Front. Biol. 2012, 7(5): 404–411
DOI 10.1007/s11515-012-1239-6

REVIEW

Microglia activation-induced mesencephalic dopaminergic neurodegeneration — an *in vitro* model for Parkinson's disease

Bin XING[1], Guoying BING (✉)[2]

[1] *Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA*
[2] *Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536, USA*

Abstract **Uncontrolled and chronic microglia activation has been implicated in the process of dopaminergic neuron degeneration in sporadic Parkinson's disease (PD). Elevated proinflammatory mediators, presumably from activated microglia (e.g., cytokines, PGE_2, nitric oxide, and superoxide radical), have been observed in PD patients and are accompanied by dopaminergic neuronal loss. Preclinical studies have demonstrated the deleterious effects of proinflammatory mediators in various *in vivo* and *in vitro* models of PD. The use of *in vitro* studies provides a unique tool to investigate the interaction between neurons and microglia and is especially valuable when considering the role of activated microglia in neuronal death. Here we summarize findings highlighting the potential mechanisms of microglia-mediated neurodegeneration in PD.**

Keywords dopaminergic neurons, microglia activation, nitric oxide, cytokines, PGE_2, p38 MAPK

Introduction

Parkinson's disease (PD) is the second most common neurological disorder and is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Epidemiological data suggests that the prevalence of PD is about 0.1% in the global population. More than one million individuals are affected in North America alone, and approximately 50000 new cases arise every year. The majority of the PD cases are sporadic / idiopathic with clinical symptoms beginning late in life and progressing over decades (Olanow and Tatton, 1999).

Clinically, the symptoms of PD do not become apparent until at least 50% of the dopaminergic neurons in the SNpc are lost, and there is an 80% reduction in dopamine levels in the striatum (Kirik et al., 1998; Lozano et al., 1998). While there are a number of treatments options aimed at symptom modification currently available, there is no effective treatment that can slow down the progressive dopaminergic neuronal loss that is observed in PD.

As of now, a number of hypotheses have been proposed to explain the selective dopaminergic neuron loss in PD. Among these putative pathophysiological mechanisms, chronic neuroinflammation and oxidative stress have been implicated by playing an important role in the pathogenesis of PD. Microglia, the immune resident macrophages in the brain, are believed to be the primary contributor to brain neuroinflammation and oxidative stress in PD. Under normal condition, microglia play a homeostatic function by scavenging excessive neurotoxins, clearing cellular debris and silently removing dying cells (Nakamura, 2002; Ransohoff and Perry, 2009). However, when microglia become aberrantly and chronically activated there is a potential for neurotoxic effects. Activated microglia produce a variety of highly soluble mediators which can have unintended effects on otherwise healthy bystander neurons. A subset of microglia-derived mediators with the potential to induce neuronal injury and death include: proinflammatory cytokines such as tumor necrosis factor (TNFα) and interleukin 1-beta (IL-1β), nitric oxide, prostaglandins, and reactive oxygen species and reactive nitrogen species (ROS/RNS) (Hunot et al., 1996; Arimoto and Bing, 2003; Arimoto et al., 2007; Hunter et al., 2007). Importantly, without a resolution of the inflammatory process a feed-forward cycle can persist (Zhang et al., 2005). Ultimately, the interaction between activated microglia and injured neurons can propel the formation of a vicious pathological cycle.

Received May 20, 2012; accepted June 27, 2012
Correspondence: Guoying BING
E-mail: gbing@uky.edu

A variety of preclinical models of PD have been generated that recapitulated aspects of the pathological, behavioral and biochemical changes observed clinically. These *in vitro* and *in vivo* preclinical models have been useful at elucidating underlying mechanism of nigral dopaminergic degeneration, and to develop and screen neuroprotective strategies. Historically, many of the preclinical models relied on toxins (described in detail in following section) such as 6-OHDA, MPTP, paraquat, rotenone, and lipopolysaccharide (LPS). More recently there has been a push for developing and characterizing preclinical models of PD genetic risk factors. This new and exciting direction of PD preclinical research has been led in part by the work of the Michael J. Fox Foundation, which maintains a database of research models (http://www.pdonlineresearch.org/MJFFResources). Moreover, the *in vitro* platform is invaluable for addressing mechanistic questions related to dopaminergic neuron cell death.

The most widely used and well-described PD research models use toxins that induce a dopaminergic neuron death. 6-hydroxydopamine (6-OHDA) and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are both excellent models to induce dopaminergic cell death. As the hydroxylated analog of dopamine, intracerebral injection of 6-OHDA into substantia nigra, medial forebrain bundle, and striatum effectively induces the dopaminergic neuron loss, dopamine depletion, and neurobehavioral deficits (Perese et al., 1989; Przedborski et al., 1995). MPTP animal model was developed after found as a neurotoxic contaminant used by a group of drug addicts in the 1980s (Langston et al., 1983). After systemic administration, MPTP can easily cross the blood-brain barrier and enter astrocyte in which it is converted into 1-methyl-4-phenylpyridinium (MPP^+), and then it is released and taken up into dopaminergic neurons via dopamine transporter (DAT), ultimately causing neuronal loss and motor impairment.

Paraquat, rotenone, and LPS are environmental toxins, which have been used as models of PD. Paraquat is a type of herbicide similar to MPP^+ structurally, and the study on its systematic administration demonstrated the deleterious effects on the survival of dopaminergic neurons in the substantia nigra and the density of striatal dopamine nerve terminal (Brooks et al., 1999). Rotenone, which has been used as herbicide and insecticide, was recently found that it could induce the damage to the dopaminergic neurons and reproduce crucial pathological features of PD (Betarbet et al., 2000; Sherer et al., 2003). LPS is an endotoxin from Gram-negative bacteria. Bing et al. and Castaño et al. independently demonstrated that intranigral LPS injection causes a selective inflammation-mediated dopaminergic neuronal death (Bing et al., 1998; Castaño et al., 1998; Herrera et al., 2000). More studies reported the consistent deleterious results with various routes including intrapallidal and intrastriatal injection (Zhang et al., 2005; Choi et al., 2009; Hunter et al., 2009).

Currently, no animal models can perfectly reproduce all the major pathological, biochemical, and behavioral features of PD patients. However, each animal model has unique aspects that can be utilized for developing new therapeutic strategies or compound screening test [for review, see (Blandini and Armentero, 2012)]. For example, although 6-OHDA and MPTP do not obviously induce the proteinaceous aggregates and Lewy-body inclusion, they can be effectively used in screening therapy test due to the robust neuronal loss and motor impairment induced by them. Recent studies suggested that the rotenone neurotoxicity might not be specific to the nigrostriatal dopamine system (Lapointe et al., 2004). Nevertheless, rotenone is valuable to study the role of α-synuclein in the nigral dopaminergic neuronal loss since rotenone can stably induce its aggregation using multiple routes (Sherer et al., 2003; Cannon et al., 2009).

Evidence of activated microglia-mediated dopaminergic neuronal loss

Is it possible that dopaminergic neurons themselves are more vulnerable to the microglia-induced toxicity than other neuron cell types? Data suggests this might be the case, as neurons in the SNpc have lower levels of intracellular glutathione, and subsequently reduced antioxidant ability (Loeffler et al., 1994). However, there may be region specific differences in microglia that could also impart the selectivity of microglia-induced neurotoxicity in the SNpc. It has been determined that compared to other brain regions the SNpc has four to five times more microglia (Kim et al., 2000).

Clinical data supports the involvement of microglia in PD. In the SNpc of postmortem PD patient extensive proliferation of reactive microglia is found surrounding dopaminergic neurons (McGeer et al., 1988a, 1988b). An increase in pro-inflammatory molecules such as TNFα, IL-1β, and inducible nitric oxide synthase (iNOS) have been found in activated glial cells in the SNpc of postmortem PD patient brains (Hunot et al., 1996). However, clinical data only provides a static view of the disease progression. It is therefore difficult to assert that increased microglial activation is a cause of the dopaminergic neuron loss and not a consequence of the neuron loss. To this point, inflammation along with increased oxidative stress in the midbrain appears to precede the eventual loss of dopaminergic neurons (McGeer et al., 1988a; Jenner and Olanow, 1996). While this correlation does not prove the involvement of microglia in the disease progression, it at least suggests that microglial activation does exist prior to the neuron loss.

With all the caveats associated with animal studies, there is a body of research in a number of animal models of PD (MPTP, 6-OHDA and LPS) that supports the notion that microglial activation plays an active role in the pathological process of dopaminergic neurodegeneration (He et al., 2001; Gao et al., 2002; McGeer et al., 2003; Vijitruth et al., 2006; Arimoto et al., 2007; Hunter et al., 2007). While it is beyond

the scope of this review to cover all the clinical and preclinical studies, there does appear to be a consensus that uncontrolled microglia activation is essential in the process of dopaminergic neuronal loss via releasing proinflammatory cytotoxic molecules.

The knowledge gained by *in vitro* studies has generated some potential mechanisms by which microglia could induce dopaminergic neuron loss. The purpose of this review is to describe the key neurotoxic molecules released from activated microglia and their potential signaling pathways related to dopaminergic neuronal death observed mainly in *in vitro* models.

Potential mechanisms of microglia-activation mediated dopaminergic neurodegeneration

ROS

Superoxide radical is a main ROS molecule produced by the activated microglia, which can readily react with nitric oxide and form the highly reactive oxidative molecule – peroxynitrite. Ultimately, peroxynitrite can lead to dopaminergic injury and death as a result of the modification of intraneuronal nucleic acids, lipids, and proteins (Hald and Lotharius, 2005).

Clinical relevance of ROS involvement in PD is provided by immunostaining of dopaminergic neurons, which demonstrates increased oxidation of lipids, DNA and proteins in SNpc of PD patients (Zhang et al., 1999). As a significant source of ROS, the subunit $gp91^{phox}$ of NADPH oxidase (PHOX) in microglia was also found upregulated in PD patients compared to normal control, and the increased PHOX was coincident with microglial activation and dopaminergic neuronal loss in MPTP animal model (Wu et al., 2003).

The important roles for PHOX have been further investigated *in vitro*. By either pharmacological or genetic inhibition of PHOX activity the role of PHOX in microglia-mediated neuronal death was tested. Using mesencephalic neuron/glia mixed cultures insulted with LPS, suppression of PHOX was found to be neuroprotective (Qin et al., 2004; Zhang et al., 2010). This data suggests that PHOX is a critical molecule in the activated microglia-induced oxidative stress-mediated dopaminergic neurodegeneration. However, potential non-oxidative mechanism of PHOX also exists. Increasing evidence suggested that PHOX in microglia is not only responsible for the oxidative production but also actively takes part in the regulation of other proinflammatory signaling pathways. For example, it has been shown that PHOX inhibition abolishes the production of proinflammatory mediators such as TNFα, prostaglandin E2 (PGE_2), iNOS, and suppresses the MAPK signaling pathways such as p38 MAPK, JNK, and ERK (Pawate et al., 2004; Qin et al., 2004; Block and Hong, 2005).

Recent work further showed that high-mobility group box 1 (HMGB1) treated in the primary microglia binds to macrophage antigen complex I (MAC-1) receptor and activates PHOX, in contrast, neutralizing HMGB1 and genetic ablation of MAC-1 blocked the production of neurotoxic molecules and the progressive dopaminergic neurodegeneration, suggesting that HMGB1 and MAC-1 may act as the upstream mediator of the LPS-induce PHOX activation and consequent production of superoxide in microglia (Gao et al., 2011).

Proinflammatory cytokines

The clinical relevance for the role of proinflammatory cytokines has also been established. A line of postmortem studies demonstrated that proinflammatory cytokines such as TNFα, IL-1β, and IL-6 are significantly increased in the striatum of PD brains (Mogi et al., 1994 a,b). Moreover, it has been demonstrated that TNFα is secreted by microglia surrounding degenerating dopaminergic neurons in the SN of PD patients (Boka et al., 1994; Hunot et al., 1999). Furthermore, enhanced expression of IL-1β, IL-6 and TNFα has also been shown in CSF of PD patients (Nagatsu et al., 2000). In addition, increased levels of TNFR1 receptor have been shown to be elevated in the SN of PD patients (Mogi et al., 2000). All the above findings suggested that proinflammatory molecules released from activated microglia have a deleterious role in the dopaminergic neurodegeneration seen in PD.

Recent studies report that neutralizing antibodies to either TNFα or IL-1β and IL-1β type 1 receptor (IL-1R) can significantly rescue dopaminergic neurons against LPS-induced toxicity in mesencephalic neuron/glia cultures and in primary ventral mesencephalic neuron-enriched cultures (Gayle et al., 2002; Long-Smith et al., 2010). Blocking soluble TNFα signaling attenuates the loss of dopaminergic neurons in primary mesencephalic/glia mixed cultures stimulated with both LPS and 6-OHDA (McCoy et al., 2006). It has been shown both *in vitro* and by using TNRa receptor (TNFR1 and TNFR2) knockout mice that activation of TNFR1 contributes the neuronal death, whereas activation of TNFR2 has an neuroprotective effect in a ischemia-reperfusion-induced retinal damage model (Fontaine et al., 2002). This is consistent with the findings in the primary cortical neuronal cultures insulted with glutamate and beta-amyloid (Li et al., 2004; Marchetti et al., 2004). Whether TNFR1 and TNFR2 have differential roles in microglia-induced dopaminergic neuronal death and the intraneuronal mechanisms need to be clarified.

iNOS and COX-2

In microglia the primary enzyme responsible for the production of nitric oxide is iNOS. Clinical relevance has also been shown for the involvement of iNOS in PD.

Postmortem studies have found an upregulation of iNOS in the SN of PD patients but not in control subjects (Hunot et al., 1996; Knott et al., 2000). Further, preclincal animal models demonstrated that iNOS inhibition protects neurons in different PD animal models (Liberatore et al., 1999; Dehmer et al., 2000; Iravani et al., 2002; Wu et al., 2002; Arimoto and Bing, 2003).

Recent animal studies have suggested that microglial p38 MAPK may act as the upstream signaling of iNOS since inhibition of p38 MAPK pathway reduced the production of nitric oxide and rescued dopaminergic neurons against MPP^+ and LPS insult (Du et al., 2001; Xing et al., 2008). As the product of iNOS, diffusible nitric oxide can readily form highly cytotoxic peroxynitrite, which can cause nitration of the key proteins resulting in the alteration of their structures and functions. A marker of nitration, 3-nitrotyrosine, has been shown by immunostaining to be increased in Lewy bodies in PD patients (Good et al., 1998). Several studies demonstrated an increased nitration of α-synuclein and its aggregation in human dopaminergic SH-SY5Y/microglia cell line cocultures upon LPS stimuli and in MPTP PD model (Paxinou et al., 2001; Przedborski et al., 2001; Shavali et al., 2006), and the increased α-synuclein nitration appears positively correlated with enhancing fibril formation and dopaminergic neuronal

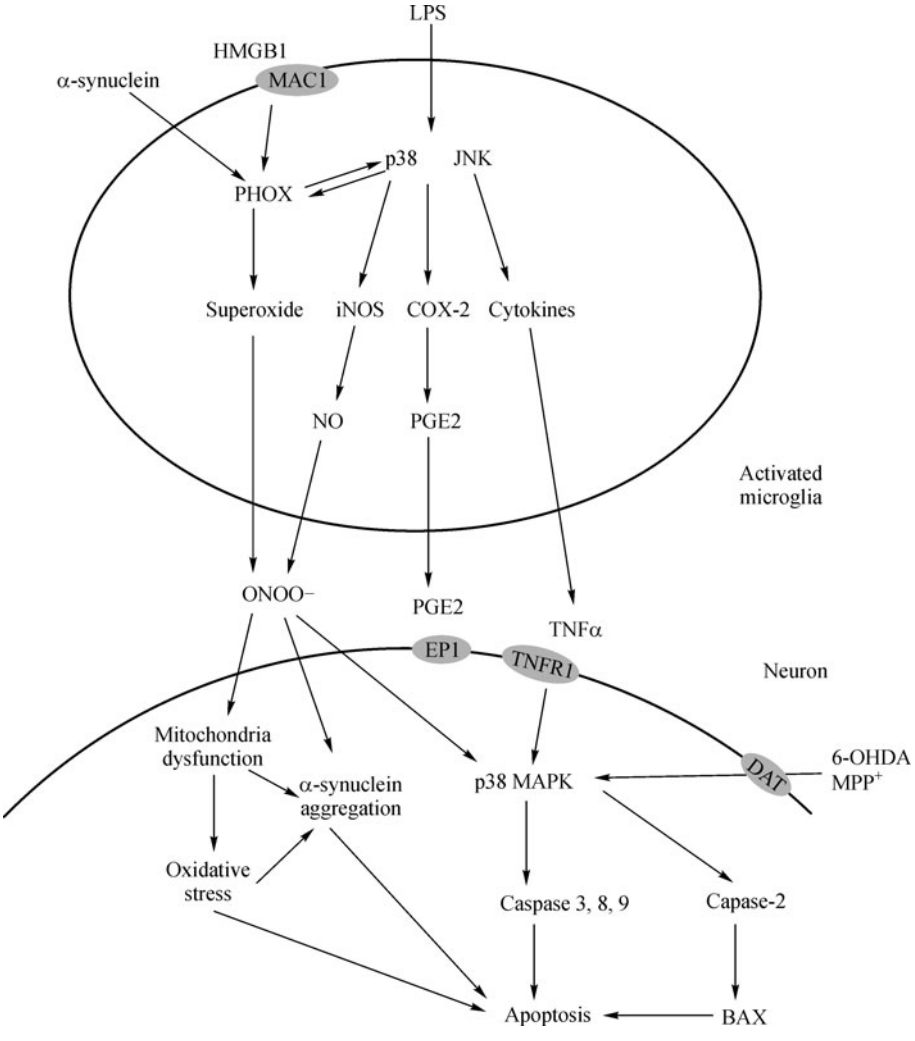

Figure 1 Schematic summary of the key molecules and their signaling cascades in the activated microglia and dopaminergic neuron. Upon the stimuli from LPS or other extracellular molecules such as HMGB1 and ones released from injured neurons such as α-synuclein, microglia are activated and release PHOX-mediated free radicals such as superoxide, and MAPKs signaling-mediated production of nitric oxide, progstagland E2, and cytokines such as TNFα. The superoxide and nitric oxide can readily react and form highly diffusible cytoxic molecules peroxynitrite (ONOO-), which can direclty cause the nitration of the key proteins such as α-synuclein and lead to its aggregation, causing neuronal apoptosis probably via inhibiting the function of ubiqutin proteasome system. In addition, ONOO- can react with mitochondrial membrane and cause its dysfunction via inhibition of complex I, enhancing the intracellular burden of oxidative stress. Microglia-derived PGE_2 induces neuronal death via its receptor EP1, though no downstream signaling has been studied in the neurons. Upon binding to its TNFR1 receptor, the overwhelming signaling from TNFα may initiate the activation of proinflammatory signaling such as p38 MAPK, leading to the activation of caspases signaling, ultimately leading to neuronal apoptosis.

death in primary neuronal/glia cultures (Hodara et al., 2004; Gao et al., 2008). Peroxynitrite can also directly react with mitochondria membranes and inhibits mitochondrial complex I (Murray et al., 2003), causing dopaminergic neuronal death (Sherer et al., 2007).

Besides direct modification on the proteins and their functions in the dopaminergic neurons, nitric oxide can also activate proinflammatory signaling cascades in neurons. The study on human neuroblastoma cells suggested that the activation of neuronal p38 MAPK induced by exogenous nitric oxide facilitates the translocation of cell death activator BAX from cytosol to the mitochondria along with neurodegeneration (Gomez-Lazaro et al., 2008). In contrast, BAX-deficiency in these neurons enables them to be resistant to nitric oxide-induced neurotoxicity (Ghatan et al., 2000). A more recent study on the rotenone *in vitro* model further demonstrated that activation of caspase-2 regulates BAX translocation and cytochrome c release from mitochondria and leads to cell death (Tiwari et al., 2011). Activation of neuronal p38 MAPK by superoxide and nitric oxide also activates the neuronal caspase- 3, -8 and-9 in the primary mesencephalic cultures (Choi et al., 2004). This is consistent with the finding that caspase 8 activation is higher in PD brains (Hartmann et al., 2001). All above studies implicated the important roles of neuronal p38 MAPK-caspases-BAX signaling cascades in the dopaminergic neuronal injury and death.

Another proinflammatory factor, cyclooxygenase-2 (COX-2), has been demonstrated in the activated microglia of PD patients but not in control subjects (Hunot et al., 1996; Knott et al., 2000). Pharmacological inhibition of COX-2 rescues dopaminergic neurons against MPTP and MPP^+ toxicity (Wang et al., 2005; Vijitruth et al., 2006). Recent study suggested that c-Jun N-terminal Kinase (JNK) might take part in the mediation of COX-2 activity since inhibition of JNK reduces the expression of COX-2 and rescues dopaminergic neurons against LPS insult in mesencephalic neuron/glia mixed cultures (Xing et al., 2007). PGE_2 is the major prostaglandin produced by COX-2. Direct neurotoxic effects have been shown for PGE_2 on dopaminergic neurons (Gao et al., 2003). Recent study on 6-OHDA-induced neurotoxicity in rat mesencephalic primary neuronal cultures showed that PGE_2 receptor EP1 on dopaminergic neurons might contribute to COX-2-mediated neuronal death since EP1 receptor agonist at nanomolar concentration killed dopaminergic neurons significantly and its selective antagonist can markedly rescue dopaminergic neurons (Carrasco et al., 2007).

Summary

Microglia activation has been considered to play an active role in the process of dopaminergic neurodegeneration and is strongly implicated in the key underlying mechanisms involved in neuroinflammtion and oxidative stress. Upon activation, microglia overproduce a variety of proinflammatory mediators and radical species which can be directly toxic to dopaminergic neurons via modification of key proteins or by initiating deleterious intraneuronal signaling cascades leading to mitochondrial dysfunction and neuronal death. It is noteworthy that there is tightly regulated cross-talk between activated microglia and injured neurons in the progress of dopaminergic neuronal death (Fig. 1). To further understand how these deleterious signaling pathways are regulated in the activated microglia and dopaminergic neurons will be very helpful in the future development of effective therapeutic strategies against progressive neuronal degeneration.

References

Arimoto T, Bing G (2003). Up-regulation of inducible nitric oxide synthase in the substantia nigra by lipopolysaccharide causes microglial activation and neurodegeneration. Neurobiol Dis, 12(1): 35–45

Arimoto T, Choi D Y, Lu X, Liu M, Nguyen X V, Zheng N, Stewart C A, Kim H C, Bing G (2007). Interleukin-10 protects against inflammation-mediated degeneration of dopaminergic neurons in substantia nigra. Neurobiol Aging, 28(6): 894–906

Betarbet R, Sherer T B, MacKenzie G, Garcia-Osuna M, Panov A V, Greenamyre J T (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci, 3(12): 1301–1306

Bing G Y, Lu N A, et al (1998). Microglia Mediaed Dopaminergic Cell Death in the *Substantia nigra*: a New Animal Model for Parkinson's Disease. Neuroscience Abstracts

Blandini F, Armentero M T (2012). Animal models of Parkinson's disease. FEBS J, 279(7): 1156–1166

Block M L, Hong J S (2005). Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol, 76(2): 77–98

Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch E C (1994). Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett, 172(1–2): 151–154

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

Cannon J R, Tapias V, Na H M, Honick A S, Drolet R E, Greenamyre J T (2009). A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis, 34(2): 279–290

Carrasco E, Casper D, Werner P (2007). PGE(2) receptor EP1 renders dopaminergic neurons selectively vulnerable to low-level oxidative stress and direct PGE(2) neurotoxicity. J Neurosci Res, 85(14): 3109–3117

Castaño A, Herrera A J, Cano J, Machado A (1998). Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. J Neurochem, 70(4): 1584–1592

Choi D Y, Liu M, Hunter R L, Cass W A, Pandya J D, Sullivan P G, Shin E J, Kim H C, Gash D M, Bing G (2009). Striatal neuroinflammation promotes Parkinsonism in rats. PLoS ONE, 4(5): e5482

Choi W S, Eom D S, Han B S, Kim W K, Han B H, Choi E J, Oh T H, Markelonis G J, Cho J W, Oh Y J (2004). Phosphorylation of p38 MAPK induced by oxidative stress is linked to activation of both caspase-8- and -9-mediated apoptotic pathways in dopaminergic neurons. J Biol Chem, 279(19): 20451–20460

Dehmer T, Lindenau J, Haid S, Dichgans J, Schulz J B (2000). Deficiency of inducible nitric oxide synthase protects against MPTP toxicity *in vivo*. J Neurochem, 74(5): 2213–2216

Du Y, Ma Z, Lin S, Dodel R C, Gao F, Bales K R, Triarhou L C, Chernet E, Perry K W, Nelson D L, Luecke S, Phebus L A, Bymaster F P, Paul S M (2001). Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci USA, 98(25): 14669–14674

Fontaine V, Mohand-Said S (2002). Neurodegenerative and neuroprotective effects of tumor necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. The Journal of neuroscience, 22(7): RC216

Gao H M, Jiang J, Wilson B, Zhang W, Hong J S, Liu B (2002). Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem, 81(6): 1285–1297

Gao H M, Kotzbauer P T (2008). Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. The Journal of neuroscience, 28(30): 7687–7698

Gao H M, Zhou H (2011). HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration." J Neurosci, 31(3): 1081–1092

Gao L, Zackert W E, Hasford J J, Danekis M E, Milne G L, Remmert C, Reese J, Yin H, Tai H H, Dey S K, Porter N A, Morrow J D (2003). Formation of prostaglandins E2 and D2 via the isoprostane pathway: a mechanism for the generation of bioactive prostaglandins independent of cyclooxygenase. J Biol Chem, 278(31): 28479–28489

Gayle D A, Ling Z, Tong C, Landers T, Lipton J W, Carvey P M (2002). Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. Brain Res Dev Brain Res, 133(1): 27–35

Ghatan S, Larner S, Kinoshita Y, Hetman M, Patel L, Xia Z, Youle R J, Morrison R S (2000). p38 MAP kinase mediates bax translocation in nitric oxide-induced apoptosis in neurons. J Cell Biol, 150(2): 335–347

Gomez-Lazaro M, Galindo M F, Concannon C G, Segura M F, Fernandez-Gomez F J, Llecha N, Comella J X, Prehn J H, Jordan J (2008). 6-Hydroxydopamine activates the mitochondrial apoptosis pathway through p38 MAPK-mediated, p53-independent activation of Bax and PUMA. J Neurochem, 104(6): 1599–1612

Good P F, Hsu A, Werner P, Perl D P, Olanow C W (1998). Protein nitration in Parkinson's disease. J Neuropathol Exp Neurol, 57(4): 338–342

Hald A, Lotharius J (2005). Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp Neurol, 193(2): 279–290

Hartmann A, Troadec J D, Hunot S, Kikly K, Faucheux B A, Mouatt-Prigent A, Ruberg M, Agid Y, Hirsch E C (2001). Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. J Neurosci, 21(7): 2247–2255

He Y, Appel S, Le W (2001). Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. Brain Res, 909(1–2): 187–193

Herrera A J, Castaño A, Venero J L, Cano J, Machado A (2000). The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. Neurobiol Dis, 7(4): 429–447

Hodara R, Norris E H, Giasson B I, Mishizen-Eberz A J, Lynch D R, Lee V M, Ischiropoulos H (2004). Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J Biol Chem, 279(46): 47746–47753

Hunot S, Boissière F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, Hirsch E C (1996). Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. Neuroscience, 72(2): 355–363

Hunot S, Dugas N (1999). FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, *in vitro*, production of nitric oxide and tumor necrosis factor-alpha in glial cells. The Journal of neuroscience, 19(9): 3440–3447

Hunter R L, Cheng B, Choi D Y, Liu M, Liu S, Cass W A, Bing G (2009). Intrastriatal lipopolysaccharide injection induces parkinsonism in C57/B6 mice. J Neurosci Res, 87(8): 1913–1921

Hunter R L, Dragicevic N, Seifert K, Choi D Y, Liu M, Kim H C, Cass W A, Sullivan P G, Bing G (2007). Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system. J Neurochem, 100(5): 1375–1386

Iravani M M, Kashefi K, Mander P, Rose S, Jenner P (2002). Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience, 110(1): 49–58

Jenner P, Olanow C W (1996). Oxidative stress and the pathogenesis of Parkinson's disease. Neurology, 47(6 Suppl 3): S161–S170

Kim W G, Mohney R P (2000). Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. J Neurosci, 20(16): 6309–6316

Kirik D, Rosenblad C, Björklund A (1998). Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp Neurol, 152(2): 259–277

Knott C, Stern G, Wilkin G P (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and-2. Mol Cell Neurosci, 16(6): 724–739

Langston J W, Ballard P, Tetrud J W, Irwin I (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science, 219(4587): 979–980

Lapointe N, St-Hilaire M (2004). Rotenone induces non-specific central nervous system and systemic toxicity. FASEB journal, 18(6): 717–719

Li R, Yang L (2004). Tumor necrosis factor death receptor signaling cascade is required for amyloid-beta protein-induced neuron death. The Journal of neuroscience, 24(7): 1760–1771

Liberatore G T, Jackson-Lewis V, Vukosavic S, Mandir A S, Vila M, McAuliffe W G, Dawson V L, Dawson T M, Przedborski S (1999). Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med, 5(12): 1403–1409

Loeffler D A, DeMaggio A J, Juneau P L, Havaich M K, LeWitt P A (1994). Effects of enhanced striatal dopamine turnover *in vivo* on

glutathione oxidation. Clin Neuropharmacol, 17(4): 370–379

Long-Smith C M, Collins L, Toulouse A, Sullivan A M, Nolan Y M (2010). Interleukin-1β contributes to dopaminergic neuronal death induced by lipopolysaccharide-stimulated rat glia *in vitro*. J Neuroimmunol, 226(1–2): 20–26

Lozano A M, Lang A E, Hutchison W D, Dostrovsky J O (1998). New developments in understanding the etiology of Parkinson's disease and in its treatment. Curr Opin Neurobiol, 8(6): 783–790

Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel U L (2004). Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. J Biol Chem, 279(31): 32869–32881

McCoy M K, Martinez T N (2006). Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. The Journal of neuroscience, 26(37): 9365–9375

McGeer P L, Itagaki S, Akiyama H, McGeer E G (1988a). Rate of cell death in parkinsonism indicates active neuropathological process. Ann Neurol, 24(4): 574–576

McGeer P L, Itagaki S, Boyes B E, McGeer E G (1988b). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology, 38(8): 1285–1291

McGeer P L, Schwab C, Parent A, Doudet D (2003). Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. Ann Neurol, 54(5): 599–604

Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, Nagatsu T (1994a). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci Lett, 180(2): 147–150

Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T (1994b). Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett, 165(1–2): 208–210

Mogi M, Togari A, Kondo T, Mizuno Y, Komure O, Kuno S, Ichinose H, Nagatsu T (2000). Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. J Neural Transm, 107(3): 335–341

Murray J, Taylor S W, Zhang B, Ghosh S S, Capaldi R A (2003). Oxidative damage to mitochondrial complex I due to peroxynitrite: identification of reactive tyrosines by mass spectrometry. J Biol Chem, 278(39): 37223–37230

Nagatsu T, Mogi M, Ichinose H, Togari A (2000). Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm Suppl, (60): 277–290

Nakamura Y (2002). Regulating factors for microglial activation. Biol Pharm Bull, 25(8): 945–953

Olanow C W, Tatton W G (1999). Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci, 22(1): 123–144

Pawate S, Shen Q, Fan F, Bhat N R (2004). Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. J Neurosci Res, 77(4): 540–551

Paxinou E, Chen Q (2001). Induction of alpha-synuclein aggregation by intracellular nitrative insult. The Journal of neuroscience, 21(20): 8053–8061

Perese D A, Ulman J, Viola J, Ewing S E, Bankiewicz K S (1989). A 6-hydroxydopamine-induced selective parkinsonian rat model. Brain Res, 494(2): 285–293

Przedborski S, Chen Q, Vila M, Giasson B I, Djaldatti R, Vukosavic S, Souza J M, Jackson-Lewis V, Lee V M, Ischiropoulos H (2001). Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. J Neurochem, 76(2): 637–640

Przedborski S, Levivier M, Jiang H, Ferreira M, Jackson-Lewis V, Donaldson D, Togasaki D M (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. Neuroscience, 67(3): 631–647

Qin L, Liu Y, Wang T, Wei S J, Block M L, Wilson B, Liu B, Hong J S (2004). NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem, 279(2): 1415–1421

Ransohoff R M, Perry V H (2009). Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol, 27(1): 119–145

Shavali S, Combs C K, Ebadi M (2006). Reactive macrophages increase oxidative stress and alpha-synuclein nitration during death of dopaminergic neuronal cells in co-culture: relevance to Parkinson's disease. Neurochem Res, 31(1): 85–94

Sherer T B, Kim J H, Betarbet R, Greenamyre J T (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. Exp Neurol, 179(1): 9–16

Sherer T B, Richardson J R, Testa C M, Seo B B, Panov A V, Yagi T, Matsuno-Yagi A, Miller G W, Greenamyre J T (2007). Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. J Neurochem, 100(6): 1469–1479

Tiwari M, Lopez-Cruzan M, Morgan W W, Herman B (2011). Loss of caspase-2-dependent apoptosis induces autophagy after mitochondrial oxidative stress in primary cultures of young adult cortical neurons. J Biol Chem, 286(10): 8493–8506

Vijitruth R, Liu M, Choi D Y, Nguyen X V, Hunter R L, Bing G (2006). Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J Neuroinflammation, 3(1): 6

Wang, T., Pei, Z., et al (2005). MPP^+-induced COX-2 activation and subsequent dopaminergic neurodegeneration. FASEB journal, 19(9): 1134–1136

Wu D C, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi D K, Ischiropoulos H, Przedborski S (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci, 22(5): 1763–1771

Wu D C, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, Przedborski S (2003). NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Proc Natl Acad Sci USA, 100(10): 6145–6150

Xing B, Liu M, Bing G (2007). Neuroprotection with pioglitazone against LPS insult on dopaminergic neurons may be associated with its inhibition of NF-kappaB and JNK activation and suppression of COX-2 activity. J Neuroimmunol, 192(1–2): 89–98

Xing B, Xin T, Hunter R L, Bing G (2008). Pioglitazone inhibition of lipopolysaccharide-induced nitric oxide synthase is associated with altered activity of p38 MAP kinase and PI3K/Akt. J Neuroinflamma-

tion, 5(1): 4

Zhang F, Shi J S, Zhou H, Wilson B, Hong J S, Gao H M (2010). Resveratrol protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory actions. Mol Pharmacol, 78(3): 466–477

Zhang J, Perry G, Smith M A, Robertson D, Olson S J, Graham D G, 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(5): 1423–1429

Zhang J, Stanton D M, Nguyen X V, Liu M, Zhang Z, Gash D, Bing G (2005). Intrapallidal lipopolysaccharide injection increases iron and ferritin levels in glia of the rat substantia nigra and induces locomotor deficits. Neuroscience, 135(3): 829–838

Zhang W, Wang T (2005). Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB journal, 19(6): 533–542