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Cerium oxide nanoparticles could ameliorate behavioral and neurochemical impairments in 6-hydroxydopamine induced Parkinson's disease in rats

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ABSTRACT

Background: Cerium oxide nanoparticles (CeO_2NPs) showed promising effects in neurodegenerative diseases including some animal models of Parkinsonism. However, the implication of CeO_2NPs in 6-hydroxydopamine (6-OHDA) induced Parkinsonism remains to be investigated.
Aim: This study was designed to assess whether CeO_2NPs treatment could alleviate neurobehavioral and neurobiochemical deficits in 6-OHDA induced neurotoxicity in rats.
Material and methods: 50 rats received left intrastriatal (IS) injection of either saline (control, n = 10) or 6-OHDA (n = 40). At the third week post-lesion, motor dysfunction was verified using neurobehavioral tests. Then diseased rats received intraperitoneal injection of 0.1, 0.5 or 1 mg/kg of CeO_2NPs or vehicle (10 rats each) for 3 weeks. Rats were subjected to behavioral assessments and then sacrificed for biochemical analyses of the striatum. Striatal dopamine levels, oxidative stress markers including total antioxidant capacity (TAC) and malondialdehyde (MDA), and caspase 3 activity as an apoptotic marker were assessed.
Results: Different doses of CeO_2NPs variably improved motor dysfunctions induced by 6-OHDA injection in open field, Rota Rod and stepping tests. In addition, the neurobiochemical derangements were almost reversed by the 0.5 mg/kg dose of CeO_2NPs, while 0.1 mg/kg dose was not sufficient to alter biochemical measurements in the striatum. Administration of 1 mg/kg of CeO_2NPs partially ameliorated striatal dopamine and decreased apoptosis without significant effect on oxidative stress.
Conclusion: The present study showed a putative therapeutic role of CeO2NPs in the treatment of 6-OHDA-induced Parkinsonian rats, and suggested their antioxidant and antiapoptotic effects as possible mechanisms for elevated striatal dopamine level and improved motor performance.

1. Introduction

Parkinson's disease (PD) is regarded as the most common neurodegenerative disease after Alzheimer's disease (Bové et al., 2005). The cardinal biochemical abnormality in PD is the profound deficit in brain dopamine level attributed to the loss of neurons of the nigrostriatal dopaminergic pathway. The concept of free radical–mediated neuronal injury has been suggested as the main hypothesis for PD associated degeneration of dopaminergic neurons (Fahn and Cohen, 1992; Hwang, 2013). Conventional antioxidants have been attempted to alleviate the pathological changes in PD but with limited success (Etminan et al., 2005).

One of the main challenges in neurological disorders is to develop an effective therapeutic modality that can overcome the blood brain barrier (BBB) (Saraiva et al., 2016). Nanoparticles have emerged as a revolutionary treatment for neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and strokes due to their targeted delivery and ability to pass

Abbreviations: 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; CeO_2NPs, cerium oxide nanoparticles; ROS, reactive oxygen species; TAC, total antioxidant capacity; MDA, malondialdehyde; IS, intra-striatal.

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through biological barriers (Spuch et al., 2012). However, recent studies have shown some types of nanomaterials have intrinsic therapeutic potential and could actively mediate molecular processes to regulate cell functions such as anti-angiogenic and antioxidant mechanisms (Kim and Hyeon, 2014).

Cerium oxide nanoparticles (CeO_2NPs) have distinctive properties that could be effective in nanotherapeutics. As CeO_2NPs can reversibly bind oxygen and shift between oxidation states (Ce^{3+}/Ce^{4+}) (Das et al., 2013), they are able to scavenge reactive oxygen species (ROS) (Heckert et al., 2008; Korsvik et al., 2007). CeO_2NPs also exhibit superoxide dismutase and catalase enzymes mimetic activities (Heckert et al., 2008). Therefore, they are expected to scavenge almost all types ROS, making them superior to other antioxidants. As opposed to traditional antioxidants, the radical scavenging property of CeO_2NPs is regenerative, allowing extended activity for long durations (Andrievsky et al., 2009; Rzigalinski et al., 2016). Thus CeO_2NPs could be helpful in the treatment of chronic oxidative stress conditions.

Researches have shown that CeO_2NPs protected neurons and other cell types from ROS-induced damage (Niu et al., 2011; Rzigalinski et al., 2011; Schubert et al., 2006). The antioxidant effects of CeO_2NPs were used to prevent macular degeneration and neovascular lesions in the retina (Kong et al., 2011; Zhou et al., 2011). They also proved valuable to inhibit adipogenesis, reduce weight gain and ameliorate obesity-associated metabolic derangements (Rocca et al., 2015); accelerate wound healing activity (Davan et al., 2012); and enhance vascularization of bone grafts (Xiang et al., 2016) Concerning neurological diseases, CeO_2NPs, showed promising therapeutic potential in some neurodegenerative diseases in which oxidative stress plays a key role such as Alzheimer's disease, multiple sclerosis and traumatic brain injury (Bailey et al., 2016; D'Angelo et al., 2009; Heckman et al., 2013; Sandhir et al., 2015). Furthermore, CeO_2NPs has been proposed as a disease modifying therapy for 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Frey et al., 2014).

The neurotoxin 6-Hydroxydopamine (6-OHDA), a hydroxylated analog of dopamine, has been found to increase ROS generation and initiate mitochondrial-mediated apoptotic cascade events in dopaminergic neurons that trigger pathological changes similar to PD (Smith and Cass, 2007). Therefore, the aim of the present work was to investigate the impact of CeO_2NPs on rats subjected to unilateral intra-striatal (IS) 6-OHDA lesion with the main focus on behavioral and neurobiochemical changes.

2. Materials and methods

2.1. Experimental animals

Adult male Wistar rats weighing 200–220 g (procured from Experimental Animal house in Medical Physiology Department, Alexandria University) were used. Animals were maintained at room temperature under standard conditions of a 12 h light-dark cycle with food and water ad libitum. They were allowed to acclimatize for 1 week prior to experimentation. Efforts were made to minimize animal suffering and to reduce the number of the animals used. The procedures involving animals and their care were carried out in accordance with ethical guidelines of Alexandria University on laboratory animals and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 80-23, revised 1978), and the protocol was approved by the Faculty of Medicine, Alexandria University Ethics Committee.

The animals were divided into the following groups: 10 rats received a single left IS stereotaxic injection of 2 μl of vehicle (0.9% saline with 0.1% ascorbic acid, pH 5.5) followed after 3 weeks by daily intraperitoneal injection of 0.5 ml sterile PBS for another 3 weeks, to serve as the control group. A cohort of rats received a single dose of left IS injection of 10 μg of 6-OHDA-hydrobromide (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 2 μl of the same vehicle to induce PD model. Ascorbic acid was used to stabilize 6-OHDA-hydrobromide and prevent its oxidation to an inactive form (Smith and Cass, 2007). At the third week after surgery, Parkinsonism like manifestations had been confirmed versus the control group using a battery of neurobehavioral tests as described below. Rats that showed positive findings revealed by ~50% reduction in their locomotor scores than controls were selected for further experimentation. Animals were categorized into mild, moderate or severe according to their neurological deficits and then allocated into 4 balanced groups of 10 rats each. These groups were treated with either 0.5 ml of sterile PBS or CeO_2NPs at different doses (0.1 mg/kg, 0.5 mg/kg or 1 mg/kg), daily by intraperitoneal injection for 3 weeks. (Kim et al., 2012). CeO_2NPs were freshly dispersed in 0.5 ml of sterile PBS by sonication for 15 min before administration. Fig. 1 shows the experimental design of the study.

2.2. Intrastriatal injection of 6-OHDA

Rats were anesthetized (by ketamine 100 mg/kg and xylazine 5 mg/kg, intraperitoneally) and fixed in a stereotaxic frame (David kopf instrument, Germany). 6-OHDA or vehicle was slowly injected into left striatum at a rate of 0.4 μl/min using 5 μl Hamilton syringe at the stereotaxic coordinates 1 mm anterior to bregma, 2.6 mm left of midline and 5.0 mm below the surface of the skull according to the Atlas of (Paxinos and Watson, 1998). The needle was left in place for an additional 5 min and then slowly withdrawn. The burr hole was then filled with Gel-foam and the incision was closed with sutures. Animals were individually placed in a heated recovery chamber until recovered from anesthesia. Then, rats were housed together in a group of four animals per cage. After surgery, all rats received gentamycin (5 mg/kg, intraperitoneally) for 3 days to prevent sepsis, and meloxicam (1 mg/kg, subcutaneously) for analgesia. Saline injections (500 μl, subcutaneously) continued until the animals became hydrated and regained their pre-surgery weight.

2.3. Cerium oxide nanoparticles

CeO_2NPs were purchased from Sigma (code 544841, Sigma-Aldrich, St. Louis, MO, USA) and were characterized in previous work (Ciofani et al., 2013, 2014). For further confirmation before biological testing, nanoparticles were dispersed through a mild sonication in phosphate-buffered saline (PBS) (pH 7.4), and particle morphology was analyzed by Transmission electron microscopy (TEM; JEM-1 400; Jeol Ltd., Tokyo, Japan). The samples were prepared by placing a drop of nanosuspension on a paraffin sheet. Then, carbon coated grid was placed on sample and left for 1 min to allow nanoparticles to adhere on the carbon substrate. The remaining suspension was removed and samples were air dried before microscopic examination. Besides, particle size distribution, polydispersity index (PDI) and zeta potential of the dispersion were determined by dynamic light scattering (DLS) technique using Zetasizer Nano ZS. (Malvern, Instruments Ltd., Malvern, UK).

2.4. Locomotor and behavioral tests

During the seventh week of experimental period, all groups were re-subjected to the following locomotor and neurobehavioral tests to assess the responsiveness of diseased rats to treatment. All experimental procedures were carried out in a separate room between 8:00 a.m. and 13:00 p.m. by an investigator blinded to the