

Review Article

Lipopolysaccharide Animal Models for Parkinson's Disease

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Lipopolysaccharide (LPS), an endotoxin from Gram-negative bacteria, acts as a potent stimulator of microglia and has been used to study the inflammatory process in the pathogenesis of Parkinson's disease (PD) and anti-inflammatory therapy for PD treatment. Here, we review the growing body of literature on both *in vitro* and *in vivo* LPS PD models. Primary cell cultures from mesencephalic tissue were exposed to LPS *in vitro*; LPS was stereotaxically injected into the substantia nigra, striatum, or globus pallidus of brain or injected into the peritoneal cavity of the animal *in vivo*. In conclusion, the LPS PD models are summarized as (1) local and direct LPS treatment and (2) systemic LPS treatment. Mechanisms underlying the PD models are investigated and indicated that LPS induces microglial activation to release a variety of neurotoxic factors, and damaged neurons may trigger reactive microgliosis, which lead to progressive dopaminergic neurodegeneration.

1. Introduction

Parkinson's disease (PD) is the most prevalent neurodegenerative movement disorder. In PD, clinical symptoms including tremor, rigidity, and bradykinesia are primarily resulted from the loss of dopamine-containing neurons in the substantia nigra pars compacta. Although the etiology and pathogenesis of PD remain not fully elucidated, many interacting pathological processes appear to contribute to dopaminergic neuron degeneration in the disease. Recently, inflammatory processes have been implicated as one of the active contributors to dopaminergic neuron damage in the development and progression of the disease [1, 2]. In the central nervous system, microglia, the resident innate immune cells, play a major role in the inflammatory process. Typically microglia exist in a resting state characterized by ramified morphology and monitor the brain environment [3]. In response to various pathogenic stimuli including inflammation, microglia are readily activated and undergo a transformation to amoeboid morphology with an upregulated catalogue of surface molecules [3–5]. Activated microglia can serve diverse beneficial functions essential to neuron survival, which include cellular maintenance and innate immunity [6]. However, uncontrolled activated microglia produces a variety of neurotoxic factors such as proinflammatory cytokines (interleukin-1 (IL-1), tumor

necrosis factor alpha (TNF- α), interleukin-6 (IL-6)), nitric oxide (NO), prostaglandin E2, and superoxide, which lead to neuronal damage or death [1, 7–10]. Additionally, damaged neurons may emit injury signals to cause microglia activation, which used to be defined as reactive microgliosis [11]. This microglial-neuronal interaction will be reinforced and become a self-amplifying cycle of neuronal injury and microglial activation, which finally leads to more neuronal damage and death. Importantly, clinical researches have reported that microglial activation was found in the nigrostriatal system of PD patients [12–14]. Therefore, it is essential to study the inflammatory process in PD, which may help us understand the pathogenesis of the disease and eventually develop an effective therapeutic strategy.

Over the last two decades, studies in animal models have demonstrated that inflammation induced by lipopolysaccharide (LPS) can replicate some characteristics of PD, including extensive activation of microglia and selective loss of dopaminergic neurons in the nigrostriatal system [15–19]. The history of understanding LPS starts in the late nineteenth century. LPS is found in the outer membrane of Gram-negative bacteria and acts as endotoxin. LPS from many Gram-negative bacteria species initiates acute inflammatory responses in mammals and induces a diverse range of effects, ranging from pyrexia to Gram-negative septic shock [20]. Thus, using different serotypes of LPS and their different

application routes may cause different outcomes [21]. Moreover, LPSs from different bacteria species share common features in their basic architecture, which consists of three covalently linked segments, a surface carbohydrate polymer (O-specific chain), a core oligosaccharide featuring an outer and inner region, and an acylated glycolipid (termed lipid A). The O-specific chain shows the most diversity and is the basis for serological specificity, while lipid A, which anchors the LPS molecule in the Gram-negative outer membrane, is the most conserved biochemical structure across different bacterial species [20]. There is wide acceptance that the lipid A moiety is the innate immune stimulating or endotoxic component of LPS [22]. In addition, it is documented that LPS-associated pathology results from the stimulation of host cell responses, in which LPS binds to specific receptors in order to elicit the release of cytokines and other inflammatory mediators. Several membrane-bound and soluble proteins have been shown to bind LPS; the most important appear to be CD14 and LPS-binding protein (LBP) and the toll-like receptor (TLR) family which is a recently discovered group of transmembrane receptors [23, 24]. In the central nervous system, it is found that systemic LPS injection upregulated its membrane CD14 receptor within specific cellular populations including microglia in the brain [25]. Thereafter, microglia were identified as the major LPS-responsive cell in the brain. LPS binds to TLR4 on microglia and induces microglial activation that results in neuronal damage [26, 27].

LPS acts as an endotoxin and elicits multiple pathological effects in human beings. One case report may uncover a potential link between LPS infection and the development of Parkinsonism. A 22-year-old laboratory worker was accidentally exposed to 10 μg *Salmonella minnesota* LPS through an open wound and developed Parkinson's syndrome with bradykinesia, rigidity, tremor, and cogwheel phenomenon three weeks later; damage to the substantia nigra and cerebral cortex was shown by positron emission tomography a few years after the accident [28]. However, it is known that LPS from many bacterial species such as *Salmonella*, *Pseudomonas*, *Vibrio*, and *Rhizobium* can initiate acute inflammatory responses in mammals and induce a large and diverse range of effects, ranging from pyrexia and Gram-negative septic shock [29]. There is another case report regarding the *Salmonella* endotoxin exposure. One middle-aged laboratory worker was self-administered intravenously a single large dose of endotoxin (1 mg *Salmonella minnesota* LPS) and immediately developed a severe septic shock syndrome with multiple-organ dysfunction. The patient was successfully rescued in the emergency room, and there has been no follow-up report to date [30]. Thus, further investigation and more epidemiologic data are needed to exploit the relationship between endotoxin and PD.

In the current paper, we present a summary of a variety of LPS PD models and discuss their strengths and limitations, which may be helpful for the future LPS PD study.

2. In Vitro Studies of LPS PD Model

2.1. LPS Treatment to Cell Culture from Mesencephalic Tissue. Bronstein et al. in 1995 reported the comparison study

between the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) and LPS in rat mesencephalic cultures [31]. Investigators found that, in the neuron-enriched cultures, 6-OHDA killed 89% of the tyrosine hydroxylase- (TH-) immunopositive neurons, but LPS (50 $\mu\text{g}/\text{mL}$) was not neurotoxic; however, in the mixed neuron-glia cultures, 6-OHDA killed only 27% of the TH-immunopositive neurons, but LPS killed 70% of the TH-immunopositive neurons. This early experiment suggested that the dopaminergic neurotoxicity of LPS is dependent on the presence of microglia. Subsequently, dopaminergic neurotoxicity of LPS was confirmed by the other groups on rat mesencephalic mixed neuron-glia cultures and demonstrated that LPS induced microglial activation, and activated microglia released the proinflammatory and cytotoxic factors: NO, TNF- α , and IL-1 β , which lead to dopaminergic neuron damage [32, 33]. In addition, the dopaminergic neurotoxicity of LPS was studied on mouse mesencephalic neuron-glia culture, and it was found that the neurotoxicity was mainly mediated through LPS-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation on microglia, which generated reactive oxygen species production, which are neurotoxic factors [34].

In the studies of LPS-treated primary cultures generated from forebrains of embryonic day 17 mice, the investigators found that the LPS neurotoxicity occurred through binding the signal-transducing receptor, TLR4; microglia are the major cells in the central nervous system that express TLR4. However, it is emphasized that the toxic effect of LPS on neurons is a general phenomenon, independent of neuronal subtype [26]. Thus, LPS may cause neurotoxicity without selectivity for neuronal types. Since previous studies from primary microglia cultures have found LPS treatment induced the release of proinflammatory and cytotoxic factors from microglia [35, 36], the investigators suggested that the activation of TLR4 on microglia may initiate the intracellular signaling pathway of microglia, and result in the release of proinflammatory mediators which cause neuronal damage [26].

3. In Vivo Studies of LPS PD Model

3.1. Intranigral Injection of LPS. In order to study the response of the nigrostriatal system to inflammation, Bing et al. and Castaño et al. independently reported the PD model of LPS intranigral injection in 1998 [15, 16]. After LPS was stereotaxically injected into the nigral area of rats, investigators found that LPS induced microglia activation and dopaminergic neuron loss in the substantia nigra [15, 16]. In a following study, it was reported that LPS-induced dopaminergic neuronal damage was permanent, as observed one year postinjection. Moreover, there was no detectable damage to either the GABAergic or the serotonergic neurons in the striatum and nigra after LPS injection, indicating that LPS selectively induced dopaminergic neuron death in the nigrostriatal system [37]. Thereafter, more studies confirmed the results and also found the increased level of proinflammatory cytokines including IL-1 β , TNF- α , IL-6, and NO in the substantia nigra after LPS injection,

which may be causal factor for LPS-induced neuronal damage [38–40]. In addition, the effects of intranigral LPS injection on behavior and dopamine content and turnover were investigated and showed that LPS treatment enhanced locomotor activity 2- to 3-fold and increased dopamine turnover ratios in comparison with control subjects. This suggests that LPS insult may induce a compensatory response of dopaminergic system [41].

3.2. Intrapallidal Injection of LPS. The globus pallidus is a major integrative nucleus within the basal ganglia, with neurons projecting to striatum, subthalamic nucleus, entopeduncular nucleus, and substantia nigra. Thus, the globus pallidus is positioned to influence the nigrostriatal pathway and function of the basal ganglia as a whole. LPS was injected into the globus pallidus of young and middle-aged rats. The results showed that microglial activation was found in both globus pallidus and substantia nigra, dopaminergic neurons were significantly and progressively decreased in the substantia nigra, and locomotor deficits were detected in animal after LPS injection [17]. Moreover, the following study reported an increased level of proinflammatory cytokines including IL-1 β , TNF- α , and IL-6, the elevated expression of inducible nitric oxide synthase, and the enhanced α -synuclein nitration and oligomerization in the substantia nigra of LPS-injected animal [42]. Interestingly, the above pathological changes were much severer in middle-aged animals when compared with the younger animals after LPS treatment, supporting the view that aging itself is a risk factor for PD development [42]. Inflammation promotes the release of neurotoxic factors and the development of synucleinopathy lesions that finally lead to dopaminergic neurodegeneration in PD model of LPS intrapallidal injection. Additionally, the finding of abnormal α -synuclein may help us to explore the mechanisms underlying progressive loss of dopaminergic neurons in LPS PD models. It is reported that aggregated α -synuclein induced microglial activation in a primary mesencephalic neuron-glia culture system [43], thus the pathological process of reactive microgliosis may be triggered and microglial activation may become uncontrolled, which eventually result in progressive dopaminergic neurotoxicity.

3.3. Intrastratial Injection of LPS. In the nigrostriatal system, the cell bodies of dopaminergic neurons are located in the substantia nigra and their dopamine-containing terminals are distributed in the striatum. After LPS was injected into the striatum of rats, we detected a progressive degeneration of dopamine cell bodies in the substantia nigra and their axonal terminals in the striatum, a depletion of dopamine content in the striatum, cytoplasmic accumulation of α -synuclein and ubiquitin in the nigral dopamine neurons, and behavioral deficits assessed by cylinder test and amphetamine-induced rotational behavior behavioral test [19, 44–46]. Molecular mechanisms underlying the neurotoxicity of LPS intrastratial injection included activation of microglia, impairment of mitochondria state III and state V respiration, and an increased release of proinflammatory mediators: IL-1 β , TNF- α , IL-6, IL-1 α , and NO, in both the substantia nigra and the striatum. This indicates that

the inflammatory insult or stimuli in the striatum not only directly damaged the terminals of dopaminergic neurons in the striatum, but also indirectly damaged the cell bodies of dopaminergic neurons in the substantia nigra through an unknown retrograde signal transduction pathway [19, 44, 45].

3.4. Intraperitoneal/Systemic Injection of LPS. To study how infectious disease through blood transmission affects the development of neurodegenerative disease in the central nervous system, LPS was systemically injected into animals. Early work reported that after systemic (intraperitoneal or intravenous) injection of LPS, LPS has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations including microglia, which is likely to be responsible for the transcription of proinflammatory cytokines: first within accessible structures from the blood and thereafter through scattered parenchymal cells during severe sepsis [25]. In addition, early work also showed that intraperitoneal endotoxin even at a high dose (2 mg/kg of LPS, which has cardiovascular effects, e.g., a decrease in blood pressure) into rats did not disrupt blood-brain barrier (BBB) permeability, suggesting that intraperitoneal LPS administration is unlikely to contribute to the observed central nervous system mediated effects of endotoxin [47]. However, other studies have found that some cytokines including TNF- α and IL-1 can be transported across the BBB by saturable transport systems, which are able to directly affect central nervous system functions [48, 49]. Using the intraperitoneal injection of LPS in mice, Qin et al. reported that increased cytokine TNF- α due to LPS insult was critical for the transfer of inflammation from the periphery to the central nervous system to induce microglial activation and dopaminergic neuron loss in the substantia nigra at 7 and 9 months posttreatment [18]. Nevertheless, Byler et al. found that systemic LPS injection alone did not affect dopamine levels or Parkinsonian behavioral tests in mice whereas systemic LPS plus MPTP in combination induced the depletion of dopamine in the striatum and Parkinsonian behavioral deficits (reduced stride length) at 4 months postinjection [50]. In addition, MPTP treatment alone reduced striatal dopamine levels quickly but they recovered to normal levels later, addressing the point that nigrostriatal dopamine neurons may succumb after time to multiple toxic agents [50].

4. Implication Using the Animal LPS PD Models

A number of studies have suggested that microglial activation plays a key role in the initiation and progression of PD [8, 12, 13, 51, 52]. LPS PD models provide us with a good tool to investigate the inflammatory process in PD development and anti-inflammation therapy for PD treatment. For example, naloxone, an antagonist of opioid receptors, provided the dopaminergic neuroprotective effects against LPS damage [32, 38, 53]. Interleukin-10, a natural immune modulator, reduced LPS-induced dopaminergic neurotoxicity by inhibiting microglial activation [40, 54]. Pioglitazone, an agonist of peroxisome proliferator-activated

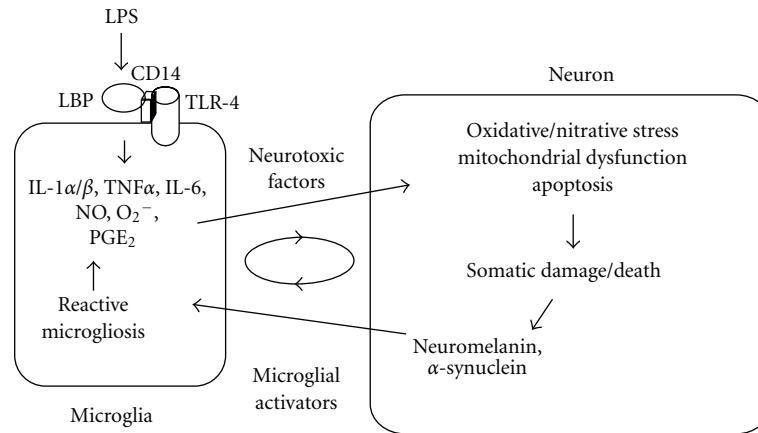


FIGURE 1: LPS induces progressive neurotoxicity. In response to LPS stimuli, microglial cells are readily activated. It is demonstrated that LPS binds to specific receptors, for example, CD14/TLR4/LBP receptor complex on the microglia, to induce microglial activation. Uncontrolled microglial activation produce a variety of neurotoxic factors such as proinflammatory cytokines (IL-1, TNF- α , IL-6), NO, PGE₂, and O₂⁻, which lead to neuronal damage or death through a cascade of events such as oxidative/nitrative stress, mitochondrial dysfunction, and apoptosis. Moreover, damaged neurons may emit injury signals to cause microglia activation, which is defined as reactive microgliosis. The injury signals could be neuromelanin and α -synuclein released by injured dopaminergic neurons. This microglial-neuronal interaction will be reinforced and become a self-amplifying cycle of neuronal injury and microglial activation, which may finally result in the neurodegenerative disease.

receptor gamma, improved dopaminergic neuron survival by restoring mitochondrial function, decreasing the release of proinflammatory mediators and suppressing the oxidative stress [33, 45, 55, 56]. Minocycline, a semisynthetic second-generation tetracycline, exerts potential neuroprotective effects by reducing the inflammatory response and inhibiting apoptotic cell death [57–59]. Among all of these, minocycline is receiving a great deal of attention for its potent antiinflammatory and anti-apoptosis effects. It has been demonstrated that minocycline has few safety concerns and that it should be considered for a large phase III efficacy trial after phase II clinical trials in early PD patients [60, 61]. Currently, minocycline is used in many ongoing clinical trials for various diseases including PD [62].

5. Discussion of LPS PD Models

Mechanisms underlying the LPS PD models are investigated and indicated that LPS induces microglial activation, activated microglia release proinflammatory and neurotoxic factors such as IL-1, TNF- α , IL-6, and NO to cause neuronal damage [40, 42, 63], and damaged neuron may emit injury signals such as neuromelanin and abnormal α -synuclein to trigger reactive microgliosis [43, 64, 65]. This neuronal-microglial interaction may be reinforced and become a self-amplifying cycle to result in progressive dopaminergic neurodegeneration (Figure 1). Based on the application routes in these LPS PD models, we summarize them as follows: (1) LPS is directly and locally applied into the nigrostriatal system and its related structures, such as LPS treatment in mesencephalic cell culture systems in vitro and stereotaxic injection of LPS in nigra, striatum or globus pallidus in vivo; (2) LPS is systemically administered and selectively affects the nigrostriatal system, such as intraperitoneal injection of

LPS in vivo. First, let us discuss the local and direct LPS treatment of PD models. Many studies have suggested that dopaminergic neurons are more vulnerable than others in the nigrostriatal system to inflammation-mediated neurotoxicity owing to their precarious redox equilibrium and colocalization with a large population of microglia [2, 66]. Thus, inflammatory responses induced by direct and local LPS treatment may selectively cause dopaminergic neuron damage in mesencephalic tissue in vitro and in the nigrostriatal system in vivo. For stereotaxic injection of LPS in nigra, striatum, or globus pallidus in vivo, because of the smaller size of the nigral area compared with the striatum/globus pallidus area and the dense distribution of dopaminergic neurons in the nigra, intranigral injection itself may cause severe mechanical injury to neurons and glial cells in the nigral area whereas intrastriatal/intrapallidal LPS injection has the advantage of keeping intact the structure of the nigra for enabling the study of the toxic effect of inflammation on neurons. Moreover, intrastriatal/intrapallidal LPS treatment not only induces progressive dopaminergic neuron loss, but also leads to behavioral deficits in animal studies. Thus intrastriatal/intrapallidal LPS injection may be a better PD model in vivo. Next, let us discuss the systemic LPS treatment of PD model. There remains a puzzle how systemic treatment of LPS selectively induced dopaminergic neuron death in the nigrostriatal system of brain. We know that LPS acts as a potent stimulator of microglia and microglia density varies by brain region in human and animals. It has been reported that the level of microglial cells was high in the medulla oblongata and pons in comparison with that in the substantia nigra, hippocampus, thalamus, basal ganglia, and pedunculus cerebri in an adult normal human brain study [67]. Likewise, microglial cells are not uniformly distributed in the normal adult mouse brain. Lawson et al.

reported that microglial densely populated areas include the hippocampus, olfactory telencephalon, basal ganglia, and substantia nigra in the adult mouse brain [68]. Importantly, these studies demonstrate that the density of microglial cells in the substantia nigra is similar to that in the hippocampus, basal ganglia, and so on for both the human and mouse brains. In other words, microglia activation and subsequent proinflammatory cytokines release due to LPS insult may occur in several brain regions, but not in nigral area alone. For example, LPS is also widely used in experimental in vitro and in vivo models of inflammation and amyloidosis for Alzheimer's disease [69, 70]. Thus, it needs further investigation for the selective dopaminergic neurodegeneration in the substantia nigra after systemic LPS treatment. In summary, bacterial endotoxin LPS used as a potent stimulator of glial cells, especially microglia, help us to study the molecular mechanism underlying inflammatory processes in neurodegenerative diseases in the central nervous system. Direct and local LPS treatment in the nigrostriatal system and its related structures may be better PD models to study the etiology and therapeutic strategies for inflammation in PD.

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