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Thymoquinone exerts neuroprotective effect in animal model of Parkinson's disease

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ABSTRACT

Oxidative stress plays an important role in both the initiation and progression of Parkinson's disease (PD). Rotenone, an environmental toxin, induces oxidative stress and impact mitochondrial dynamics, including fission and fusion. Thymoquinone (TQ) has been reported to have antioxidant and anti-inflammatory characteristics in vitro and in vivo. TQ scavenges free radicals so prevents cell damage against oxidative agents. To evaluate the efficacy of TQ in the management of PD, male Wistar rats (8–10 months) received rotenone. Pre-treatment with TQ (7.5 and 15 mg/kg/day, po) one hour prior to the rotenone injection, changed motor tests results (rotarod, rearing and bar tests). Dopamine levels of dopaminergic areas in the substantia nigra (SN) and striatum (ST) measured using high-performance liquid chromatography. Western blot analysis employed to determine the protein contents of Parkin and Drp1 (dynamin-related protein-1) in these areas and in order to identify tyrosine hydroxylase positive cells, Immunohistochemical assays performed. The results indicated that TQ significantly prevented rotenone-induced motor defects and changes in the Parkin, Drp1, dopamine and TH levels in both studied areas. These findings show that TQ effects on ameliorating the PD symptoms induced by rotenone might be associated with the neuroprotective and antioxidant effects of this compound.

1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. The main pathological mechanism of this disease is damage of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). These neurons are required for appropriate motor function, and their loss is associated with symptoms, such as tremor, rigidity, bradykinesia and postural instability (Sanders and Greenamyre, 2013). The abnormal protein aggregation, oxidative stress and inhibition of mitochondrial complex I have been identified as the primary mechanisms involved in the development of Parkinson's disease (Morán et al., 2012; Sanders and Greenamyre, 2013).

Several lines of evidence have indicated that exposure to environmental toxins, such as pesticides and pollutants, greatly increased the risk of PD (Goldman, 2014). Environmental toxins, such as rotenone, induce clinical and pathologic features in rats similar to those induced by PD, including the selective degeneration of the nigrostriatal dopaminergic system and movement disorders (Zhou et al., 2016).

The association between Parkinson's disease and mitochondrial dysfunction has been revealed (Elgass et al., 2013). Mitochondria are important cellular organelles with a highly dynamic nature, undergoing frequent fission and fusion. The dynamic balance between fission and fusion plays critical roles in mitochondrial functions, and neurons are also extremely dependent on normal mitochondrial function (Han et al., 2011). It has been shown that parkin could normally function to limit mitochondrial fusion to help with the selective isolation and elimination of damaged mitochondria by mitophagy(Glauser et al., 2011), also stabilizes microtubules through strong interactions with microtubule-binding domains, generating neuroprotective effects in parkinsonian animals (Yasuda and Mochizuki, 2010). In addition, Drp1 is a cytosolic and fission factor that assembles with mitochondria to promote mitochondrial fission (Whitworth and Pallanck, 2009). Oxidative stress is an imbalance between productions of reactive oxygen species (ROS) and antioxidant defense mechanism. It stimulates the translocation of Drp1, resulting in enhanced mitochondrial fission (Eckermann, 2013).

Thymoquinone (TQ) was shown to exert antioxidant and anti-inflammatory effects (Radad et al., 2014). This agent is the primary component of the oil extracted from Nigella sativa seeds, and many of the antioxidant and anti-inflammatory properties of the seeds have been attributed to this oil (Alhebshi et al., 2014). TQ shows beneficial

Abbreviations: TQ, thymoquinone; Rot, rotenone; SN, substantia nigra; ST, striatum; TH, tyrosine hydroxylase; V, vehicle; Apo, apomorphine; SEM, standard error of the mean

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effects in cancer and inflammation through different modes of action. This compound is also a potent free radical and superoxide radical scavenger (Akhtari et al., 2014) that preserves the activity of various antioxidant enzymes, such as catalase, glutathione peroxidase and glutathione-S-transferase (Woo et al., 2012).

Moreover, the neuroprotective potential of TQ against MPP+ (1-methyl-4-phenylpyridinium) and rotenone-induced cell death in primary dopaminergic cell cultures relevant to PD has previously been described (Radad et al., 2009). Considering the role of oxidative stress in the pathophysiology of PD and the antioxidant properties of TQ, in the present study, we evaluated the effects of TQ on motor function and the nigrostriatal pathway content of Parkin, Drp1 and dopamine in an animal model of rotenone-induced Parkinson's disease.

2. Materials and methods

2.1. Materials

Rotenone (Sigma-Aldrich) was dissolved in dimethylsulfoxide (DMSO) as a solvent and diluted in sunflower oil to obtain a final concentration of 1 mg ml^{-1} rotenone for each subcutaneous injection. Thymoquinone (Sigma-Aldrich), prepared using polyethylene glycol 200 (Sigma-Aldrich) at two different concentrations (7.5 and 15 mg ml^{-1}) and administered orally. All solutions were freshly prepared prior to administration. For western blotting Antibodies against β-Actin and Parkin were purchased from Abcam (Cambridge, MA), anti Drp1 antibody was purchased from Bio-Science (Franklin Lakes, NJ). Antibody against TH and Goat Anti-Rabbit IgG H & M for Immunohistochemical study was purchased from Abcam (Cambridge, MA).

2.2. Animals & experimental groups

Forty male Wistar rats (purchased from the Pasteur Institute of Iran) weighing 300 ± 50 g, aged 8–10 months, were randomly rendered, housed in standard condition. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985) and approved by the Research Ethics Committee of Kurdistan University of Medical Sciences.

The animals randomly divided into 5 groups (n = 8):

- One group received rotenone vehicle (1 ml kg^{-1} 0.48 h, sc) + TQ vehicle (1 ml kg^{-1} 0.48 h, po)
- One group received rotenone (1 mg kg^{-1} 0.48 h, sc) + TQ vehicle (1 mlkg^{-1} 0.48 h, po) (control group)
- Two groups received rotenone (1 mg kg^{-1} 0.48 h, sc) and TQ (7.5 or 15 mg kg^{-1} 0.48 h, po)
- One group received TQ (15 mg kg^{-1} 0.48 h, po)

2.3. Induction of parkinson's disease in rats

Based on previous studies (Cannon et al., 2009), Rotenone (1 mg kg^{-1}) was administered at 48-h intervals to induce Parkinson's disease in rats. The injections continued until motor signs were observed in rotarod tests of the control group. Briefly, animals were evaluated every 48 h, and Parkinson's disease induction was considered as the day at which a significant decline in the latency time was observed in the rotarod test.

2.4. Movement tests

2.4.1. Rotarod test

This test was performed at baseline (prior to rotenone injection on the first day) and every 48 h from the 6th day of injection and reported for 1, 6, 12, 18, 24, and 30 days (Rogers et al., 1997; Urbach et al., 2010). Prior to the initiation of injections and experiments, a 5-day training program was conducted to obtain a stable performance. The rotation speed was initiated at 11 rpm on the first day and reached 15 rpm by the end of the training program (Rahimmi et al., 2015; Vandeputte et al., 2010).

2.4.2. 2 bar test

To assay rigidity and catalepsy, Rats were placed on a bar with both forepaws at 10 cm above the base in half rearing position. Subsequently, the time of removal of one or both paws was recorded as the bar test time. A latency time of 180 s was considered as the cut-off time (Xiong et al., 2011). This test was performed prior to the first administration and one day after Parkinson model induction, as approved by the rotarod test.

2.4.3. Rearing test

Animals were placed in a clear Plexiglas cylinder (20-cm diameter, 40 cm height) for 5 min in the dark under a red light. The healthy animals were normally reared and engaged in exploratory behavior, placing their forelimbs on the wall of the cylinder. This behavior was recorded by a person blinded to the experimental groups after counting the number of rears scored when the animal raised its forelimbs above shoulder level and leaned against the cylinder wall with either one or both forelimbs. This test was also performed prior to the first injection and one day after the induction of the model approved using the rotarod test (Fleming et al., 2004).

2.4.4. Apomorphine test

To detect whether motor dysfunctions were dopamine-dependent, on the 30th day (after model confirmation by movement tests in control group), apomorphine, a dopamine agonist, was administered (1 mg kg^{-1}, sc), and the rotarod test was conducted after 10 min. Previous studies have shown that apomorphine temporarily restores movement signs of Parkinson's disease (Cannon et al., 2009).

2.5. Body weight measurement

Body Weight was considered an index for monitoring the well-being and peripheral toxicity induced by the toxin rotenone. Indeed, a significant decrease in body weight reveals a rotenone-induced unspecific effect (Xiong et al., 2011). The animals were weighed prior to each injection daily.

2.6. High-performance liquid chromatography (HPLC) analysis of the dopamine levels in the substantia nigra

Animals (n = 4-5) were anesthetized (ketamine–xylazine cocktail) and immediately (< 1 min) sacrificed by decapitation. Subsequently, the substantia nigra and striatum were dissected, cleaned and frozen in liquid nitrogen and stored at −70 °C until further analysis. The samples were homogenized in 100 µl of lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% w/v NP-40) and a complete Protease Inhibitor Cocktail Tablet (Roche Cat # 04 693 132 001), per 50 mg of tissue using a Dounce homogenizer. The samples were centrifuged at 10,000 rpm for 10 min at 4 °C. Subsequently, 20 µl of supernatant was injected onto a C18 reverse-phase HR-80 catecholamine column (KNAUER Eurospher II Germany). The mobile phase contained A-0.05% aqueous TFA-methanol (97.5:2.5, v/v) and B −0.05% aqueous TFA-methanol (40:60, v/v). A flow rate of 1 ml/min was used over 20 min with the following gradient: 0.00–1.00 min 100% A; 16 min, 50% A and 50% B (linear gradient from 1 to 16 min); and 16.05 min, 100% A to return the column to initial conditions within 20 min. The native fluorescence, excitation at 220 nm and emission at 320 nm(Singh et al., 2010) were used for detection.