Neurochemistry International 80 (2015) 79–86

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Neuroprotective effects of bee venom acupuncture therapy against rotenone-induced oxidative stress and apoptosis

Wagdy K.B. Khalil [a], Naglaa Assaf [b], Shaimaa A. ElShebiney [c], Neveen A. Salem [c,*]

[a] Department of Cell Biology, National Research Centre (NRC), Cairo, Egypt
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Misr University for Science and Technology, Cairo, Egypt
[c] Department of Toxicology and Narcotics, NRC, Cairo, Egypt

ARTICLE INFO

Article history:
Received 6 May 2014
Received in revised form 17 November 2014
Accepted 28 November 2014
Available online 3 December 2014

Keywords:
Bee venom
Acupuncture therapy
Rotenone
Parkinson's disease
Oxidative stress
Apoptosis

ABSTRACT

Parkinson's disease (PD), the most common neurodegenerative movement disorder, is characterized by dopaminergic neurodegeneration, mitochondrial impairment, and oxidative stress. Exposure of animals to rotenone induces a range of responses characteristic of PD, including reactive oxygen species production and dopaminergic cell death. Although L-dopa is the drug of choice for improving core symptoms of PD, it is associated with involuntary movements. The current study was directed to evaluate the neuroprotective effect of bee venom acupuncture therapy (BVA) against rotenone-induced oxidative stress, neuroinflammation, and apoptosis in PD mouse model. Forty male Swiss mice were divided into four groups: (1) received saline solution orally and served as normal control, (2) received rotenone (1.5 mg/kg, s.c. every other day for 6 doses), (3) received rotenone concomitantly with L-dopa (25 mg/kg, daily, p.o. for 6 days), and finally (4) received rotenone concomitantly with BVA (0.02 ml once every 3 days for two weeks). Rotenone-treated mice showed impairment in locomotor behavior and a significant reduction in brain dopamine, serotonin, norepinephrine, GSH levels, and paraoxonase activity, whereas a significant increase was observed in brain malondialdehyde, tumor necrosis factor-α, interleukin-β levels besides DNA damage, and over-expression of caspase-3, Bax, and Bcl-2 genes. Significant improvement of the aforementioned parameters was demonstrated after BVA compared to L-dopa therapy. In conclusion, bee venom normalized all the neuroinflammatory and apoptotic markers and restored brain neurochemistry after rotenone injury. Therefore, BVA is a promising neuroprotective therapy for PD.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease affecting middle aged and elderly people (Braak et al., 1995). It is characterized by loss of dopaminergic neurons of the substantia nigra (SN) and depletion of dopamine in the striatum (STR) that results in the typical symptoms of parkinsonism, including resting tremor, rigidity, and bradykinesia (Fahn and Przedborski, 2005). Neuroinflammation plays a critical role in the pathogenesis of PD (Hirsch et al., 2003). The primary mediators of neuroinflammation are activated microglia, which are innate immune cells of the central nervous system (CNS) found in and around the degenerating dopamine neurons (Block and Hong, 2005; Kreutzberg, 1996; McGeer and McGeer, 2008). Microglia are dramatically activated in response to neuronal damage and produce several potentially neurotoxic substances, including reactive oxygen species (ROS) and/or proinflammatory cytokines (Block et al., 2007).

Rotenone, a commonly used natural insecticide or fish poison, is highly lipophilic and readily passes to the brain (Talpade et al., 2000), where dopaminergic neurons are very susceptible to the induced mitochondrial complex I inhibition (Betarbet et al., 2006; Sherer et al., 2002). Chronic exposure of rats to rotenone recapitulates key features of parkinsonism, including selective loss of dopaminergic neurons and locomotor deficits (Deng et al., 2010). Effective experimental PD models should present dopaminergic degeneration, cytoplasmic inclusions, and motor malfunction (Sherer et al., 2002). According to our study and other previous reports, rotenone reproduced many features of PD, including systemic mitochondrial impairment, oxidative damage, nigrostriatal dopaminergic degeneration, L-dopa responsiveness, and cytoplasmic inclusions (Cannon et al., 2009; Mao et al., 2007; Sherer et al., 2002).

The crucial role of the neuroinflammatory process in PD suggests that inhibition of the microglial reaction might be a therapeutic avenue for reducing neuronal degeneration. There are no proven neuroprotective therapies for PD, and only symptomatic treatments are available. These include drugs therapy, such as L-dopa and dopamine agonists, MAO-B and COMT inhibitors, surgery, and physiotherapy (Hunot and Hirsch, 2003). L-dopa is the drug of choice in PD due to its ability to initially improve core symptoms by

* Corresponding author. National Research Center, ElBuhouth st., Dokki, P.O. 12311, Cairo, Egypt. Tel.: +20 33371362; fax: +20 33370931.
E-mail address: dr_nsalem@yahoo.com (N.A. Salem).

http://dx.doi.org/10.1016/j.neuint.2014.11.008
0197-0186/

increasing basal ganglia dopamine activity. However, after 5 years of therapy, 50% of patients experience motor response complications, and the benefit from each dose becomes weaker ("wearing off" fluctuations), more unpredictable ("on–off" fluctuations), and associated with involuntary movements (dyskinesias). In addition, patients continue to suffer from fluctuations in motor function that are inherent to the disease itself (Verhagen Metman, 2002).

In the light of significant limitations of conventional therapy, complementary and alternative therapies represent an attractive strategy. Previous studies have suggested that bee venom (BV) results in antinociceptive and anti-inflammatory effects on arthritis, neuralgia, and chronic inflammation in humans (Lee et al., 2005) and in an animal model of inflammatory disease (Baek et al., 2006). Therefore, BV has been widely used in oriental medicine to treat some immune-related diseases, such as rheumatoid arthritis in humans and experimental animals (Kwon et al., 2002; Son et al., 2007). Recently, clinical trials have suggested that BV might be beneficial in the treatment of neurodegenerative diseases of the CNS, including PD (Cho et al., 2012; Mirshafiey, 2007). Several studies have shown that BV produced a marked suppression of leukocyte migration in the inflammatory-mediated animal models (Kwon et al., 2003) and a significant inhibition of microglia or macrophage activation (Han et al., 2007; Moon et al., 2007; Park et al., 2007). In addition, BV acupuncture (BVA) has been shown to protect dopaminergic neurons effectively against MPTP-induced toxicity due to inhibition of microglial activation in mice (Doo et al., 2010; Kim et al., 2011). Moreover, BV has a direct neuroprotective effect on SH-SY5Y human neuroblastoma cells from MPP^+-induced apoptotic cell death (Doo et al., 2012). However, such therapeutic uses are not supported by convincing evidence to date, and the underlying mechanism of BV amelioration of neurodegenerative diseases of the CNS, such as PD, remains to be elucidated. Our focus was to evaluate the neuroprotective role of BVA therapy against rotenone-induced oxidative stress, neuroinflammation, and apoptosis in mouse model of PD.

2. Materials and methods

2.1. Animals

Swiss male albino mice 20–22 g of body weight (age: 5–6 weeks) were obtained from animal house colony of the National Research Centre (NRC, Cairo, Egypt). Mice were housed under standardized conditions with free access to standard laboratory food and water. Animal procedures were performed in accordance with the Ethics Committee of the NRC and followed the recommendations of the National institutes of health guide for care and use of laboratory animals (Publication No. 85-23, revised 1985).

2.2. Experimental design

Animals were divided into four groups (10 mice each). Group (1) received saline solution orally and served as normal control. Group (2) received rotenone (Sigma–Aldrich Chem. Co, MA, USA), 1.5 mg/kg, s.c., dissolved in DMSO every other day at 6 doses for induction of parkinsonian behavior (Gawad et al., 2004). Group (3) received rotenone concomitantly with L-dopa (Sinemet® tab (carbidopa/L-dopa, 25/250), Merk & Co. Inc., Whitehouse Station, NJ, USA), 25 mg/kg, daily, p.o., for 6 days (De Leonibus et al., 2009). Group (4) received rotenone concomitantly with BVA (Sigma–Aldrich Chem. Co, USA) with 0.02 ml (1:2000 w/v) to acupoint GB34 bilaterally once every 3 days for 2 weeks (Doo et al., 2010).

2.3. Neurobehavioral measures

At the end of the experimental period, all mice were screened for motor behavioral impairment using the wire hanging test and the cylinder test.

2.3.1. Wire hanging test

Neuromuscular strength was evaluated through the wire hang test, where mouse was placed with its forelimbs on a wire mounted horizontally of 20 cm length, 50 cm above the surface. Latency time to fall was recorded. 30 seconds cut off time was taken. Soft padding was placed on the landing area to avoid injury of the mice (Sanberg et al., 1996).

2.3.2. Cylinder test

Cylinder test uses rearing frequency to assess the locomotor activity. Spontaneous movement was measured by placing the animal in a small transparent cylinder (height, 15.5 cm; diameter, 12.7 cm) for 5 min. The number of rears was recorded after each treatment. A rear was counted when an animal made a vertical movement with both forelimbs removed from the ground. This test has been successfully used previously to assess behavioral deficits in the rats receiving subcutaneous or intravenous rotenone (Fleming et al., 2004).

2.4. Biochemical analysis

Mice were killed by decapitation under ether anesthesia, brains were excised, a part of the harvested brains was kept in 10% formol-saline for histopathological investigation, while the other part was washed with ice-cold saline (0.9%), weighed, and stored at –80 °C for further biochemical and molecular analyses. The brain was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays.

2.4.1. Oxidative stress markers

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) in brain samples according to the method of Placer et al. (1966) and the results were expressed as nmole malondialdehyde (MDA)/g wet tissue. In addition, brain glutathione (GSH) was evaluated by the method of Ellman (1959) and expressed as μmol/g wet tissue.

2.4.2. Brain paraoxonase -1 (PON1) activity

The determination of PON1 activity was carried out in accordance with the method of Gatica et al. (2006). This assay involves the hydrolysis of phenylacetate (substrate) by PON1/arylesterase activity releasing phenol. The phenol formed after the addition of a 40-fold diluted homogenate sample was spectrophotometrically measured at 217 nm. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. The activity of PON1 was expressed in K unit/g wet tissue. One unit was defined as the enzyme quantity that disintegrates 1 nmol phenylacetate per minute.

2.4.3. Brain cytokine levels

Brain TNF-α and IL-1β were determined by enzyme linked immunosorbent assay (ELISA) following the methods of Kitaura et al. (2004) and Tamaoki et al. (1999), respectively, using commercial ELISA kits (Invitrogen Corporation Camarillo, California,USA) and microtiter plate reader (Fisher Biotech, Germany). An aliquot of sample or calibrator containing the antigen to be quantified is allowed to bind with a solid phase antibody. After washing, enzyme labeled antibody is added to form a sandwich complex of solid phase Ab-Ag-Ab enzyme. Excess (unbound) antibody is then washed away, then enzyme substrate is added. The enzyme catalytically