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The Lipopolysaccharide Parkinson's disease animal model: mechanistic studies and drug discovery

Garima Dutta^a, Ping Zhang^a, and Bin Liu^{a,b,*}

^aDepartment of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA

^bThe McKnight Brain Institute, University of Florida, Gainesville, FL 32610, USA

Abstract

Research in the last two decades has unveiled an important role for neuroinflammation in the degeneration of the nigrostriatal dopaminergic pathway that constitutes the pathological basis of the prevailing movement disorder, Parkinson's disease (PD). Neuroinflammation is characterized by the activation of brain glial cells, primarily microglia and astrocytes that release various soluble factors that include free radicals (reactive oxygen and nitrogen species), cytokines, and lipid metabolites. The majority of these glia-derived factors are proinflammatory and neurotoxic and are particularly deleterious to oxidative damage-vulnerable nigral dopaminergic neurons. As a proof of concept, various immunologic stimuli have been employed to directly induce glial activation to model dopaminergic neurodegeneration in Parkinson's disease. The bacterial endotoxin, lipopolysaccharide (LPS), has been the most extensively utilized glial activator for the induction of inflammatory dopaminergic neurodegeneration. In this review, we will summarize the various in vitro and in vivo LPS PD models. Furthermore, we will highlight the contribution of the LPS PD models to the mechanistic studies of PD pathogenesis and the search for neuroprotective agents for the treatment of PD.

Keywords

dopamine; lipopolysaccharide; microglia; neuroinflammation; neuroprotection

Introduction

Parkinson's disease (PD) is primarily an age-related debilitating neurodegenerative disorder of the extrapyramidal motor neurons characterized by a selective and gradual loss of dopaminergic (DA) innervations from the substantia nigra pars compacta (SNpc) to the striatum (caudate and putamen) of the basal ganglia [1,2]. Progressive degeneration of the nigrostriatal DA pathway eventually leads to the development of clinical symptoms that include bradykinesia, rigidity, tremor and defective gait, mostly in people over the age of 60. Currently, there are no therapies for modifying the course of neurodegeneration and no biomarkers for making an early diagnosis. Postmortem confirmative diagnosis often detects a massive loss of SNpc DA neurons and the presence of the characteristic cytoplasmic inclusions called Lewy bodies in survived neurons. Except for a small fraction of early onset cases of PD that are linked to mutations in a dozen genes, most cases of PD are idiopathic [3,4]. Risk factors for idiopathic

PD include age, genetic predisposition, and exposure to agents such as pesticides, metals, and infectious agents.

Advance in modeling idiopathic PD in the last three decades has provided us with powerful animal PD models that are created by exposure to toxins such as 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), rotenone, and the combination of paraquat and maneb [5]. Significant insights continue to be gained through the analysis of these toxin-induced as well as genetic models of PD. However, none of the current animal models of PD appears to encompass all the prevailing pathologies of the disease, thus hampering the development of effective neuroprotective treatment strategies and the discovery of early stage diagnostic biomarkers.

Findings from epidemiological studies and analysis of postmortem PD brains and animal PD models have provided increasing evidence to support a role for inflammation in the brain in the pathogenesis of PD [6,7]. Neuroinflammation primarily involves the activity of glial cells that have been categorized into macroglia and microglia. Macroglia include astroglia, oligodendrocytes and ependymal cells in the CNS. Astroglia are the most abundant glia and are critical to the survival of neurons by regulating their chemical microenvironment and providing trophic support. Upon neuronal injury, astroglia proliferate and increase their trophic support to neurons [8]. Microglia, a term coined by del Rio Hortega around the 1920s and initially studied independently by Nissl and Robertson [9,10], are derived from hematopoietic stem cells and play a crucial role as specialized macrophages in safeguarding CNS against infections and injury. As resident phagocytes in the brain, microglia serve to clear cellular debris. Equally important, microglia respond to slight disturbances in the microenvironment and readily become activated [11]. Microglial activation involves characteristic morphological transformation from resting (ramified) to the activated (amoeboid) state and upregulation of MHC antigens and complement receptors [9,12-14]. Furthermore, activated microglia, as well as activated astroglia to a lesser extent, release various proinflammatory and neurotoxic factors such as tumor necrosis factor- α (TNF α), interleukin-1 beta (IL-1 β), IL-6, eicosanoids, proteinases, and reactive nitrogen and oxygen species [15,16]. Sustained activation of glia, especially microglia, and accumulation of proinflammatory and neurotoxic factors are believed to contribute to the progressive DA neurodegeneration.

As a proof of concept, lipopolysaccharide (LPS) has been used to determine whether direct activation of glia, in particular microglia, would result in a progressive and selective degeneration of DA neurons in rodents (Table I). LPS is an endotoxin found in the outer membrane of gram-negative bacteria. It is composed of three components: O-antigen with multiple repeating units of monosaccharides, a polysaccharide core with an unusual sugar (2-keto-3-deoxyoctonate), and lipid A consisting of a unique diglucosamine backbone to which six fatty acid chains are attached (Fig. 1). LPS associates with the soluble LPS binding protein (LBP) and CD14 which is anchored in the outer leaflet of the plasma membrane. Signal transduction across the plasma membrane is made possible through the interaction of the LPS-CD14 complex with the transmembrane Toll-like receptor-4 (TLR-4) and the extracellular accessory protein MD-2 (Fig. 1). Members of the TLR family receptors are important for the detection of various pathogens by cells in the innate immune system and cell-cell communications [17,18]. In the case of TLR-4, association with the LPS-CD14 complex leads to the activation of various intracellular signaling pathways kinases and upregulation of gene transcription for a variety of proinflammatory factors and free radical-generating enzymes (Fig. 1). LPS is a potent stimulator of both peripheral immune cells (macrophages and monocytes) and CNS glia (microglia and astrocytes) and causes their release of various immunoregulatory and proinflammatory cytokines and free radicals [19-22]. In contrast, LPS does not seem to have a direct effect on neurons most likely due their lack of functional expression of TLR4 [23], making it an excellent tool to study inflammation-mediated DA neurodegeneration [24].

LPS models of PD

Cell culture-based in vitro LPS PD model

The in vitro cell culture LPS PD model is based on the mesencephalic mixed neuron-glia culture system. Ventral midbrain tissues that contain the emerging nigrostriatal DA neurons, as well as glial precursor cells are harvested from 14-day old rat embryos [25,26]. The embryonic precursor cells (neurons and glia) are allowed to differentiate and mature in a serum-containing media. Various cell types go through a gradual proliferation and/or maturation process. By 6-8 days in vitro (DIV), the proliferation of astroglia has led to the formation of a complete “feeder” layer and the maturation of various neurons has resulted in the formation of an intricate neuronal network (Fig. 2). Tyrosine hydroxylase (TH)-positive neurons are well differentiated and exhibit extensive dendritic processes (Fig. 2). Microglia retains the characteristic in vitro resting morphology: round and small [27]. Generally, cultures are ready for treatment with agents of interest at DIV 7 when they are composed of approximately 40% neurons with ~1% being DA neurons, 10% microglia and 50% astroglia. Treatment of the neuron-glia cultures with LPS (0.1-10 ng/ml) for up to 10 days leads to a selective and progressive degeneration of DA neurons [27]. Several important characteristics of the inflammation-mediated DA neurodegeneration have been revealed through studies using the in vitro LPS PD model. First, LPS-induced neurodegeneration is primarily observed in DA neurons and non-DA neurons are mostly spared [27-30]. Second, microglial activation precedes DA neurodegeneration. For example, significant microglial activation was observed 1 day after treatment with 0.1 ng/ml LPS but DA neurodegeneration was not observed until 10 days later [27]. Third, microglia play a more prominent role than astroglia in the release of various neurotoxic factors that cause DA neurodegeneration [31].

The in vitro LPS PD model is a powerful system for mechanistic studies of inflammation-mediated DA neurodegeneration. This is best exemplified by the use of enriched neurons, glia and reconstituted neuron-glia cultures to dissect the cellular and molecular mediators of DA neurodegeneration [27,31]. Application of this strategy to enriched/reconstituted cultures from gene knockout mice has helped pinpoint the contribution of factors to DA neurodegeneration [27,31]. Finally, the in vitro LPS PD model is an economic and efficient system very suitable for the initial screening of neuroprotective agents prior to embarking on the more costly and labor-intensive whole animal studies.

SN single injection LPS PD model

To extend the observations made in the in vitro LPS PD model to a physiologically more relevant setting, the single SN injection model was developed. A single injection of a bolus of low microgram quantities of LPS to the SN region of Wistar, Fisher or Sprague Dawley rats indeed leads to a marked loss (50-85%) of SNpc DA neurons [32-34]. Compared to the in vitro LPS PD model, the SN single injection model made possible the comparison of the relative vulnerability to inflammatory damage of DA neurons in the SN versus those in the VTA, DA versus non-DA neurons in the SN, and DA versus non-DA neuronal projections in the striata.

Injection of LPS to the SN region results in a progressive, preferential and irreversible loss of the SNpc DA neurons. In one study, unilateral SN injection of 2 µg LPS to Wistar rats decreased DA levels in both the striatum and SN (~50%) up to 21 days after LPS injection [32]. Non-DA neurons in the SN and DA neurons in the adjacent VTA region were spared of the LPS-induced damage [32,33]. The preferential degeneration of SNpc DA neurons was further corroborated by studies that employed fluorogold retrograde labeling of the striatonigral DA pathway prior to LPS injection [35]. Subsequent studies showed that 5-HT levels remained unchanged 7 days after an injection of 30 µg LPS or even one year after an injection of 2 µg of LPS to the SN region [36,37].

In the context of neuroinflammation-mediated DA neurodegeneration, the SN single injection LPS PD model made it possible to elucidate, in vivo, the temporal relationship between activation of glia (microglia and astrocytes) and DA neurodegeneration. LPS induced a rapid activation of microglia within hours as demonstrated by morphological transformation of OX-42-positive microglia, upregulation of proinflammatory cytokines, and generation of free radicals [33,36,38]. In contrast, loss of SNpc DA neurons was not observed until 1 week after LPS injection [33,37]. Compared to the rapid microglial activation, activation of astroglia occurred at a slower rate and became significant around 1 week after LPS injection [37]. Therefore, LPS-induced activation of glia, especially microglia, and their release of proinflammatory and neurotoxic factors precede the degeneration of the nigrostriatal DA pathway.

SN chronic infusion LPS PD model

The successful demonstration of SN LPS injection-induced DA neurodegeneration prompted further examination on whether a less intense and chronic period of inflammation in the SN would lead to a delayed and progressive nigrostriatal DA neurodegeneration. To this end, nanogram quantities of LPS packaged in an osmotic mini-pump was slowly delivered for a period of two weeks to the SN of Fisher F344 rats [27]. Chronic infusion of LPS to the SN induced an increasing degree of microglial activation in the SN. At the earliest time point studied (3 days after the start of LPS infusion), robust activation of SN microglia was detected. Between 1-2 weeks after the start of LPS infusion, SN microglia became fully activated exhibiting the characteristic amoeboid morphology [27]. In contrast, no apparent loss of SNpc DA neurons was observed during the two-week period when LPS was being delivered. Loss of SNpc DA neurons began to occur between 2-4 weeks but did not become significant until 6 weeks after the start of LPS infusion [27,30]. By 10 weeks after the start of LPS infusion (i.e., 8 weeks after the termination of LPS infusion), the loss of SNpc DA neurons had reached approximately 60% compared to the control [27,30]. The specificity of SNpc DA neurodegeneration was ascertained by confocal immunofluorescent analysis of DA neurons and neurons in general as well as Nissl body labeling [27,30]. Compared to the massive loss of SNpc DA neurons, DA neurons of the VTA region or non-DA neurons such as GABAergic neurons in the SN remained mostly unaffected [27,30]. These findings suggest that a brief episode (2 weeks) of neuroinflammation that occurs early in life is capable of inducing significant glial activation accompanied by a delayed, progressive and preferential degeneration of SNpc DA neurons. Continued research on the consequence of chronic LPS supranigral infusion on the degeneration of the entire nigrostriatal pathway, specifically, the depletion of striatal DA and the motor capacity of the infused animals should significantly complement this model.

Systemic LPS injection PD model

Systemic inflammation has been suspected to influence the activities of the immune cells in the brain and consequently contribute to the chronic neurodegenerative process for diseases such as PD [39]. A recent study reported that chronic microglial activation and progressive DA neurodegeneration were observed following an intraperitoneal injection of 5 mg/kg of LPS in C57 mice [39]. Loss of nigral DA neurons reached 23% and 43% at 7 and 10 months respectively. Similar to that observed in the single SN injection and chronic SN infusion LPS PD models, DA neurons in the adjacent VTA region were relatively spared of the LPS-induced damage. Microglial activation was detected in the SN and other brain regions at very early stages following LPS administration, coinciding with increased levels of TNF α and IL-1 β message and/or protein. Levels of TNF α protein in the brain, in the meantime, remained elevated for the entire course of the study (10 months) [39].

In utero LPS injection PD model

During pregnancy, a fraction of women suffer from vaginal or cervical bacterial infections and there may be a risk for bacterial toxins including LPS to impact the fetal development [40, 41]. One of the potential targets for endotoxin assault may be the developing nigrostriatal DA pathway. To test the effects of bacterial endotoxin on the developing DA system, LPS (10,000 endotoxin units) was administered by an in utero injection to gravid Sprague Dawley rats at gestation day 10.5, a critical time point during embryonic DA neuron development [42]. In 3 week-old pups from in utero LPS-exposed rat mothers, a significant reduction in the number of SNpc DA neurons (27%) and striatal DA content (29%) was observed when compared to pups from saline-injected rat mothers [42]. In utero LPS exposure did not appear to affect DA-neurons in the VTA region and non-DA neurons in the SN [43]. LPS administration resulted in significant microglial activation and a sustained elevation of TNF α in both the SN and striata [42]. In addition to causing a reduction in SNpc DA neurons, in utero LPS exposure appears to predispose the nigrostriatal DA system of the pups to enhanced susceptibility to neurotoxins such as rotenone and 6-OHDA [43,44].

Intrapallidal LPS injection PD model

Rather than delivering LPS directly to the supranigral region, in one study, LPS was injected to the globus pallidus, an integral component of the basal ganglia that is important in movement regulation [45]. Bilateral intrapallidal injection of LPS (10 μ g) was administered to young (3 month-old) and middle aged (16 month-old) Fisher F344 rats. Microglial activation was evident in both groups of rats 4 weeks after LPS injection. At the same time point, compared to the control rats, LPS injection resulted in a greater loss of SNpc DA neurons in the older (70%) than the younger rats (~20%). In addition, α -synuclein-positive intracellular inclusions were detected in the SN DA neurons of the LPS-injected middle-aged rats but not in the LPS-injected younger rats. Furthermore, LPS-injection induced locomotor deficits in both age groups. However, while the aged rats exhibited slowed locomotor movement throughout the entire 4-week period of the study, the young rats resumed normal locomotor movement within 2 weeks after the LPS injection [45].

Key Mediators of LPS-Induced DA Neurodegeneration

LPS is capable of activating glial cells to release a wide array of proinflammatory and neurotoxic factors that include oxygen and nitrogen-centered free radicals, cytokines and lipid mediators. Results from studies thus far with enzyme inhibitors, neutralizing antibodies, and gene knockout animals have identified the following factors as major contributors to the DA neurodegeneration induced by LPS-activated glia.

Nitric Oxide (NO)

Excessive production of NO by activated glia, especially microglia, has been attributed to contribute to DA neurodegeneration [24]. Several lines of evidence support a role for over production and accumulation of NO in the LPS-induced DA neurodegeneration. First, LPS causes the upregulation of the inducible nitric oxide synthase (iNOS) and release of NO in microglial, astroglial but not neuronal cultures [46-48]. Furthermore, after SN LPS injection, an increase in nitric oxide synthase activity could be observed [49,50]. Second, LPS-induced NO production precedes DA neurodegeneration [51]. Third, blockade of NO production by inhibiting the activity of iNOS reduces LPS-induced DA neurodegeneration in cultures and in animal models [35,49,50]. Mechanistically, NO has been suggested to contribute to LPS-induced DA neurodegeneration through several mechanisms. NO can react with superoxide free radical to form peroxynitrite radicals that are highly damaging to neurons due to their extreme reactivity [52]. NO has also been shown to inhibit complex 1 and complex 4 of

mitochondria and cause reduction in ATP synthesis [53]. NO can also reverse glial glutamate transporter and hence increase glutamate levels to cause excitatory toxicity to neurons [54].

Reactive oxygen species

Besides NO, ROS generated by activated glia, especially microglia are major mediators of the inflammation-mediated DA neurodegeneration. ROS can cause lipid peroxidation, protein oxidation, DNA damage, and mitochondrial dysfunction [55]. LPS-induced ROS production in microglia is mediated by NADPH oxidase, a multi-subunit enzyme system [27,56]. Pharmacologic inhibition of NADPH oxidase affords protection against LPS-induced neurotoxicity and NADPH oxidase knockout mice are resistant to LPS-induced loss of SNpc DA neurons [56]. LPS-induced activation of NADPH oxidase and induction of iNOS in microglia leads to the formation of the more toxic intermediate peroxynitrite and the formation of peroxynitrite and its toxicity are abolished in microglia from NADPH oxidase knockout mice [57]. While ROS released from activated microglia can be directly deleterious to neurons, intracellular ROS in microglia have been shown to upregulate the gene expression of TNF α , IL-1 β and cyclooxygenase-2 (COX-2) to enhance the collective inflammatory insult on neurons [58-60].

Cytokines

Of the variety of cytokines that are released by LPS-activated glia, the proinflammatory IL-1 β and TNF α may be the major cytokines involved in the LPS-induced DA neurodegeneration [61-63]. Compared to astroglia, microglia appear to possess a larger repertoire of cytokine production [16]. For example, one study showed that LPS stimulated IL-1 β release in microglia but not astrocytes [61]. Furthermore, differential kinetics exist for LPS-stimulated microglial release of TNF α and IL-1 β [16]. In cell culture studies, significant TNF- α release was detected as early as several hours following LPS stimulation and significant IL-1 β release was not observed until 24 hours later [29,59]. Interestingly, in rats prenatally exposed to LPS, a sustained elevation of TNF α was observed in the striatum and mesencephalon [44]. The contribution of these cytokines to DA neurodegeneration was supported by studies showing that neutralizing antibodies against TNF α or IL-1 β markedly reduced the LPS-induced loss of DA neurons [64]. Furthermore, in the chronic LPS SN infusion PD model, loss of SNpc DA neurons was significantly reduced by blockade of TNF α signaling pathway [30].

Part of the challenge to sort out the contribution of individual cytokines to neurodegeneration may be a result of the complex interplay among various cytokines, pro- and anti-inflammatory cytokines alike. Not only microglial TNF α upregulates its own production in an autocrine fashion [22,59], TNF α can also increase the surface expression of the neuronal TNF α death receptor (TNFR1), thus exacerbating the LPS-induced neurotoxicity [65]. On the other hand, while LPS activates microglia to release TNF α IL-1 β , and IL-6 [59], microglial TNF α secretion, however, is inhibitable by IL-6 or IL-10 [66]. IL-10, an anti-inflammatory cytokine has been shown to reduce LPS-induced microglial activation and loss of SNpc DA neurons [67,68].

Cyclooxygenase-2 (COX-2)

LPS is known to upregulate the expression of COX-2 and increase the release of lipid mediators such as prostaglandin E₂ (PGE₂) in microglia [60,69]. Intrastriatal injections of LPS resulted in a significant upregulation of the striatal protein expression of COX-2 as well as the activation of microglia. In contrast, the expression of COX-1 protein was not affected by LPS administration [70]. Pharmacological inhibition of COX-2 activity protected DA neurons in midbrain slice cultures or neurons in cortical neuron-glia cultures from LPS-induced neurodegeneration [71,72]. Administration of celecoxib, an inhibitor of COX-2, markedly

reduced the loss of SNpc DA neurons induced by intrastrially injected LPS in rats. Interestingly, the LPS intrastriatal injection-induced decrease in striatal DA content and increase in striatal DA turnover were not restored by the administration of celecoxib [70].

While the precise molecular mechanisms of action responsible for the neurotoxicity of individual factors released from activated glia remain to be completely understood, it is clear that a complex interplay exists among various proinflammatory and neurotoxic factors. First, TNF α and IL-1 β released from LPS-stimulated microglia are capable of upregulating, in an autocrine fashion their own release [59]. Second, LPS-induced microglial ROS production appears to have two consequences: a) an increased release of extracellular ROS such as superoxide that can combine with NO to form more damaging peroxynitrite and b) a rise in intracellular ROS that can further enhance the gene expression of TNF α , IL-1 β , and COX-2 [22,60]. Third, one factor released from LPS-activated microglia can regulate, in a paracrine fashion, the expression of another factor. For example, IL-1 β acts as an important mediator of COX-2 upregulation, a phenomenon not observed in IL-1 β -null mice [73,74]. Furthermore, proinflammatory and neurotoxic factors released from activated glia are known to work in concert to induce neurotoxicity. Further knowledge of the interplays will aid the development of glia activity-modulating therapies for the treatment of PD.

Neuroprotective Drug Discovery Using LPS PD Models

Since the establishment of the various in vitro and in vivo LPS PD models, a variety of agents have been evaluated for their potential neuroprotective activities. Perhaps, due to the versatility of the in vitro LPS PD model, a great many of the neuroprotection studies have been carried out using in vitro LPS PD model. Agents that show great promise in the in vitro studies have, in several instances, been evaluated using the various in vivo models. In this section, we highlight the potential neuroprotective agents that have been tested in both the in vitro and in vivo LPS PD models (Table II).

Nicotine

A number of epidemiological studies have found an inverse correlation between cigarette smoking and incidence of PD in the general populations [75-77]. Nicotine is one of the major components of tobacco smoke and it has been shown to have a modulatory effect on the nigrostriatal DA pathway [78,79], possibly through its interaction with the α -4 or α -7 subunits of the nicotinic receptor complex [79,80].

More importantly, interaction of nicotine with microglial α -7 nicotinic receptor has been shown to suppress the LPS-induced release of TNF α [81-83]. Therefore, nicotine may possess anti-inflammatory activity that could protect DA neurons against inflammatory damage. Indeed, micromolar concentration of nicotine significantly reduced the LPS-stimulated release of TNF α and loss of DA neurons in cell culture LPS PD model [80]. In Sprague Dawley rats, administration of nicotine (1 mg/kg, 5 times a day) significantly reduced the release of TNF α and the loss of SNpc DA neurons induced by intranigral LPS injection [80]. Interestingly, the neuroprotective effect of nicotine could only be observed with relatively low doses of nicotine as high doses of nicotine seemed to desensitize nicotinic receptors, hence reducing its potential anti-inflammatory activity [78]. Furthermore, considering the addictive and harmful effects associated with nicotine, a drug that targets a specific subtype of nicotinic acetylcholine receptor may be more suitable as a potential neuroprotective agent.

Minocycline

Minocycline is a member of the broad-spectrum tetracycline antibiotics and it has been shown to have anti-inflammatory activities. In the cell culture LPS PD model, nanomolar

concentrations of minocycline significantly protected DA neurons against-LPS induced neurotoxicity [84]. The underlying mechanism of action responsible for the neuroprotective effect of minocycline has been attributed to its inhibition of LPS-stimulated NO production, TNF α release, and caspase-3 activation [84]. Administration of minocycline (45 mg/kg) significantly reduced SN microglial activation induced by intranigral LPS injection in Wistar rats [85]. The LPS-induced expression of proinflammatory factors such as IL-1 β and TNF α and the production of peroxynitrites were also inhibited by minocycline administration. Moreover, LPS-induced loss of SNpc DA neurons was significantly decreased in minocycline-dosed rats [85].

Naloxone Stereoisomers

Although studies have reported the presence of functionally relevant opioid receptors on microglia [86,87], naloxone, a structural analog of morphine and a non-selective opioid receptor antagonist has been found to reduce microglial activation-mediated DA neurodegeneration in a seemingly opioid receptor-independent manner.

In the cell culture LPS PD model, LPS-induced microglial release of TNF α and IL-1 β , production of ROS and NO, and loss of DA neurons were significantly reduced by treatment with either (-)-naloxone, the opioid receptor antagonist or (+)-naloxone which is not an opioid receptor antagonist [29]. In the SN single injection LPS PD model, systemic administration of either naloxone isomers effectively reduced LPS-induced microglial activation in the SN and the loss of SNpc DA neurons [33,34]. While naloxone isomers were effective in reducing ROS production in LPS-activated microglia [29], they did not appear to possess anti-oxidant properties since neither was capable of affecting the xanthine-xanthine oxidase-driven superoxide free radical generation [6]. In addition, naloxone in combination with indomethacin, a non-selective inhibitor of COX-1 and COX-2 reduced the LPS-induced striatal DA depletion [88].

Dexamethasone

Glucocorticoids have been used for the treatment of brain inflammation and spinal cord injury due to their anti-inflammatory properties [89,90]. In the single SN injection LPS PD model, systemic administration of dexamethasone (2 mg/kg) to Wistar rats significantly inhibited LPS-induced microglial activation [91]. Furthermore, dexamethasone significantly attenuated LPS-induced reduction in striatal DA content and the loss of SNpc DA neurons [91]. In Sprague–Dawley rats, an intraperitoneal injection of dexamethasone (4 mg/kg) significantly inhibited microglial activation in the SN and protected SNpc DA neurons from degeneration induced by an intranigral injection of LPS [49]. These studies demonstrated the efficacy of dexamethasone in affording protection of DA neurons against inflammatory damage at least in animal LPS PD models. However, the side effects associated with long-term use of steroids in humans need to be kept in mind.

Peroxisome proliferator activated receptors gamma (PPAR- γ) agonist

Peroxisome proliferator activated receptors (PPAR) exist in three forms (α , β and γ) and are activated by natural ligands such as fatty acids and eicosanoids. PPAR- γ is predominantly expressed in macrophages and cells of the intestine and adipose tissue. PPAR- γ ligands have been shown to inhibit TNF α , IL-6 and IL-1 β expression at the transcription level in monocytes and macrophages [92-94]. A recent study showed that pioglitazone, a PPAR- γ agonist, at a concentration of 20 mg/kg, significantly attenuated the loss in SNpc DA neurons and partially restored the depletion of striatal DA content induced by intrastratial LPS injection in Sprague–Dawley rats [70]. The neuroprotective effects of pioglitazone-induced PPAR- γ activation were attributed to its partial restoration of mitochondrial function and reduction of oxidative stress and its inhibition of microglial activation in the nigrostriatal DA pathway following LPS

injection [70]. Interestingly, pioglitazone also reduced the LPS-induced loss of insulin receptors that are highly expressed in SN of non-PD brains but are significantly reduced in the SN of PD brains [70].

Conclusion and Perspective

Neuroinflammation has been increasingly associated with the development of PD in humans and animal models. However, a direct cause-effect relationship has not been firmly established for bacterial and/or viral infection-induced development of PD in humans. Epidemiological studies on early life occurrence of encephalitis lethargica and later development of parkinsonism remain intriguing and yet controversial [95,96]. Experimentally, administration of Japanese encephalitis virus reproduces several features of PD in rats [97,98]. Injection of the bacteria *Norcardia asteroides* has been shown to induce Parkinsonism in mice. However, its relevance to human PD development requires further investigation [99,100].

Nevertheless, utilization of bacterial endotoxin LPS as a potent stimulator of glial cells, especially microglia, has taught us important lessons in modeling inflammation-mediated DA neurodegeneration for PD in animals. First, directly evoking an episode of neuroinflammation in the nigrostriatal region of the rodent brains does result in a delayed, progressive and preferential degeneration of the nigrostriatal DA pathway, reminiscent of DA neurodegeneration in PD. Second, various neurotoxic factors released from LPS-activated microglia and astroglia are sufficient to initiate and drive the progressive DA neurodegenerative process. Third, the LPS PD model has provided us with an important tool to delineate the precise contribution of various proinflammatory and neurotoxic factors to DA neurodegeneration. This type of information will help us design specific agents that may modify the DA neurodegenerative process and/or identify early stage-diagnostic biomarkers. Fourth, the LPS PD model, being purely inflammation-driven, is a unique addition to the current pool of animal PD models. The development and validation of various therapeutic strategies in most, if not all of the animal PD models may be necessary in order to provide effective therapeutic intervention that will slow or halt neurodegeneration in PD [101].

In addition to the employment of multiple animal PD models for the screening of neuroprotective agents for treating PD patients [101], experimental designs that test the efficacy of agents that can afford neuroprotection when applied well after the onset of DA neurodegeneration may also be particularly desirable under the current situation [102,103]. The rationale is based on the current inability to clinically diagnose PD before a person has lost more than half of the dopamine-releasing capacity in the nigrostriatal dopamine pathway. A better understanding of the disease progression process, the availability of early stage diagnostic biomarkers, and the identification of specific causative factors of PD in the near future should help resolve the issue.

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Figure 1.

Schematic representation of LPS-induced and glial activation-mediated DA neurodegeneration. LPS binding protein (LBP) works as a chaperon that enhances the binding of LPS to its intermediate receptor CD14. The Toll like receptor-4 (TLR-4) is a transmembrane protein. Association of the LPS-CD14 complex with TLR-4, together with the accessory adaptor protein MD2 initiates a plethora of downstream signaling events that involves mitogen-activated protein kinases (MAPK) and transcription factors such as nuclear factor-kappa B (NF- κ B). Upregulation of gene transcription leads to the production and release of cytokines such as TNF α and IL-1 β . Induction of COX-2 and iNOS expression results in the biosynthesis and release of prostaglandins (PGs) and nitric oxide (NO). Activation of the multi-subunit phagocyte oxidase complex (PHOX), also called NADPH oxidase generates superoxide anion that combines with NO from iNOS to form the more damaging peroxynitrite ($ONOO^-$) free radical. The collective insult of microglia-released cytokines, ROS and lipid metabolites eventually leads to the demise of the oxidative stress-vulnerable dopamine (DA) neurons.

Figure 2.

Immunocytochemical analysis of the maturation of various cell types of the in vitro LPS PD model. Ventral mesencephalic brain tissues were harvested from embryos of gestation day 14 Fisher F344 rats. Dissociated cells were plated into poly-D-lysine-coated 24-well plates and cultured in a serum-containing media. At day in vitro (DIV) 2, 4, 6 and 8, cultures were fixed with formaldehyde and immunochemically stained for neuronal cell bodies and dendrites with an antibody against microtubule-associated protein-2 (MAP-2). Dopamine neurons (arrow heads) in the culture were immunostained with an anti-tyrosine hydroxylase (TH) antibody. Astrocytes were identified with an antibody against glial fibrillary acidic protein (GFAP). Scale bars: 100 μ m.

Table I

Characteristics of various LPS PD animal models

LPS administration	Rodent	SNpc DA neuron	ST DA	Key factors studied	Reference
SN injection	Rat	Significant loss	Significant depletion	ROS, iNOS, TNF α , IL-1 β	32-37
SN infusion	Rat	Significant loss	Significant depletion	TNF α	26,29
In utero	Rat	Significant loss	Significant depletion	TNF α	42-44
Systemic	Mouse	Significant loss		TNF α , IL-1 β	39
Intrapallidal injection	Rat	Significant loss			45

Table II

Drug discovery using LPS PD models

Drug	Neuroprotection	Potential mechanism	Reference
Nicotine	Attenuates DA neuron loss	Reduces microglial activation and TNF α release	80-83
Minocycline	Attenuates DA neuron loss	Reduces activation of iNOS and release of TNF α & IL-1 β	84,85
Naloxone	Attenuates DA neuron loss	Reduces microglial activation, production of ROS, TNF α and IL-1 β	32,33
Dexamethasone	Attenuates DA neurodegeneration	Reduces microglial activation	49,91
Pioglitazone	Attenuates DA neurodegeneration	Reduces activation of microglia, iNOS and COX-2	70