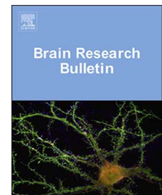
Brain Research Bulletin 131 (2017) 117–122

Contents lists available at ScienceDirect

Brain Research Bulletin

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



Research report

Neuroprotective effect of cerium oxide nanoparticles in a rat model of experimental diabetic neuropathy

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ARTICLE INFO

Keywords:
Dorsal root ganglion
CeO_2 nanoparticles
Diabetic neuropathy
High glucose
Oxidative stress

ABSTRACT

Objective: Neuropathies are a nerve disorders that caused by diabetes. Neuropathy affects over 50% of diabetic patients. High blood glucose and their toxic byproducts are the main causes for nerve dysfunction. In the present study, we examined the neroprotective effects of cerium oxide (CeO_2) nanoparticles in diabetic rats.
Method: Rats divided into four groups: control group, diabetic group, the diabetic group treated with CeO_2 nanoparticle at a dose of 65 mg/kg and diabetic group received CeO_2 nanoparticle at a dose of 85 mg/kg. Diabetes was induced by single intraperitoneal injection of 65 mg/kg streptozotocin (STZ). 8 weeks after the induction of diabetes, body weight and pain sensitivity in all groups were measured. The blood sample was collected for biochemical analysis. The dorsal root ganglion (DRG) neurons were isolated for histopathological stain and morphometric parameters studies.
Results: Reduction of body weight, total thiol molecules (TTM), total antioxidant power (TAP) and ADP/ATP ratio in diabetic rat was reversed by CeO_2 nanoparticles administration. We showed that lipid peroxidation (LPO) and nociception latency were significantly increased in STZ-treated rats and decreased after CeO_2 nanoparticles administration. DRG neurons showed obvious vacuole and various changes in diameter, area and the count of A and B cells in STZ-diabetic rat. CeO_2 nanoparticles improved the histopathology and morphological abnormalities of DRG neurons.
Conclusion: Our study concluded the CeO_2 nanoparticles have a protective effect against the development of DN.

1. Introduction

Diabetes mellitus (DM) is a major health problem characterized by defects in insulin secretion, insulin resistance, or both (Whalen et al., 2015). DM is a metabolic disorders that induces microvascular complication such as nephropathy, retinopathy and neuropathy and macrovascular complication including ischemic heart disease (Sayin et al., 2015).

High glucose – induced oxidative stress has been shown as one of the main links between diabetes and its complication. In chronic hyperglycemia increases the generation of reactive oxygen species (ROS) through glucose auto- oxidation and protein glycosylation (Mrowicka, 2005).

Once increase the production of ROS, they react with macromolecules including carbohydrate, lipid, protein and DNA leading to the loss of their function (Sheikh et al., 2010). In addition, ROS induce various cellular signaling pathways that lead to development of complication of diabetes. NF-kB in response to an increase in ROS generation, enhance the transcription of pro-inflammatory cytokines and chemokines such as MCP-1, IL-1β, IL-6 and TNF-α (Oyenihi et al., 2014).

Diabetic neuropathy (DN) may be the most common complication that present in 60–70% of diabetic patients. DN occurs when there is an imbalance between nerve damage and repair. The mechanism responsible for DN includes oxidative stress, polyol pathway, nonenzymatic glycolation and the protein kinase C pathway. Therefore, it is seen that antioxidant therapy may help prevent or delay neuropathy as well as other diabetes complication (Vincent et al., 2004).

Nanoparticls due to having unique mechanical, electrical, chemical, optical and biological properties are an ideal therapeutic agent for treatment of various diseases (Bodapati, 2011). Some nanoparticles have antioxidant properties and act by scavenging free radicals. Among them, CeO_2 nanoparticles play a major role because of the high

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http://dx.doi.org/10.1016/j.brainresbull.2017.03.013
Received 2 February 2017; Accepted 29 March 2017
Available online 31 March 2017
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potential in free radical scavenging (Mohammad et al., 2008). CeO_2 nanoparticles present in both trivalent (Ce^{3+}) and tetravalent (Ce^{4+}) state that allowing the CeO_2 nanoparticles release and store oxygen. In surface of CeO_2 nanoparticle, oxygen vacancies interact with free radical, mimicking the antioxidant enzymes activities including catalase and super oxide dismutase (Dunnick et al., 2015; Rubio et al., 2015).

The present study was designed to examine the protective effects of CeO_2 nanoparticles on diabetic neuropathy in rats.

2. Materials and methods

2.1. Experimental animals

A total of 28 Male Wistar rats with 2–3 months of age and weighing 180–250 g were obtained from the animal facility of the Iran University of Medical Science. Rats were housed in individual stainless steel cages at a 45–55% humidity and temperature of 20–22 °C under a 12 h light/dark cycle. All rats were fed with standard rodent water and diet. This study was approved by the Ethical Committee of Iran University of Medical Sciences base on National Institutes of Health Principles of Laboratory Animal Care (NIH publication no. 85–23, revised 1985).

2.2. Experimental design

Diabetes induced by a single intraperitoneal injection of 65 mg/kg STZ in citrate buffer. The control group received citrate. Blood samples were obtained 3 and 7 days after the STZ injection via tail vein and glucose levels were measured using a glucometer. Rats with a blood glucose level of > 200 mg/dl was considered diabetic. The fasting blood glucose level in the nondiabetic rats was 85 ± 5 mg/dl. Diabetic rats were randomized into 3 groups (n = 7): group 1, diabetic control; group 2, treated with 65 mg CeO_2 nanoparticles/kg body weight orally; group 3, treated with 85 mg CeO_2 nanoparticles/kg body weight orally. Group 2 and 3 received CeO_2 nanoparticles for one week before and one week after of STZ injection. 8 weeks after conformation of diabetes, DN occurred (Hosseini et al., 2011). All experiments were performed 8 weeks after injection of STZ. Blood glucose and body weight were examined before and at the end of the experimental period.

2.3. CeO_2 nanoparticles characterization

CeO_2 nanoparticles was obtained from Navarrean Nenoproducts Technology (Spain). Field emission scanning electron microscopy was performed by FE-SEM, Hitachi, Japan S-4160. The zeta potential and particle size distribution were measured by a Nano Z-Sizer (Malvern Instrument Zen 3600).

2.4. Hot plate test

A hot plate test used to measure pain sensitivity (Socrel Hot plate model DS37, Ugo Basile, Italy). Rats were placed on the plate with the diameter of 19 cm, height of 30 cm and temperature of 52 °C ± 2 °C. Response time to thermal pain was calculated from the onset of the test and front legs licking or jumping. Maximum time was considered 60 s (Karami et al., 2011).

2.5. Sample preparation

The animals were anesthetized with intraperitoneal injection of ketamine and xylazin and blood sample were collected from the heart and centrifuged at 1200*g* for 10 min at 4 °C for separating the plasma. The plasma samples were frozen at −80 °C for biochemical analysis.

For tissue preparation the DRG was isolated and fixed into 10% paraformaldehid and then embedded in paraffin. The 40 μm sections were stained with Hematoxylin-Eosin (H & E) and assessed by light microscope. We used four sections per sample.

2.6. Morphometry of DRG neurons

DRG neurons were defined as A or B cell (Hosseini et al., 2011). A cell has a light nucleus with one large nucleolus in central and granular cytoplasm. The nucleus of B cell is light and contains multiple nucleolus that peripherally located. The cytoplasm of B cell is dark, more homogenous and intensively stained compare to A cell. In this study we determined the diameter, number and area of A and B cells in all groups.

2.7. Measurement of intracellular ADP/ATP ratio

The frozen DRG was homogenized in ice and centrifuged at 12,000*g* for 15 min at 4 °C. The supernatant was neutralized with 3 M KOH in 1.5 M Tris, and centrifuged. The supernatants were separated using reverse-phase HPLC. Chromatographic separation was done by column (SUPELCO- SILTMLC-18-T) with a flow rate of 1.2 ml/min and ammonium dihydrogen phosphate (75 mM) as mobile phase. The adenine nucleotides were assessment at 254 nm (μg/ml per mg of tissue) and changes in energy was reported as the ADP/ATP ratio (Najafi et al., 2015).

2.8. Measurement of lipid peroxidation (LPO)

LPO was assessed by the reaction of thiobarbituric acid with malondialdehyde (MDA). The sample was mixed with Trichloroacetic Acid (20%), centrifuged at 1500*g* for 10 min the precipitate was dissolved in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate) was added and the sample was incubated in a boiling water bath for 30 min. At the end, LPO was extracted by *n*-butanol and its absorbance was measured at a wavelength of 532 nm (Ghaznavi et al., 2015).

2.9. Measurement of total thiol molecules (TTM)

TTM was measured by the reaction of DTNB with thiol molecules that forms a yellow complex. Samples were mixed with Tris-EDTA buffer and subsequent mixed with DTNB (10 mM). After 20 min the absorbance of samples were determined at 412 nm (Hosseini et al., 2015).

2.10. Measurement of total antioxidant power (TAP)

Reduction of ferric ions (Fe^{+3}) to ferrous ions (Fe^{+2}), by the samples represents of antioxidant power. TAP was assessed by FRAP test. In this method, reaction of Fe^{+2} and 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ) generates a blue color with a maximum absorbance at 593 nm (Najafi et al., 2015).

2.11. Statistical analysis

Data were presented as the mean ± standard error of the mean (SEM). The significance of the differences between groups was analyzed using one-way ANOVA and Tukey's posthoc tests by Stats Direct version 2.7.8. A $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. CeO_2 nanoparticles characterization

The Result from Dynamic light scattering (DLS) confirmed size distribution of CeO_2 nanoparticles were ranged from 18.2 to 50.7 (Fig. 1a). Zeta potentials of CeO_2 nanoparticles were 6.25. SEM imaging confirmed poly- dispersion of these nanoparticles (Fig. 1b).

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