α-synuclein binding, both events that are hypothesized to cause cell death.

transfection, however, protected against this cell death, and JNK was inactivated due to increased expression of JNK-interacting protein (JIP). Moreover, α-synuclein was shown to co-localize with JIP. This proposes a protective role of α-synuclein against oxidative stress due to its ability to inactivate the JNK pathway via upregulation of JIP (Hashimoto et al., 2002). The protective activity of the α-synuclein may decrease if the protein is mutated, misfolded, or aggregated, and therefore unable to interact with JIP (Figure 1). Further studies involving oxidative stress conditions and the JNK pathway may bolster the connection between pathological forms of α-synuclein and oxidative stress. The formation of Lewy bodies within surviving nigrostriatal neurons of PD patients (Spillantini et al., 1997) has led to two theories regarding α-synuclein toxicity: aggregates are precursors to cytotoxic events in these neurons (Goedert, 1999), or aggregates are cytoprotective against smaller toxic forms of misfolded α-synuclein (McNaught et al., 2002). A study examining dopamine's interaction with α-synuclein provides evidence for the latter. This study revealed that dopamine quinones inhibited the fibrillization of α-synuclein by reacting with the protein's amino groups to form toxic, oligomeric, cross-linked, dopamine-quinone adducts (Li et al., 2005). Because oxidative stress conditions are conducive to the formation of dopamine quinones, this study creates a strong connection between dopaminergic neuronal death, α-synuclein, and oxidative stress.

The function of α-synuclein has also been implicated in dopamine transport. Dopamine transporter (DAT) facilitates the reuptake of dopamine from the synaptic cleft back into the presynpatic neuron (Reith et al., 1997). α-Synuclein has been shown to bind to DAT and assist it in clustering within the membrane to accelerate dopamine uptake (Lee et al., 2001). Damaged or aggregated α-synuclein may lead to the accumulation of dopamine and its subsequent oxidation into harmful metabolites, thus increasing oxidative stress. The role of α-synuclein in the JNK pathway, lipid binding, and dopamine transport all provide hypotheses for its involvement with oxidative stress conditions that may cause the neuronal degeneration seen in PD.

Conclusion

Although a great deal of progress has been made in delineating the mechanisms by which neuronal degeneration occurs in PD, there are still many gaps in knowledge that plague the field. Oxidative stress is undoubtedly a key player in neuronal degeneration in PD, but targeting the pathways by which it induces cell death cannot be considered until the mechanisms are fully understood.

In reviewing studies that focus on oxidative stress and PD, it is apparent that α-synuclein, oxidative stress, and apoptosis are all related by complex

mechanisms that may or may not be linked. Examining the effects of PD-inducing agents reveals new findings that supports mechanisms by which oxidative stress is involved in the activation of specific apoptotic pathways. The JNK pathway is seemingly important as DJ-1, rotenone, and α-synuclein can all be implicated in the activation or inactivation of this pathway, which can lead to or prevent apoptosis (Figure 1).

α-Synuclein, itself, seems to be involved in several cellular process due to its lipid binding abilities. This study has mentioned two such abilities: its association with JIP and its regulation of dopamine transport. Because α-synuclein may have several functions, it may also be responsible for a great deal of cellular dysfunction when damaged (Figures 1 & 2). Whether oxidative stress is a cause or effect of α-synuclein aggregation is a major question that remains in the field and must be answered before any therapy can target either of these phenomena.

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References

Alam, Z.I., Daniel, S.E., Lees, A.J., Marsden, D.C., Jenner, P., and Halliwell, B. 1997a. A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J Neurochem 69(3): 1326-9.

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(3):1196-203.

Andreassen, O.A., Ferrante, R.J., Dedeoglu, A., Albers, D.W., Klivenyi, P., Carlson, E.J., Epstein, C.J., and Beal, M.F. 2001. Mice with a partial deficiency of manganese superoxide dismutase show increased vulnerability to the mitochondrial toxins malonate, 3-nitropropionic acid, and MPTP. Exp Neurol 167(1): 189-95.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., and Greenamyre, J.T. 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3(12): 1301-6.

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J.C., Brice, A., van Duijn, C.M., Oostra, B., Meco, G., and Heutink, P. 2003. DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. Neurol Sci 24(3): 159-60.

Bowling, A.C., and Beal, M.F. 1995. Bioenergetic and oxidative stress in neurodegenerative diseases. Life Sci 56(14): 1151-71.

Bretaud, S., Lee, S., and Guo, S. 2004. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. Neurotoxicol Teratol 26(6): 857-64.

Brooks, A.I., Chadwick, C.A., Gelbard, H.A., Cory-Slechta, D.A., and Federoff, H.J. 1999. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 823(1-2): 1-10.

Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M., and Kopin, I.J. 1983. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. PNAS 80(14): 4546-50.

Carrasco, E., Casper, D., and Werner, P. 2005. Dopaminergic neurotoxicity by 6-OHDA and MPP+: differential requirement for neuronal cyclooxygenase activity. Journal of Neuroscience Research 81(1): 121-31.

Casper, D., Yaparpalvi, U., Rempel, N., and Werner, P. 2000. Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro. Neuroscience Letters 289(3): 201-4.

Chen, H., Jacobs, E., Schwarzschild, M.A., McCullough, M.L., Calle, E.E., Thun, M.J., and Ascherio, A. 2005. Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. Annals of Neurology [Epub ahead of print].

Corasaniti, M.T., Bagetta, G., Rodino, P., Gratteri, S., and Nistico, G. 1992. Neurotoxic effects induced by intracerebral and systemic injection of paraquat in rats. Hum Exp Toxicol 11(6): 535-9.

Coulom, H., and Birman, S. 2004. Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. J Neurosci 24(48): 10993-8.

Dauer, W., and Przedborski, S. 2003. Parkinson's disease: mechanisms and models. Neuron 39(6): 889-909.

Davidson, W.S., Jonas, A., Clayton, D.F., and George, J.M. 1998. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. J Biol Chem 273(16): 9443-9.

Delacourte, A., Defossez, A., Ceballos, I., Nicole, A., and Sinet, P.M. 1988. Preferential localization of copper zinc superoxide dismutase in the vulnerable cortical neurons in Alzheimer's disease. Neurosci Letters 92(3): 247-53.

Dexter, D., Carter, C., Agid, F., Agid, Y., Lees, A.J., Jenner, P., and Marsden, C.D. 1986. Lipid peroxidation as cause of nigral cell death in Parkinson's disease. Lancet 2(8507): 639-40.

Eliezer, D., Kutluay, E., Bussell, R. Jr., and Browne, G. 2001. Conformational properties of alpha-synuclein in its free and lipid-associated states. J Mol Biol 307(4): 1061-73.

Farin, F.M., Hitosis, Y., Hallagan, S.E., Kushleika, J., Woods, J.S., Janssen, P.S., Smith-Weller, T., Franklin, G.M., Swanson, P.D., and Checkoway, H. 2001. Genetic polymorphisms of superoxide dismutase in Parkinson's disease. Mov Disord 16(4): 705-7.

Goedert, M. 1999. Filamentous nerve cell inclusions in neurodegenerative diseases: tauopathies and alpha-synucleinopathies. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 354(1386): 1101-18.

Hartley, A., Stone, J.M., Heron, C., Cooper, J.M., and Schapira, A.H. 1994. Complex I inhibitors induce dose-dependent apoptosis in PC12 cells: relevance to Parkinson's disease. J Neurochem 63(5): 1987-90.

Hashimoto, M., Hsu, L.J., Rockenstein, E., Takenouchi, T., Mallory, M., and Masliah, E. 2002. alpha-Synuclein protects against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells. J Biol Chem 277(13): 11465-72. Epub 2002 Jan 14.

Hastings, T.G., and Zigmond, M.J. 1997. Loss of dopaminergic neurons in parkinsonism: possible role of reactive dopamine metabolites. J Neural Transm Suppl 49: 103-10.

Heikkila, R.E., and Cohen, G. 1973. 6-Hydroxydopamine: evidence for superoxide radical as an oxidative intermediate. Science 181(98): 456-7.

Hinerfeld, D., Traini, M.D., Weinberger, R.P., Cochran, B., Doctrow, S.R., Harry, J., and Melov, S. 2004. Endogenous mitochondrial oxidative stress: neurodegeneration, proteomic analysis, specific respiratory chain defects, and efficacious antioxidant therapy in superoxide dismutase 2 null mice. J Neurochem 88(3): 657-67.

Jaattela, M., Benedict, M., Tewari, M., Shayman, J.A., and Dixit, V.M. 1995. Bcl-x and Bcl-2 inhibit TNF and Fas-induced apoptosis and activation of phospholipase A2 in breast carcinoma cells. Oncogene 10(12): 2297-305.

Javoy, F., Sotelo, C., Herbet, A., and Agid, Y. 1976. Specificity of dopaminergic neuronal degeneration induced by intracerebral injection of 6-hydroxydopamine in the nigrostriatal dopamine system. Brain Res 102(2): 201-15.

Jones, A.C., Yamamura, Y., Almasy, L., Bohlega, S., Elibol, B., Hubble, J., Kuzuhara, S., Uchida, M., Yanagi, T., Weeks, D.E., and Nygaard, T.G. 1998. Autosomal recessive juvenile parkinsonism maps to 6q25.2-q27 in four ethnic groups: detailed genetic mapping of the linked region. Am J Hum Genet 63(1): 80-7.

Junn, E., Taniguchi, H., Jeong, B.S., Zhao, X., Ichijo, H., and Mouradian, M.M. 2005. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. PNAS 102(27): 9691-6. Epub 2005 Jun 27.

Kim, K.S., Hwang, H.A., Chae, S.K., Ha, H., and Kwon, K.S. 2005. Upregulation of Daxx mediates apoptosis in response to oxidative stress. J Cell Biochem 96(2): 330-8.

Kitayama, S., Shimada, S., and Uhl, G.R. 1992. Parkinsonism-inducing neurotoxin MPP+: uptake and toxicity in nonneuronal COS cells expressing dopamine transporter cDNA. Ann Neurol 32(1): 109-11.

Kitayama, S., Wang, J.B., and Uhl, G.R. 1993. Dopamine transporter mutants selectively enhance MPP+ transport. Synapse 15(1): 58-62.
Knott, C., Stern, G., and Wilkin, G.P. 2000. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Molecular and Cellular Neurosciences 16(6): 724-39.

Krueger, M.J., Singer, T.P., Casida, J.E., and Ramsay, R.R. 1990. Evidence that the blockade of mitochondrial respiration by the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) involves binding at the same site as the respiratory inhibitor, rotenone. Biochem Biophys Res Commun 169(1): 123-8.

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(50): 51783-92. Epub 2004 Oct 6.

Lee, F.J., Liu, F., Pristupa, Z.B., and Niznik, H.B. 2001. Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J 15(6): 916-26.

Li, H.T., Lin, D.H., Luo, X.Y., Zhang, F., Ji, L.N., Du, H.N., Song, G.Q., Hu, J., Zhou, J.W., and Hu, H.Y. 2005. Inhibition of alpha-synuclein fibrillization by dopamine analogs via reaction with the amino groups of alpha-synuclein. Implication for dopaminergic neurodegeneration. FEBS J 272(14): 3661-72.

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): 1-21.

McDaniel ML, Kwon G, Hill JR, Marshall CA, Corbett JA. Cytokines and nitric oxide in islet inflammation and diabetes. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine. 1996 Jan;211(1):24-32.

McNaught, K.S., Shashidharan, P., Perl, D.P., Jenner, P., and Olanow, C.W. 2002. Aggresome-related biogenesis of Lewy bodies. Eur J Neurosci 16(11): 2136-48.

Mendez, J.S., and Finn, B.W. 1975. Use of 6-hydroxydopamine to create lesions in catecholamine neurons in rats. J Neurosurg 42(2): 166-73.

Mizuno, Y., Ohta, S., Tanaka, M., Takamiya, S., Suzuki, K., Sato, T., Oya, H., Ozawa, T., and Kagawa, Y. 1989. Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. Biochem Biophys Res Commun 163(3): 1450-5.

Nishinaga, H., Takahashi-Niki, K., Taira, T., Andreadis, A., Iguchi-Ariga, S.M., and Ariga, H. 2005. Expression profiles of genes in DJ-1-knockdown and L 166 P DJ-1 mutant cells. Neurosci Letters 390(1): 54-9.

Olanow, C.W., and Tatton, W.G. 1999. Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci 22: 123-44.

Outeiro, T.F., and Lindquist, S. 2003. Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302(5651): 1772-5.

Parker, W.D. Jr, Boyson, S.J., and Parks, J.K. 1989. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Annals of Neurology 26(6): 719-23.

Rae, T.D., Schmidt, P.J., Pufahl, R.A., Culotta, V.C., and O'Halloran, T.V. 1999. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. Science 284(5415): 805-8.

Ramsay, R.R., Krueger, M.J., Youngster, S.K., Gluck, M.R., Casida, J.E., and Singer, T.P. 1991. Interaction of 1-methyl-4-phenylpyridinium ion (MPP+) and its analogs with the rotenone/piericidin binding site of NADH dehydrogenase. J Neurochem 56(4): 1184-90.

Reith, M.E., Xu, C., and Chen, N.H. 1997. Pharmacology and regulation of the neuronal dopamine transporter. Eur J Pharmacol 324(1): 1-10.

Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., and Deng, H.X. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362(6415): 59-62.

Sahgal, A., Andrews, J.S., Biggins, J.A., Candy, J.M., Edwardson, J.A., Keith, A.B., Turner, J.D., and Wright, C. 1984. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) affects locomotor activity without producing a nigrostriatal lesion in the rat. Neurosci Letters 48(2): 179-84.

Schapira, A.H., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B., and Marsden, C.D. 1989. Mitochondrial complex I deficiency in Parkinson's disease. Lancet 1(8649): 1269.

Sharon, R., Goldberg, M.S., Bar-Josef, I., Betensky, R.A., Shen, J., and Selkoe, D.J. 2001. alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. PNAS 98(16): 9110-5.

Sherer, T.B., Betarbet, R., Stout, A.K., Lund, S., Baptista, M., Panov, A.V., Cookson, M.R., and Greenamyre, J.T. 2002. An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 22(16): 7006-15.

Simantov, R., Blinder, E., Ratovitski, T., Tauber, M., Gabbay, M., and Porat, S. 1996. Dopamine-induced apoptosis in human neuronal cells: inhibition by nucleic acids antisense to the dopamine transporter. Neuroscience 74(1): 39-50.

Singer, T.P., Castagnoli, N. Jr,, Ramsay, R.R., and Trevor, A.J. 1987. Biochemical events in the development of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Neurochem 49(1): 1-8.

Slivka, A., and Cohen, G. 1985. Hydroxyl radical attack on dopamine. J Biol Chem 260(29): 15466-72.

Slot, J.W., Geuze, H.J., Freeman, B.A., and Crapo, J.D. 1986. Intracellular localization of the copper-zinc and manganese superoxide dismutases in rat liver parenchymal cells. Lab Invest 55(3): 363-71.

Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., and Goedert, M. Alpha-synuclein in Lewy bodies. Nature 388(6645): 839-40.

Sundstrom, E., Fredriksson, A., and Archer, T. 1990. Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: a model for Parkinson's disease. Brain Res 528(2): 181-8.

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(2): 213-8. Epub 2004 Jan 23.

Volles, M.J., Lee, S.J., Rochet, J.C., Shtilerman, M.D., Ding, T.T., Kessler, J.C., and Lansbury, P.T. Jr. 2001. Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry 40(26): 7812-78129.

Watabe, M., and Nakaki, T. 2004. Rotenone induces apoptosis via activation of bad in human dopaminergic SH-SY5Y cells. J Pharmacol Exp Ther 311(3): 948-53. Epub 2004 Jul 27.

Willingham, S., Outeiro, T.F., DeVit, M.J., Lindquist, S.L., and Muchowski, P.J. 2003. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. Science 302(5651): 1769-72.

Wu, Y., Blum, D., Nissou, M.F., Benabid, A.L., and Verna, J.M. 1996. Unlike MPP+, apoptosis induced by 6-OHDA in PC12 cells is independent of mitochondrial inhibition. Neuroscience Letters 221(1): 69-71.

Yang, E., Zha, J., Jockel, J., Boise, L.H., Thompson, C.B., and Korsmeyer, S.J. 1995. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 80(2): 285-91.

Yang, X., Khosravi-Far, R., Chang, H.Y., and Baltimore, D. 1997. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell 89(7): 1067-76.

Zhou, W., and Freed, C.R. 2005. DJ-1 upregulates glutathione synthesis during oxidative stress and inhibits A53T alpha -synuclein toxicity. J Biol Chem. [Epub ahead of print].