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Protective role of apigenin on rotenone induced rat model of Parkinson's disease: Suppression of neuroinflammation and oxidative stress mediated apoptosis

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A B S T R A C T

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra which is associated with oxidative stress, neuroinflammation and apoptosis. Apigenin (AGN), a non-mutagenic flavone found in fruits and vegetables, exhibits a variety of biological effects including anti-apoptotic, anti-inflammatory, and free radical scavenging activities. The current study was aimed to investigate the neuroprotective effects and molecular mechanisms of AGN in a rat model of PD induced by rotenone (ROT). Unilateral stereotaxic intranigral infusion of ROT caused the loss of tyrosine hydroxylase (TH) immunoreactivity in striatum and substantia nigra. AGN treatment (10 and 20 mg/kg, i.p.) showed a significant improvement in behavioral, biochemical and mitochondrial enzyme activities as compared to ROT exposed rats. The mRNA expression of inflammatory markers and neurotrophic factors was quantified by reverse transcriptase polymerase chain reaction (RT-PCR). Administration of AGN significantly attenuated the upregulation of NF-κB gene expression in ROT induced group and prevented the neuroinflammation in substantia nigra pars compacta (SNpc). Further, AGN inhibited the release of pro-inflammatory cytokines TNF- α, IL-6 and pro-inflammatory enzyme iNOS-1 induced by ROT. Additionally, AGN prevents the reduction of neurotrophic factors BDNF and GDNF mRNA expression in ROT lesioned rats. Immunoblot results illustrated that AGN treatment downregulated α-synuclein aggregation and upregulated the TH protein expression as well as dopamine D2 receptor (D2R) expression in ROT lesioned rats. Thus, the present findings collectively suggest that AGN exerts its neuroprotection in ROT model of PD and may act as an effective agent for treatment of PD.

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1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra, subsequent decrease of the dopamine level in the striatum and the presence of Lewy bodies (LBs), in which specific proteins including modified α-synuclein are deposited [1]. The symptoms of PD include movement disorders such as resting tremors, postural abnormalities, rigidity, and akinesia, all of which develop as a result of the loss of 50–70% of dopaminergic neurons [2]. The neurochemical features of PD include reactive oxygen species (ROS) generation, mitochondrial dysfunction, inflammation, accumulation of misfolded proteins, nitric oxide production and ubiquitin-proteasome system dysfunction [1].

Environmental factors, such as pesticide exposure, have long been suggested as etiologic factors in PD [3–5]. Rotenone (ROT) is the most potent member of the rotenoids, a family of isoflavonoids extracted from Leguminosae plants, commonly used as a natural pesticide and a classical mitochondrial complex-I inhibitor [6]. Since mitochondrial complex I inhibition is a well-documented feature of idiopathic PD [7], ROT was suspected to be a dopaminergic neurotoxin. Many studies have demonstrated that systemic administration of ROT accurately recapitulates many other features of PD including: selective degeneration of the nigrostriatal dopaminergic system, ROS generation, accumulation and aggregation of α-synuclein, Lewy bodies, Lewy neuritis, activation of microglia, neuroinflammation and movement disorders [8,9]. Nian Xiong and

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colleagues reported that stereotaxical infusion of ROT into the rat substantia nigra as a reliable rodent model for Parkinson's disease [10].

Neuroinflammation has recently been implicated as a critical mechanism involved in the PD pathogenesis [11]. Inflammatory activation of microglial cells may contribute to the neurodegenerative process through structural invasion and the release of pro-inflammatory cytokines, reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and the levels of nitric oxide (NO) and superoxides, which have deleterious effects on dopaminergic neurons [12,13]. In addition, suppressing neuroinflammation with anti-inflammatory drugs alleviates dopaminergic neurodegeneration in various experimental models of PD [14].

Recently, there has been intense interest in the potential of flavonoids to modulate neuronal function and prevent against degenerated diseases like PD. Flavonoids such as tangeretin, has been observed to maintain nigro-striatal integrity and functionality following lesioning with 6-hydroxydopamine, suggesting that it may serve as a potential neuroprotective agent against the underlying pathology associated with Parkinson's disease [15]. Apigenin (4′, 5, 7-trihydroxyflavone) (AGN) is a member of the flavone subclass of flavonoids present in fruits and vegetables such as onions, oranges [16]. AGN has been shown to possess various biological activities such as antioxidant [16], anti-inflammatory [17], and anti-tumorigenic [18] properties in various cell types. It is reported that AGN protects brain neurovascular coupling against amyloid-β_{25-35} induced neurotoxicity in mice [19]. Furthermore, AGN was found to prevent the harmful microglial mediated inflammation and maintains proper glia-neuron interactions [20]. AGN had neuroprotective role against the MPTP induced parkinsonian mouse model [21]. There are no reported studies of the neuroprotective effects of AGN in a ROT rat model of PD. Therefore, in the present study, we investigated the neuroprotective activity of AGN against nigrostriatal dopaminergic degeneration, neuroinflammation and oxidative stress in a rat model of PD induced by ROT.

2. Materials and methods

2.1. Chemicals and reagents

Rotenone (ROT) and Apigenin (AGN) were purchased from Sigma –Aldrich Chemicals Private Ltd. Glutathione (GSH), glutathione oxidized (GSSG), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro- 2,4-dinitrobenzene (CDNB), 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), thiobarbituric acid and ethylene diamine tetra acetic acid (EDTA) were purchased from Sisco Research Laboratories (SRL). RNA Primers (β-actin, BDNF, GDNF, i-NOS1, TNF-α, NF-κB and IL-6), primary antibodies (Tyrosine hydroxylase, Dopamine receptor D2 (D2R) and α-synuclein) and Horseradish Peroxidase (HRP) conjugate rabbit anti goat were purchased from Santacruz biotechnology Inc.

2.2. Animals and treatments

Healthy adult male Sprague-Dawley rats (3 months old) weighing about 200–250 g were obtained from Central Animal House (Dr. ALMPGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil Nadu, India). Rats were housed in clean polypropylene cages and maintained in an air-conditioned animal house with constant 12-h light/dark cycle. Rats were permitted free access to drinking water throughout the experimental period. The animals were fed with standard rat pellet diet (Lipton India Ltd., Mumbai, India). Experiment was approved by the Institute Animal Ethical Committee (IAEC No. 01/11/13). All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC No. 01/11/13) of Dr.ALMPGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil Nadu, India. After 1 week of acclimation, the animals were randomly divided into five groups of six animals each: Group I (Control):Rats were given 2 μl of vehicle (DMSO and PEG in the ratio of 1:1) through intranigral injection, considered as controls for 14 days. Group II (ROT): Rats were given 2 μl of ROT (6 μg/rat) through intranigral injection on 1st day. Group III (ROT + AGN 10 mg): Rats were given 2 μl of ROT (6 μg/rat) through intranigral injection on 1st day followed by intraperitoneal administration of AGN (10 mg/kg in DMSO) for 14 days. Group IV (ROT + AGN 20 mg): Rats were given 2 μl of ROT (6 μg/rat) through intranigral injection on 1st day followed by intraperitoneal administration of AGN (20 mg/kg in DMSO) for 14 days. Group V (AGN 20 mg): Rats were given AGN (20 mg/kg in DMSO) intraperitoneally for 14 days.

2.3. Intranigral rotenone administration

Rats were anaesthetized with ketamine and xylazine intraperitoneally and placed on a small animal stereotaxic frame (Instruments and Chemicals, Ambala, New Delhi). ROT dissolved in DMSO: PEG (1:1) was injected (2 μl) into the right substantia nigra pars compacta (SNpc) at a flow rate of 0.2 μl/min. The stereotaxic coordinates were: lateral = 0.20; anterio-posterior = 0.53; and dorso-ventral = 0.75, from the Bregma point [22]. In sham control animals, 2 μl of the vehicle (DMSO and PEG in the ratio of 1:1) was infused into the right SNpc. Proper post-operative care was taken till the animals recovered completely.

2.4. Post operative care

Recovery of anesthesia took approximately 4–5 h. The rats were kept in a well-ventilated room at 25 ± 3 °C in individual cages until they gained full consciousness; they were then housed together in groups of 4 animals per cage. Food and water was kept inside the cages for the first week, allowing animals' easy access, without physical trauma due to overhead injury. Animals were then treated normally; food, water, and the bedding of the cages were changed twice per week.

2.5. Behavioral assessments

All the behavioral parameters were performed at room temperature without any outside interference. All of the behavioral parameters were performed between 10.00 a.m. and 5.00 p.m.

2.5.1. Rotarod testing

The behavior of each rat was assessed by the rotarod test, as described previously [23]. The rotarod treadmill consists of a bar with a diameter of 2.5 cm, subdivided into four compartments by disks, 25 cm in diameter which enables four rats to walk on the rod at the same time. In the present study, the accelerating rotor mode was used (10-grade speeds from 2 to 20 r.p.m. for 5 min). The performance time was recorded while rats were running on the rod. Each rat was subjected to three different trials with 3 min of interval between each trial, and the mean of their falling latency values was used in the statistical analysis as actual value.

2.6. Tissue preparation

On the 15th day, the animals were sacrificed by cervical decapitation and the brains were dissected out. The striatum and substantia nigra were separated and placed on ice. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer saline

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