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Modulatory effects of resveratrol on endoplasmic reticulum stress-associated apoptosis and oxido-inflammatory markers in a rat model of rotenone-induced Parkinson's disease

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ABSTRACT

The mechanisms leading to neuronal death in Parkinson's disease (PD) are not fully elucidated; however, mounting evidence implicates endoplasmic reticulum (ER) stress, oxidative damage, and inflammatory changes are the crucial factors in its pathogenesis. This study was undertaken to investigate the modulatory effects of resveratrol on ER stress-mediated apoptosis, inflammatory and oxidative stress markers in a rat model of rotenone-induced PD. mRNA expression levels of ER stress markers; C/EBP homologous protein (CHOP) and glucose-regulated protein 78 (GRP78), were estimated in the rat brain using quantitative real-time PCR. Caspase-3 activity, IL-1β levels and Nuclear Factor Erythroid 2-related factor (Nrf2) DNA-binding activity were estimated by ELISA, while glutathione peroxidase and Xanthine oxidase activities, as well as protein carbonyl contents in the rat brain were evaluated spectrophotometrically. Our data revealed that Resveratrol ameliorated rotenone-induced ER stress by down-regulating CHOP and GRP78 genes expression and hampered caspase-3 activity in the brain of rotenone exposed rats. It also restored redox balance as evident by suppressing Xanthine oxidase activity and protein carbonyls formation; in addition to preservation of intracellular antioxidants status via activating glutathione peroxidase and Nrf2 signaling pathway. In conclusion; our study launched promising avenues for the potential use of resveratrol as a neuroprotective therapeutic agent in Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting from the selective loss of dopaminergic neurons in the substantia nigra [36]. PD patients show progressive motor disturbances such as muscle rigidity, resting tremors and bradykinesia [35].

Although the mechanisms leading to the selective neuronal death in PD are not fully elucidated, compelling experimental evidence implicates endoplasmic reticulum (ER) stress, oxidative damage, mitochondrial dysfunction, and inflammation as the crucial factors in PD pathogenesis [23]. Also, an association between environmental factors and occurrence of PD has been recorded. Rotenone, a commonly used pesticide that inhibits mitochondrial complex I, has been found to induce neuropathological hallmarks of PD in exposed organisms [30].

The ER is a complex intracellular membranous network which orchestrates proteins synthesis, folding, modifications and transport to their final destination. Altered ER homeostasis initiates a condition called ER stress [44]. To cope with this stress, cells activate the unfolded protein response (UPR) which is an integrated signal transduction pathway consisting of two components: the adaptive and the apoptotic UPR. The adaptive UPR seeks to restore normal ER function while the apoptotic UPR eliminates irreversibly damaged cells through activation of cell death cascades [12].

The ER-resident chaperone GRP78 is a key regulatory protein that plays an important role in the recognition of unfolded proteins. On triggering ER stress, the unfolded proteins make complex with free GRP78 lead to its activation, and trigger the UPR [15].

The transcription factor; C/EBP homologous protein (CHOP) has attracted significant attention due to its role in neurodegenerative disorders. CHOP is induced by ER stress at the transcriptional level

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[11]. CHOP over-expression promotes apoptosis, while its deficiency can protect the cells from ER stress-induced apoptosis [54].

Mounting evidence suggested a crosstalk between ROS generation and ER stress response. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a key coordinator in expression of genes required for maintenance of redox potential and xenobiotics detoxification [29]. Under basal conditions, Nrf2 interacts with Kelch-like erythroid cell-derived protein 1 (Keap-1), a cytosolic repressor protein, limiting Nrf2-mediated gene expression. Upon activation, the Keap-1-Nrf2 complex is dissociated and thus triggering Nrf2-mediated gene expression [8].

Recently, there is great interest in the development of neuroprotective drugs from natural sources as a therapeutic approach for PD. Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic phytoalexin found in grapes, cranberries and peanuts with multiple biological activities. Recent studies have reported the ability for resveratrol to offer protective effects against a number of neurodegenerative and cardiovascular diseases, and cancer [4].

Therefore, we aimed to investigate the modulatory effects of resveratrol on ER stress mediated apoptosis and oxidative stress in a rat model of rotenone-induced PD in order to provide new mechanistic insights and to launch promising avenues for the potential use of resveratrol as a neuroprotective therapeutic agent in PD.

2. Materials and methods

2.1. Chemicals

Rotenone, resveratrol and other chemicals used were purchased from Sigma-Aldrich Chemicals (St. Louis, MO).

2.2. Animals and experimental design

The present work was conducted on fifty adult male Wistar albino rats (3–4 months old, 200–250 g weight), they were housed in wire mesh cages under standard laboratory conditions with free access to standard pellet diet and tap water, constant room temperature of 22 °C, 50–60% humidity, and a natural day–night cycle through the whole period of the experiment. The study protocol was approved by the local ethical Committee at Faculty of Medicine, Tanta University. The experiment was conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication No.85-23, revised in 1996).

The animals were randomly allocated to four groups. Group I (10 rats) received vehicle of 1% dimethylsulfoxide (DMSO) in a dose of 0.1 ml/100 g; S.C and served as a control group. Groups II (10 rats) received only resveratrol (freshly dispersed in 0.9% saline solution) via oral gavage at a dose of 20 mg/kg/day; in a total volume of 1 ml for 3 weeks. Groups III (15 rats) received eleven S.C injections of rotenone every other day at a dose of 1.5 mg/kg dissolved in 1% DMSO [50] for 21 days. Groups IV (15 rats) received both rotenone (S.C s injections; every other day at a dose of 1.5 mg/kg dissolved in 1% DMSO for 21 days) and resveratrol via oral gavage at a dose of 20 mg/kg/day; in a total volume of 1 ml; daily for 3 weeks and 1 h before rotenone administration on the days of rotenone injection [26].

2.3. Assessment of motor function

2.3.1. Catalepsy test

The bar and grids test were used for measuring catalepsy 24 h after the last injection of rotenone to estimate the degree of akinesia signifying the development of parkinsonism. For the bar test, rats were placed with both fore paws on bars 9 cm above and parallel from the base and were in a half-rearing position. The time taken for the animals to show a cataleptic posture, which was defined as an immobile posture while keeping both forelimbs on the bar, was measured in seconds [46]. For the grid test, rats were hung by its paws on a vertical grid (25.5 cm wide and 44 cm high with a space of 1 cm between each wire), and the time for the rats to move its paws or any sort of first movement was recorded as descent latency. The maximum cut off time was fixed at 180 s for both tests [2].

2.3.2. Rotarod test

The rotarod test was performed to assess motor function using a rotarod apparatus (3 cm in diameter and rotating at a constant speed of 20 rpm). At the start, animals were pre-trained to maintain posture on the rotarod apparatus by giving two training sessions of 5 min each with a gap of 10 min between the two sessions. In the final test, animals were allowed to move over the rotarod and their falling time was recorded using a cutoff limit of 120 s [24].

2.4. Tissue sampling

After sacrificing rats by decapitation under isoflurane anesthesia, brains were immediately excised and perfused in situ with ice-cold 0.9% (w/v) NaCl solution. Striata of both hemispheres were isolated and stored at −70 °C till used for preparation of tissue homogenate and nuclear extracts.

2.4.1. Preparation of brain tissue homogenate

One piece of each specimen was weighed and homogenized in 0.15 M KCl, at a ratio of 100 mg of tissue protein to 1 ml of buffer, using a Potter-Elvenhjem tissue homogenizer. Homogenates were centrifuged at 7700×*g* for 30 min at 4 °C and the resultant supernatant was used for further analysis.

2.4.2. Preparation of brain nuclear extracts

A nuclear extract of brain cells was prepared using the Nuclear/Cytosol Fractionation Kit (Cat #K266-25, BioVision, Inc., CA, USA) according to the protocol of the manufacturer.

2.5. Biochemical assays

2.5.1. Estimation of striatal dopamine (DA) levels

Dopamine (DA) levels were measured in the supernatant fractions of the brain homogenate by a specific rat ELISA kit (Cat #CSB E08660r, Cusabio; China) according to the manufacturer's protocol [37].

2.5.2. Estimation of endoplasmic reticulum stress markers (CHOP and GRP78) mRNA expression levels by real-time PCR

- **RNA extraction:** Total RNA from the striatum was prepared using Qiagen RNeasy Mini Kit according to the protocol supplied by the manufacturer. RNA was eluted, its concentration was measured spectrophotometrically (260 nm) and then stored at −80 °C.
- **cDNA synthesis** was performed using the RevertAid H Minus First Strand cDNA Synthesis kit (Cat#K1632, Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions. Ten μl of random hexamer primers (Roche, Mannheim, Germany) were added to 21 μl of RNA which was denatured for 5 min in the thermal cycler (Biometra, USA). The RNA-primer mixture was cooled to 4 °C. The cDNA master mix was prepared (5 μl of first strand buffer, 10 mM of dNTPs, 1 μl of RNase inhibitor, 1 μl of reverse transcriptase Superscript™ II-RT enzyme and 10 μl of DEPC treated water) according to the kit