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Neuroprotective potential of spermidine against rotenone induced Parkinson's disease in rats

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ABSTRACT

Parkinson's disease is a leading hypokinetic disorder characterized by selective loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) region of mid-brain. Degeneration of dopaminergic neurons is considered to be due to oxidative stress, neuroinflammation, disturbed calcium homeostasis and glutamate excitotoxicity etc. Spermidine is a polyamine which counteracts age associated cell death by scavenging free radical formation, activates authophagic machinery by enhancing formation of autophagosome, and antagonizes NMDA receptor. In the current study we investigated the neuroprotective potential of spermidine against rotenone induced PD in rats. Rats were treated subcutaneously with rotenone 1.5 mg/kg daily for 28 days. Spermidine 5&10 mg/kg was administered orally 1 h prior to rotenone administration from 15 to 28. Rotenone caused significant reduction in motor functioning and elevated levels of oxidative stress markers and proinflammatory cytokines levels (IL-1β, IL6 and TNF-α). The neurochemical analysis revealed a significant decrease in serotonin, norepinephrine, dopamine and their metabolites accompanied by a significant loss of dopaminergic neurons in the SNpc following ROT injection. However, treatment with spermidine rescued DAergic neurons in SNpc and nerve terminals in the striatum following ROT insult. Spermidine treatment also attenuated oxidative stress, neuroinflammation and restored striatal neurochemistry. Results of our study suggest that spermidine has promising neuroprotective effect against degenerative changes in experimental PD, and the protective effects are mediated through its antioxidant and anti-inflammatory properties.

1. Introduction

In the new century, Parkinson's disease (PD) ranks among the most common late life neurodegenerative diseases, affecting approximately 1.5%–2.0% of the population over the age of 60. PD is a long-term degenerative disorder of the central nervous system which affects movement and speech. It is a progressive disease marked by tremor, muscular rigidity, and slow, imprecise movement associated with the degeneration of the dopaminergic neurons in substantia nigra pars compacta region of brain (Wang et al., 2015). These neurons project to the striatum and their loss leads to alterations in the activity of the neural circuits within the basal ganglia that regulate movement, in essence an inhibition of the direct pathway and excitation of the indirect pathway. The direct pathway facilitates movement and the indirect pathway inhibits movement, thus the loss of these cells leads to a hypokinetic movement disorder (Bejjani et al., 1999). Many factors are speculated to operate in the mechanism of nigrostriatal dopaminergic degeneration, including oxidative stress and cytotoxicity of reactive oxygen spices (ROS), disturbances of intracellular calcium homeostasis and excitotoxicity due to overactivation of NMDA receptor through NR2B subunit. (James, 2013).

Though levodopa-a dopamine precursor, still the most effective therapy for PD patients, however, long term use is associated with troublesome motor fluctuations such as dyskinesia, referred as LID (L-dopa Induced Dyskinesia) which decreases the motor ability of PD patients (Wan et al., 2017). Other than levodopa, dopamine receptor agonists, catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) inhibitors have also been used to relieve the motor fluctuations (Lin et al., 2017a). Surgical treatment treatments including stem cell therapy, deep brain stimulation, thalamotomy, pallidotomy, transcranial magnetic stimulation, gamma knife

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surgery has become a mainstay of late-stage management, although not all patients can afford it (Lozano et al., 2017). In recent years, gene therapy has developed some hope, but yet to prove its efficacy in PD patients (Lin et al., 2017b; Ma et al., 2013). Currently available drugs provide only the symptomatic relief but do not slow or reverse the disease pathology as well as these are associated with various side effects. Thus, there is a great need to identify potential target sites or candidate drug molecules which could modify disease pathology associated with PD (Levy et al., 2009).

Polyamines such as spermine, spermidine, putrescine and thermospermine are found in green vegetables, milk products, and meat. It belongs to a ubiquitous family of organic polycations, the polyamines, which exert diverse roles in cell proliferation, differentiation, survival and death (Büttner et al., 2014). A decrease in polyamine levels is known to play a significant role in the aging process and the pathophysiology of neurodegenerative disorders including PD. Spermidine is a polyamine that counteracts age associated cell death via activation of the autophagic machinery. It belongs to an ubiquitous family of organic polycations, the polyamines, which exert diverse roles in cell proliferation, differentiation, survival and death (Jamwal and Kumar, 2016). An age dependent decrease of polyamine levels has been described in many organisms, including the brain of drosophila, rodents and humans. Interestingly, dietary spermidine supplementation as well as genetically enforced biosynthesis of spermidine showed to protected cognitive aging (Vivó et al., 2001). It acts by scavenging the free radical formation, antagonize NMDA receptors and enhance the formation of autophagosome. Furthermore, results from our laboratory have shown that spermidine exhibit beneficial effects in 3-nitropropionic acid induced neurotoxicity in rats by combating oxidative stress, inflammation, apoptosis and mitrochondrial dysfunctioning (Jamwal et al., 2015). These observed outcomes from our earlier studies led to foundation of the current study, herein we investigated the effect of spermidine on rotenone induced motor deficits, biochemical abnormalities and neurochemical alterations in rats.

2. Materials and methods

2.1. Experimental animals

In the present study, experiments were carried out in male Wistar rats (200-250 gm) procured from Panecia biotech and kept in the central animal house of ISF College of Pharmacy, Moga, Punjab (India) with food and water ad libitium. The animals were kept in polyacrylic cages and maintained under standard husbandary conditions (room temperature 22±2 °C and relative humidity of 55–60%) with a 12-h light/dark cycle (lights turned on at 7 a.m.). All the behavioral assessments were carried between 19:00 and 23:00 h i.e., in the active phase of animals. The experimental protocol was approved (ISFCP/CPCSEA/18/313) by the Institutional Animal Ethics committee (IAEC) and experiments were carried out in accordance with Indian National Science Academy (INSA) guidelines for the use and care of experimental animals.

2.2. Drugs and chemicals

Rotenone and spermidine was purchased from (Sigma Aldrich, St. Louis, MO, USA). Rotenone was dissolved in DMSO (dimethyl sulphoxide) and administered s.c. daily for 28 days. On the other hand, spermidine was dissolved in double distilled water (Jamwal et al., 2015). It is administered p.o. from 15 to 28 days daily 1 h prior to the rotenone administeration. All other chemicals used in the study were of analytical grade. Solutions of the drugs and chemicals were freshly prepared before use.

2.3. Experimental procedure

Animals were randomly assigned into 5 groups (n = 6 for each group). Group I –Normal control, Group II – Rotenone (1.5 mg/kg, s.c. for 28 days), Group III – Rotenone + spermidine (5 mg/kg, p.o.), Group IV – Rotenone + spermidine (10 mg/kg, p.o.), Group V – Rotenone + ropinirole (0.5 mg/kg i.p.). Spermidine and ropinirole were administered 1 h prior to rotenone administration for 14 days i.e. from day 15–28. Behavioral assessments were carried out on 14, 21 and 28 day. On day 29 animals were sacrificed for neurochemical, neuroinflammatory, biochemical and histopathological assessment. The experimental procedure is summarized in (Fig. 1).

2.3.1. Experimental design

Rotenone was dissolved in 1% DMSO solution and administered subcutaneously daily for 28 days at a dose of 1.5 mg/kg. Spermidine was dissolved in double distilled water and administered at a dose of 5 and 10 mg/kg by the per oral route. It was given 1 h prior to the rotenone administration from day 15–28. Behavioral parameters like grip strength, narrow beam, rotarod, and locomotor activity were assessed on day 14th, 21st and 28th day. Terminally on 29th day, animals were sacrificed to isolate brain. Then striatum was separated and used to estimate biochemical parameters (LPO, nitrite, and reduced GSH) neurotransmitter analysis (DA, NE, serotonin, DOPAC, HVA, 5-HIAA, glutamate and GABA). The levels of pro-inflammatory cytokines (IL-1b, IL-6, and TNF-a) were estimated using ELISA kits.

2.4. Parameters

2.4.1. Measurement of body weight

The body weight of animals was measured prior to Rotenone administration (1st day) and on the last day of the study (28thday). The percentage change in body weight was calculated as follows: Change in bodyweight = bodyweight (1st day–28thday)/1st day body weight × 100.

2.4.2. Assessment of behavioral parameter

2.4.2.1. Open field test. Open field test is used to monitor spontaneous locomotor activity using wooden, rectangular, light brown-colored open field apparatus measuring 100 9100 9 40 cm. The floor of the apparatus was divided into 25 rectangular squares by pencil lines. The experimental room was illuminated by 40 W white bulb located 150 cm above the test apparatus. The animal was placed for 12 in the center and numbers of squares crossed in last 10 min were recorded. Each crossing was considered only when all

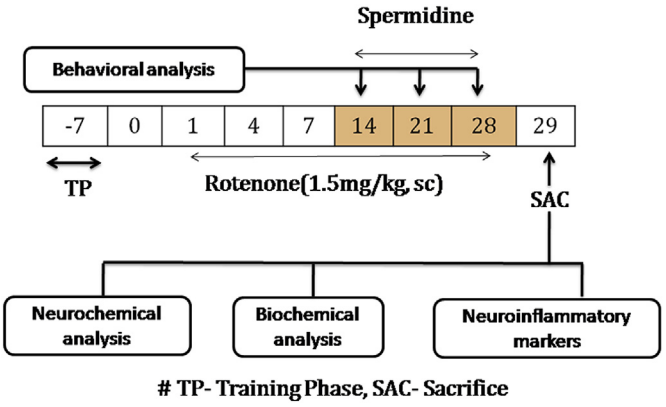

Fig. 1. Experimental design (Duration of drug administration and parameter assessment).