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Characterization of the lipopolysaccharide induced model of Parkinson's disease: Role of oxidative stress and neuroinflammation

Neha Sharma *, Bimla Nehru *

Department of Biophysics, Panjab University, Chandigarh 160014, India

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ABSTRACT

Introduction: Primary pathology underlying Parkinson's disease (PD) is the loss of dopaminergic neurons in the substantia nigra (SN). A variety of genetic and environmental factors underlie this loss of dopaminergic neurons. However, recent studies have highlighted the role of elevated oxidative stress and the pro-inflammatory responses contributing to or exacerbating the nigrostriatal degeneration.
Methods: With the establishment of neuroinflammation as an important process involved in the PD pathogenesis, in the present study this pathogenic feature was replicated in animals using lipopolysaccharide (LPS) (5 ug/5 ul PBS) infused stereotaxically into the SN of rats.
Results: LPS injected into the SN successfully replicated the pathogenic features of PD in rats as it elicited an inflammatory response via action of microglia. LPS infusion resulted in glial cell activation as depicted from immunohistochemistry (IHC) analysis of GFAP and Iba-1. Also, a significant increase in the mRNA expression of proinflammatory cytokines, i.e. TNF-α and IL-1β, was observed after 7 days of LPS infusion whereas the alterations in the oxidative stress markers, i.e ROS, lipid peroxidation, NO formation, NADPH oxidase activity, glutathione system, SOD and catalase, became highly significant after 14 days of infusion. As a consequence, after 21 days of LPS infusion we observed activation of apoptotic pathway indicated by increased expression of caspases 3 and caspase 9. This was followed by a significant decline in the expression of tyrosine hyroxylase (TH) as revealed by IHC. Further, there was a marked decrease in the level of dopamine and its metabolites enough for the production of behavioral abnormality in rats.
Conclusion: Hence, the present study provides extensive characterization of LPS induced model of PD. Study also confirms the co-existence and complex interplay between inflammation and oxidative stress contributing equally to the dopaminergic neuronal degeneration process in PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by slow and progressive degeneration of dopaminergic neurons in the substantia nigra and associated motor dysfunction. Although the pathogenesis of PD remains to be elusive, cumulative evidence supports a pivotal role for oxidative stress and neuroinflammation in initiation and progression of nigral dopamine neuronal loss (Beal, 2005; Lin and Beal, 2006). Several neurotoxic molecules such as 6-OHDA, MPTP etc that have been utilized to develop PD models, elicit an inflammatory response, but it is difficult to delineate whether neuroinflammation is the cause or consequence of injured dopaminergic neurons. However, the LPS induced PD model has provided us with an important tool to delineate the precise contribution of various proinflammatory and neurotoxic factors to dopaminergic neurodegeneration. Moreover, as revealed by recent studies, humans often get exposed to LPS as it is presently suspended in the air as a component of the air pollutant PM2.5 or as part of house dust and aerosols generated from contaminted water (Genc et al., 2012; He et al., 2013). These PM2.5, i.e particulate matter less than 2.5 μm, originate from several sources like oil refineries, metal processing facilities, tailpipe and brake emissions, residential fuel combustion, power plants, and wild fires. Furthermore, occupational exposure to LPS is common for people in agricultural settings or in textile mills as suggested by previous reports (Clapp et al., 1993). Therefore, aforementioned points highlighted the use of LPS to create an inflammatory animal model of PD.

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; CAT, catalase; DTNB, 5, 50 dithio-bis-2-nitrobenzoate; GFAP, glial fibrillary acidic protein; GPx, glutathione peroxidase; GSH, glutathione; H2O2, hydrogen peroxide; IL-1β, interleukin-1β; MDA, malondialdehyde; NFκB, nuclear factor-κB; NO, nitric oxide; LPO, lipid peroxidation; LPS, lipopolysaccharide; PD, Parkinson's disease; PMS, post mitochondrial supernatant; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor –alpha.

* Corresponding author. Department of Biophysics, Panjab University, Chandigarh, 160014, India. Tel.: +91 172 2534128.
E-mail address: jaitly_neha@rediffmail.com (N. Sharma), bnehru@pu.ac.in (B. Nehru).

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Further, LPS infusion into the SN results in microglial activation which consequently leads to the generation of proinflammatory cytokines particularly (TNF-α, IL-1β) and neurotoxic factors, i.e ROS and RNS, thus, creating an environment of increased inflammatory and oxidative stress in the SNpc (Imai et al., 2011; Pereira et al., 2012). However, oxidative stress generation following LPS infusion is primarily governed by NADPH oxidase (PHOX), a membrane-bound enzyme; its expression has been found to be up regulated in PD (Brown and Neher, 2010). This can prove to be detrimental for healthy neuronal population, resulting in a self-perpetuating cycle of microglial activation leading to neuroinflammation and ROS driven toxicity within the brain (Hines et al., 2013). Furthermore, clinical and animal studies with compounds having anti-inflammatory actions (NSAIDS, COX-2 inhibitors, iNOS inhibitors) and anti-oxidative properties especially NADPH inhibitors, have been shown to rescue dopaminergic neurons from various neurotoxic insults in LPS induced PD (Hines et al., 2013; Orr et al., 2002), hence, supporting the presence of both neuroinfllammation and oxidative stress in PD.

Also, observations made in PD patients as well as various animal models of PD suggest that PD develops unilaterally which later becomes bilateral Parkinsonism (Yagi et al., 2010). Previous reports on available PD models, suggests that microglia activation and inflammatory factors in brain microenvironment are associated with dopaminergic degeneration (Glass et al., 2010); therefore, it is when injected unilaterally into the rat substantia nigra, produced microglia activation and stable loss of nigral dopamine neurons along with a stable reduction of around 60% in the striatal dopamine level (Dutta et al., 2008; Herrera et al., 2000; Iravani et al., 2002). The study from Hoban et al. (2013) highlighted the need for behavioral characterization of the LPS model of PD and compared the impact of intrastriatal versus intracerebral LPS injection.

Therefore, keeping in view the reports from earlier studies on LPS, the present study was designed to record the sequential changes in oxidative stress markers, antioxidant enzyme system, inflammatory markers as well as behavioral parameters at different time intervals, i.e day 7,day 14 and day 21 following LPS infusion into the SN. The study also helps to understand the complex interplay between oxidative stress and neuroinflammation contributing to dopaminergic neurodegeneration process in LPS induced animal model of disease. Hence, the study suggests to target this complex interaction between oxidative stress and neuroinflammation in order to find suitable therapeutic agents to cure this progressive disease.

2. Materials and methods

2.1. Animals

Healthy male rats of the Sprague Dawley strain of 5–7 weeks age groups, weighing 250–300 grams, were procured from the central animal house of Panjab University, Chandigarh, India and were acclimatized in the department animal house for 2 weeks in polypropylene cages under hygienic conditions and were provided standard animal feed and water ad libitum throughout the treatment period. All procedures were done in accordance with ethical guidelines laid down by the Ethics Committee on the Use of Experimental Animals of the Panjab University and in general according to the NIH guidelines (Rule No 23–85, as revised in 1985).

2.2. Experimental design and methodology (Fig. 1)

Animals were divided randomly into two different groups (n = 12). Animals in the first group received single intranigral injection of 5 ul PBS and served as control. Animals in the second group received single intranigral injection of LPS at a dose of 5 ug/5 ul of PBS/animal, i.e 0.2 mg/kg b.wt. Dose selection of LPS was done on the basis of reports from earlier studies on LPS (Castaño et al., 1998, 2002; Herrera et al., 2000; Tufekci et al., 2011). Dose of LPS was also standardized in our lab. The impact of LPS administration on the neurobehavioral, neurochemical, and biochemical, histopathological and neuro-inflammatory markers was studied at different time intervals. Also, a correlation was established between the extent of dopaminergic neuronal loss and motor deficits produced.

2.3. Surgical procedures for the infusion of LPS into the SN

To achieve unilateral lesions of the nigrostriatal system, rats weighing 250–300 gms received LPS injection into the right substantia nigra. Rats were anaesthetized using thiobarbitol (45 mg/kg b.wt) and placed into a stereotactic frame with nose and ear bars specially adapted for rats. LPS (Sigma Chemical Co., St. Louis, MO, USA) was dissolved at a dose of 1 mg/1 ml of PBS. The injection needle was lowered through a drill hole 5.5 mm posterior, 1.5 mm lateral and 8.3 mm ventral to the bregma for the substantia nigra. A dose of 5 µl from the stock solution of 1 mg/1 ml of LPS was delivered using Hamilton syringe over a period of about 2 min

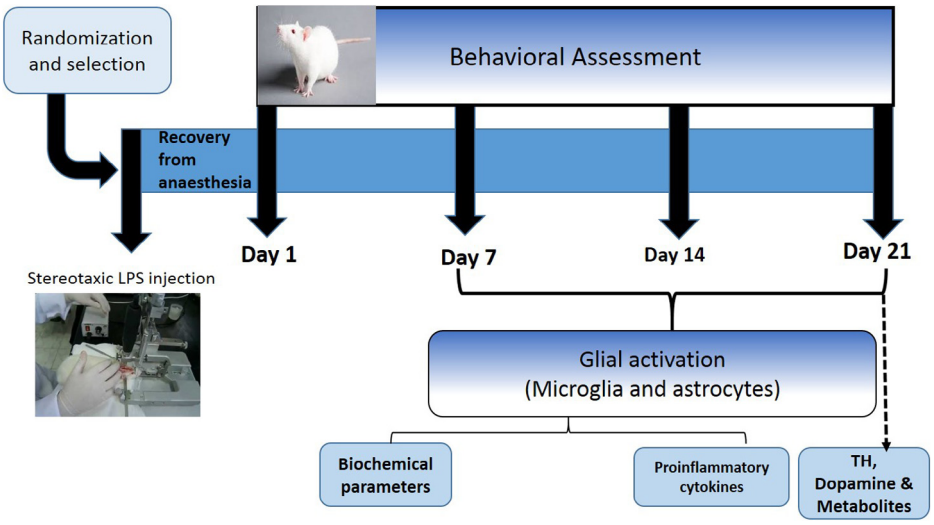

Fig. 1. Experimental design.