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Edible bird's nest improves motor behavior and protects dopaminergic neuron against oxidative and nitrosative stress in Parkinson's disease mouse model

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ABSTRACT

Parkinson's disease (PD) is a neurological disorder originated from the death of dopaminergic neurons in the midbrain. Evidences showed that oxidative and nitrosative stress play a central role in neurodegeneration. In this study, we examine the neuroprotective effects of EBN (20 mg/kg and 100 mg/kg) in 6-hydroxydopamine (6-OHDA)-treated C57BL/6J mice. Twenty eight days of EBN oral administration greatly improved locomotor activity of PD mice in terms of travel distance and balancing. EBN also protected dopaminergic neuron against 6-OHDA in the substantia nigra. EBN reversed the reduction in the expression of antioxidant enzyme glutathione peroxidase 1 and the increased microglia activation in PD mice. Our results also showed that EBN effectively reduced 6-OHDA-induced nitric oxide formation and lipid peroxidation in SH-SY5Y cells. The data altogether indicates that EBN exerted neuroprotection through enhancement of antioxidant enzyme activity and the inhibition of microglia activation, nitric oxide formation and lipid peroxidation in PD model.

1. Introduction

Parkinson's disease is a progressive neurodegenerative disease affecting more than 1% of the population over 60 years of age (Farrer, 2006). Pathologically, there is loss of dopaminergic neurons in the substantia nigra which subsequently causes dopamine depletion in the striatum (Dauer & Przedborski, 2003). Dopamine depletion ultimately leads to deterioration of motor function, as seen in most PD patients (Snyder & Adler, 2007). Patients of this disorder are manifested with clinical signs such as tremor, rigidity and slow responsiveness. In addition, abnormal α-synuclein protein aggregation known as Lewy bodies is also detected in surviving neurons (Przedborski, 2005). The disease is not fatal, but it imposes a huge economic burden to the country due to the huge cost in medical care and morbidity associated with the disease.

Substantial number of evidences has proved that interplay of oxidative stress and apoptosis is a critical determinant in the pathogenesis of PD. To name a few, accumulation of protein aggregates and the loss of dopaminergic neurons in the midbrain region are two hallmarks of PD that are known to be associated with increased level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the midbrain (Bernstein, Garrison, Zambetti, & O'Malley, 2011). The neurotransmitter dopamine in nigral neuronal cells can auto-oxidize and release toxic dopamine-quinone species, superoxide radicals and hydrogen peroxide (Fernández-Espejo & Tseng, 2009). Glutathione peroxidase (GPX) is a member of the intracellular antioxidant defence system that catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides, hence eradicates these highly reactive biomolecules (Lei, Cheng, & McClung, 2007). Under the circumstances of PD, GPX level in substantia nigra becomes lower than in healthy individual (Kish, Morito, & Hornykiewicz, 1985). In addition, microglial activation in neurodegenerative diseases was linked to up-regulation of the enzyme inducible nitric oxide synthase (iNOS) (Liberatore et al., 1999) and hence the production of nitric oxide (NO), which reacts with ROS to form the aberrant RNS including peroxynitrite and eventually give rise to hydroxyl radicals (Yokoyama, Kuroiwa, Yano, & Araki, 2008). RNS is as unfavourable as ROS in the brain because it is believed to participate in the adverse events involved in neurodegeneration. Both high

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oxidative stress and nitrosative stress within the biological system would render cells and tissues more prone to the free radicals attack. Since cells consist mostly of proteins and lipids, they belong to the labile target of these chemically-reactive electron-rich molecules. This is supported by evidence of elevated levels of oxidative adducts of lipids, proteins and DNA in substantia nigra of PD patients (Alam et al., 1997; Dexter et al., 1989; Floor & Wetzel, 1998). Also, higher levels of NOS expression and peroxynitrite were found in PD patients (Huerta et al., 2007; Kouti et al., 2013). In fact, it was recognized that pathological protein aggregation in PD was mediated by peroxynitrite, hence further emphasizes the role of RNS in the development of PD as an inflammatory mediator (Good, Hsu, Werner, Perl, & Olanow, 1998).

On a side note, lipid peroxidation is a process whereby lipid-rich components in cell are oxidized by free radicals to results in a number of highly reactive electrophilic aldehydes including malondialdehyde, 4-hydroxy-2-nonenal (HNE) and acrolein (Sultana, Perluigi, & Butterfield, 2013). Malondialdehyde adducts and HNE have been found in Lewy bodies in PD (Castellani et al., 2002; Dalfó & Ferrer, 2008). HNE modified alpha-synuclein protein by potentiating its oligomerization and led to toxicity in neuronal cultures (Qin et al., 2007). It was also demonstrated that dopamine uptake by the rat striatal synaptosomes was disrupted by HNE (Morel, Tallineau, Pontcharraud, Piriou, & Huguet, 1998). Inefficient dopamine transport and protein aggregation due to lipid peroxidation may be pivotal to neurodegeneration and progression of PD.

Edible bird nest (EBN) is the hardened salivary product of the *Aerodramus* swiftlets and is commonly prepared in sugary water before consumption. It is a highly regarded natural food popular among Asians due to traditional use in enhancing skin complexion, appetite immunity, growth, metabolism and blood circulation (Chye, Tai, Koh, & Ng, 2017; Lim & Cranbrook, 2002). EBN has been scientifically implicated for influenza-inhibiting (Guo et al., 2006); osteoporosis-improving (Matsukawa et al., 2011), neuroprotection (Hou et al., 2017; Yew, Koh, Chye, Othman, & Ng, 2014) and prevention of cardiometabolic disease (Hou et al., 2015). Although the specific bioactive compounds accounted for the effects observed were yet to be identified in these studies, complex compositional makeup of EBN may suggest a plethora of therapeutic potentials (Marcone, 2005). In our previous study, we have found that EBN treatment ameliorated neurotoxicity of 6-hydroxydopamine (6-OHDA) in PD cell model (Yew et al., 2014). The mechanism behind the neuroprotective effect of EBN was associated with apoptosis-inhibiting and ROS-reducing effects of the treatment. There was, however, lack of study to confirm the neuroprotective effects of EBN in *in vivo* system. Application of EBN to mitigate PD-related motor dysfunction, degeneration of midbrain dopaminergic neuron and neuroinflammation in whole living organisms were never investigated, hence such investigation is a potential venue to explore.

In this study, we first establish the animal toxicology profile in our animal model with the EBN doses used in previous publication, followed by investigation into the neuroprotective effects of EBN with respect to motor function and dopaminergic neuron in substantia nigra of PD mouse model. The expression of antioxidant marker GPX1 was evaluated to indicate changes in intraneuronal antioxidant state. Microglial marker, cluster of differentiation molecule 11B (CD11b), was also studied to assess the inflammatory process in the midbrain. We complemented these studies by measuring NO release and lipid hydroperoxides formation in neuronal model SH-SY5Y to better understand if EBN suppresses nitrosative and oxidative stresses. This study further explores the application of EBN in the treatment of neurodegenerative diseases especially PD.

2. Materials and methods

2.1. Materials

Antibodies used in immunostaining including anti-tyrosine hydroxylase (TH), anti-GPX1, anti-CD11b were purchased from Novus Biologicals. Alexa Fluor 488-conjugated secondary antibody and Alexa Fluor 647-conjugated secondary antibody were obtained from Abcam. Biotinylated horseradish peroxidase-conjugated secondary antibody and 3,3′-diaminobenzidine (DAB) kit were purchased from Vector Laboratories.

Bovine serum albumin (BSA), phosphate buffered saline (PBS), 6-OHDA, Triton-X, paraformaldehyde, sucrose and Griess reagent were obtained from Sigma-Aldrich. Human neuroblastoma cells SH-SY5Y was obtained from the American Type Culture Collection (ATCC no. CRL-2266). All cell culture reagents including Dulbecco's Modified Eagle's Medium and fetal bovine serum were purchased from Gibco.

2.2. Preparation of EBN

Raw EBN collected from a local bird's nest farm in Perak, Malaysia. EBN were prepared into pancreatin-digested extracts denoted as S1 (crude extract) and S2 (water extract) according to our previously established protocol (Yew et al., 2014).

2.3. Animal

Male C57BL/6J mice were housed at 3–4 per cage. Standard laboratory chow and drinking water were available *ad libitum*. The animals were kept under controlled conditions of 12-h/12-h light/dark schedule and temperature at 23 ± 2 °C. Animal experimentation has obtained ethics approval from the Monash Animal Research Platform-1 *Animal Ethics* Committee of *Monash University* (*AEC number* MARP/2014/081).

2.4. Toxicology screening

Prior to the commencement of the experiment, animals were screened for the toxicology profile of EBN administration according to the Organisation for Economic Co-operation and Development guidelines (OECD, 2008). Sub-chronic oral exposure to EBN (20 mg/kg and 100 mg/kg daily) for 28 days was selected as criteria to test the effect of EBN on the animal's overall health and well-being. The dosages selected were adopted from the study by Matsukawa et al. using rats (Matsukawa et al., 2011). Therefore in this study safety profile was reassessed using C57BL/6J mice. The animals were taken good care throughout the 28 days. Water and food intake, as well as the signs of toxic effects and/or mortality were monitored daily. Body weight was recorded weekly. At the end of 4-week feeding period, the mice were euthanized by cardiac puncture on the 29th day. Maximal volume of blood was extracted from the heart and collected into plain blood tube. The blood was transported on ice to Faculty of Veterinary at University Putra Malaysia for biochemistry tests. The test included assessment of biomarkers for toxicity in the liver (eg. alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and albumin levels), heart (eg. creatine kinase level), kidney (eg. urea and creatinine levels) and pancreas (eg. glucose level), and for lipid metabolism (eg. triglyceride and cholesterol levels). Vital organs such as lung, heart, liver, kidney and spleen were excised from the animals and examined macroscopically. The weights of the organs were recorded. Afterwards, the organs were fixed in formalin and sent for histopathological processing in Faculty of Veterinary at University Putra Malaysia. Tissue from the organs was made into slides, stained with Haematoxylin & Eosin. Images were taken under the light microscope for further histopathological analysis. All values and findings were compared between treated and control groups.

2.5. Oral gavage

EBN (S1 and S2) were prepared according to a previously established protocol (Yew et al., 2014), dissolved in distilled water and fed to