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The effect of nobiletin on performance of rats in forced swimming and elevated plus maze tests in intranigral lipopolysaccharide rat model of Parkinson's disease

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Abstract

Background and Objective: Anti-inflammatory property of nobiletin (NOB) is proven and neuroinflammation is involved in triggering and progression of neurodegenerative disorder such as Parkinson's disease (PD). PD is a neurodegenerative disorder characterized by motor and non-motor features including psychiatric symptoms such as depression and anxiety. The purpose of this study to investigate whether oral nobiletin administration at a dose of 10 mg/kg has the ability to alleviate non-motor behavioural changes including depression and anxiety-like behaviors in LPS-induced model of PD in rat.

Materials and Methods: For this purpose, 32 male Wistar rats (195-245 g) were divided into four groups (n=8) as follows: Sham-operated group, nobiletin-treated sham-operated group (sham+NOB), lesion group (LPS) and nobiletin-treated lesion group (LPS+NOB). LPS (5 µg/kg) rat was unilaterally injected into the SN of rat brains through standard stereotaxis, according to the atlas of Paxinos and Watson (to generate a neuroinflammatory model of PD), with or without NOB (10 mg/kg administrated daily for 1 week after surgery, via gavage). Behavioral assessment was carried out one week after surgery by assessment of performance in forced swimming and elevated plus maze tests.

Results: NOB-treated LPS group showed significant decrease in immobility time and insignificant increase in the percentage of open arm spending time as compared with LPS group which demonstrate the anti-depressant like effect of NOB in inflammatory model of PD in rats.

Conclusion: Taken together, this study demonstrated that nobiletin as an anxiolytic and anti-depressive agent in the LPS-induced rat model of Parkinson's disease.

Keywords: Parkinson's disease, Nobiletin, Neuroinflammation, Behavioral changes, Lipopolysaccharide

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder of the elderly and is clinically characterized by the motor symptoms of tremor, bradykinesia, and rigidity. The pathological hallmark of PD involves the presence of Lewy bodies, resulting in degeneration of dopamine (DA) neurons in substantia nigra pars compacta (SNc) and subsequently, the striatum. The pathological process begins in the dorsal motor nucleus, proceeding in an ascending fashion to the midbrain (including caudal raphe nuclei) and forebrain (1). Growing lines of evidence suggest that PD is not solely a DA-ergic disease but that there is a more diffuse pathology involving other, non-DA neurotransmitter systems, such as the serotonergic. Serotonin (5-HT) neurons in the dorsal raphe nuclei project mainly to the basal ganglia, particularly the striatum, but also to the frontal cortex and the limbic system. The serotonergic system is thought to be involved in the modulation of various cognitive and physiological processes, such

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as, mood, emotion, sleep, and appetite; thus altered serotonergic neurotransmission is likely to be implicated in both motor and non-motor disturbances observed in PD (2). PD is considered to be due to the combination of genetic and environmental factors (3, 4). The prevalence of PD increases with age (5) and the lifetime risk of PD is 20% for men and 1.3% for women (6). It is now well-recognized that the vast majority of individuals with Parkinson's disease (PD) suffers from non-motor systems that can be as disabling as motor symptoms, if not more so non-motor symptoms can be a harbinger of disease, hasten disease progression and mortality, lead to nursing home placement, and significantly diminish quality of life. This in turn affects care partners, including their stress and burden, and negatively impacts their health and mental status (7). Depression and anxiety are some of the most common comorbidities arising in patients with PD (8). 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. Several neurotoxic molecules such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (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 pro-inflammatory and neurotoxic factors to dopaminergic neurodegeneration.

LPS is a gram-negative bacterial endotoxin that activates microglia through the toll-like receptor-4, leading to the production of inflammatory cytokines and chemokines (9, 10). Microglia are the major players in the inflammatory process that mediate inflammation lipopolysaccharide (LPS)-induced neurotoxicity (11-15). Activated microglia produce a variety of pro-inflammatory factors, including nitric oxide (NO) (12, 16-21), tumor necrosis factor α (TNF-α) (20, 22, 23), interleukin-1β (IL-1β) (20, 24, 25), prostaglandin E_2 (PGE_2) (26-28) and reactive oxygen species (ROS) (29-31), all of which serve immune surveillance functions by removing foreign microorganisms (32).

Nobiletin, one of the major components of polymethoxyflavone family in citrus fruits, has been reported to have anti-inflammatory activity (33, 34). Nobiletin produces rapidly acting antidepressant-like responses in the chronic unpredictable mild stress model of depression (CUMS) through BDNF-TrkB pathway (35). Nobiletin treatment improves both motor and cognitive deficit observed in MPTP-treated mice, an effect maintained for 2 weeks after nobiletin withdrawal (36). Depression is a psychological feature of PD (37) and nobiletin reportedly has an antidepressant-like effect via stimulation of the serotonergic, noradrenergic and dopaminergic system (38). Dopamine replacement therapies, particularly L-DOPA treatment, have been highly successful in improving motor function in PD (39). However, treatment can also promote problems with dyskinesia and L-DOPA administration becomes less effective over time (40, 41).

The present assay evaluated the effect of nobiletin on performance of rats in forced swimming and elevated plus maze tests in intranigral lipopolysaccharide (LPS) rat model of PD.

3. Materials and Methods

Adult male Wistar rats (195-245 g; n=32) (Shahid Beheshti University, Tehran, Iran) were housed three to four per cage in a temperature-controlled colony room under 12 h light/dark cycle with food and water available ad libitum. The animals were held in the colony room for at least one week before being tested. The animals were randomly divided into four groups: Sham-operated group, nobiletin-treated sham-operated group (sham+NOB), lesion group (LPS) and nobiletin-treated lesion group (LPS+NOB). All behavioral experiments were carried out between 11 a.m. and 4 p.m. Protocols of the present investigation for all the animal studies were approved by the Ethical Committee of Shahed University and carried out in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (42).

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

To achieve unilateral lesions of the nigrostriatal system, rats received LPS injection into the right substantia nigra. Rats were anaesthetized by i.p. injection of a combination of ketamine and xylazine (100 and 10 mg/kg, respectively) 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 2.5 mg/1 ml of normal saline. The injection needle was lowered through a drill hole 5.30 mm posterior, 2 mm lateral and 7.6-7.7 mm ventral to the bregma for the substantia nigra, according to atlas of Paxinos and Watson (43). Two µl (5 µg) from the stock solution of LPS was delivered using Hamilton syringe over a period of about 2 min and after each, the needle was left in situ for an additional 5 min to avoid reflux along the injection track and then withdrawn at a rate of 1 mm/min. The lesion group received a single injection of 2 µl of 0.9% saline containing 2.5 µg/µL of LPS (5µg). The sham group received an identical volume of 0.9% normal saline. The LPS+NOB group received the neurotoxin in addition to nobiletin (Cayman, USA) *p.o.* (using rodent gavage) dissolved in Cremophor at a dose of 10 mg/kg. NOB was daily administered with an interval of 24 h.

2.2. Behavioral tests

Battery of behavioral experiments were carried out one week after LPS injection.

2.2.1. Immobility as a behavioral response The forced swimming test (FST) was originally introduced in 1977 by Porsolt and has been implemented and analyzed in several different ways (44, 45). In any form, the test is based on the observation that when rodents are forced with an inescapable aversive situation they can elect different strategies of coping that can be scored as either active or passive. Active strategies (climbing and swimming) predominate in the initial exposure to the swim but these are typically replaced over time with the appearance of a passive strategy (floating). The key observation that brought the test into widespread use was the discovery that effective antidepressants in humans had the ability to increase the amount of active strategies adopted by the animal in the FST. Thus, the major advantage of the FST has been its predictive validity: a drug's effectiveness in promoting active coping in the FST had potential to predict its efficacy as an antidepressant. This was a particularly important observation because it yielded a simple screen in animal models to identify similarly acting drugs (46, 47). Forced swimming test is a well-established measurement for evaluating the effects of antidepressants and the assessment is highly reliable to predict the validity of antidepressants. The procedure comprised two sections (the pretest and the test). Rats were individually placed in glass cylinders (height 40 cm, diameter 20 cm) containing water for a height of 25 cm maintained at 25˚c. During the pretest session, rats were forced to swim for 5 min. The procedure was repeated 24 h later in a 5 min swim session (test session). The total duration of immobility (time spent floating with the minimal movements to keep the head above the water) was recorded during the testing period. An animal was judged immobile whenever it ceased all active behaviors (i.e., struggling, swimming, diving, and jumping) and remained floating in the water in hunched but upright position and making only the movements necessary to keep its head above water. After each pretest and test session, the rats were taken out of the water and allowed to dry across heaters before being returned to their home cages. The cylinders were cleaned between animals.

2.2.2. Time spent on the open arms as behavioral response

The elevated-plus maze (EPM) test was performed to evaluate anxiety-like behavior in PD models (48). Behavior in the EPM is utilized to measure exploration, anxiety and motor behavior. The EPM consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm above the ground. Two arms were enclosed by walls 30 cm high and the other two arms have no walls. On the 10th day of experiments, 30 min after drug administration, each rat was placed at the juncture of the open and closed arms and the amount of time spent on the open arms was recorded during a 90 s period. After each assay, the maze was carefully cleaned with wet tissue. Trial (2) 24 h after trial (1) was carried out in the same testing room. Time spent on the open arms is an index of anxiolytic effects of drugs (49).

2.3. Statistical analysis

The animal's behavioral activities in EPM task and FST were statistically analyzed with one-way analysis of variance (ANOVA). All results are expressed as mean ± SEM. P values < 0.05 were regarded as statistically significant. Significant differences between individual groups were determined using pair–wise comparison TUKEY post-hoc test. Graphical representation was performed using Microsoft Excel 2013.

3. Results

3.1. The antidepressant and anxiolytic-like effect of oral nobiletin treatment in rats

Percent time spent in open arms of Elevated Plus Maze as a measure of anxiety level in rats has been shown in Fig. 1. A significant decrease in percent time in open arms in LPS group compared to Sham group was obtained that is indicative of higher anxiety and higher fear behavior. In contrast, animals in LPS group treated with NOB showed insignificantly higher percentage of open arms time versus LPS group (p>0.05).

In addition, immobility time was measured in FST. Rats in LPS group showed significantly higher percentage of immobility duration versus sham group (p < 0.05). In contrast, animals in LPS group treated with NOB showed a significantly lower immobility time versus animals in LPS group (p < 0.05).

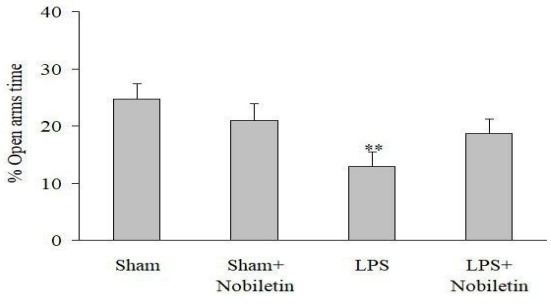

Figure 1. Characterization of Se NPs. A) The hydrodynamic size of Se NPs. TEM images of B) bare and C) BSA coated Se NPs.

3.2. Effects of Se NPs in two-dimensional microenvironment on the behavior of PC12 cells

Five days after treatment with 250 µg/mL dose of Se NPs, PC12 cells were spread out and neurite outgrowth was found that was not significant

Figure 1. Data is presented as % time spent (Mean±SEM). Rats in LPS group showed significant lower percentage of open arms time versus sham group (** P<0.01). In addition, animals in LPS group treated with NOB showed insignificantly higher percentage of open arms time versus LPS group.

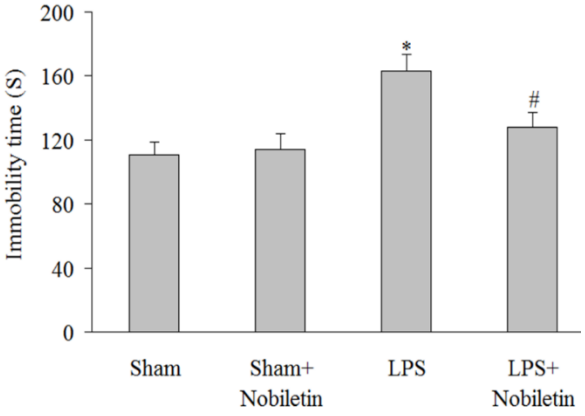

Figure 2. Representative results of the effects of nobiletin (one-week oral treatment) on immobility time as an index for depression-like behavioral changes in the FST. Rats in LPS group showed significant higher percentage of immobility time versus sham group (* P<0.05). In addition, animals in LPS group treated with NOB showed significantly lower immobility time versus animals in LPS group (# P<0.05).

4. Discussion

Anxiety and depressive disorders are common neuropsychiatric complications of Parkinson's disease (PD) (8) and there is dysfunction of both dopamine and serotonin systems and their interaction in the brain, contributing to development of complications (50). Our present observations demonstrated that bilateral injection of LPS (5 µg/rat) into the substantia nigra of the rat brain can simulate depression and anxiety-like behavior in PD. The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (51). An anxiolytic agent increases the time spent in open arms in this test. This biologic activity could be explained by an anxiolytic-like effect of the nobiletin in treated animals. The forced swimming test has been validated as a suitable tool for predicting the antidepressant properties of drugs (52, 53). When rodents are forced to swim in a confined space, after an initial period of struggling, they would become immobile, resembling a state of despair and mental depression. This inescapable stressful situation can be evaluated by assessing different behavioral strategies (54). By assessment of performance of rats in elevated plus maze (EPM) test, it was demonstrated that percentage of time spent in the open arms in nobiletin-treated sham-operated group (sham+NOB) is insignificantly lower than Sham-operated group and in lesion group (LPS) rats as compared to Sham-operated group, it was significantly lower. Furthermore, rats in nobiletin-treated lesion group (LPS+NOB) showed an insignificant increase in this parameter versus lesion group (LPS). Although intranigral LPS could stimulate anxiety-like behavior in rats but the anxiolytic effect of oral administration of nobiletin (10 mg/kg) was not significant in this model, but on the other hand as increasing in percentage of time spent in the open arms, it will be probably significant if the dose of nobiletin or treatment duration or the number of rats in each group will be increased. In the present study, after oral administration of nobiletin (10 mg/kg) for 7 consecutive days, there was no significant differences between sham-operated group and nobiletin-treated sham-operated group (sham+NOB) in terms of immobility time, but this parameter in lesion group (LPS) versus Sham-operated group was significantly increased and moreover significant decrease in the immobility time of the nobiletin treated LPS lesion rats was seen compared to lesion group (LPS) (Fig. 2).

To conclude, these results suggest an anti-depressant-like effect of the nobiletin in response to an inescapable stress in experimental LPS-induced model of PD in the rat and proper supplementation with nobiletin may protect against the neurodegeneration involved in PD.

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Conflict of Interest:

The authors report no conflicts of interest.

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