Biomedicine & Pharmacotherapy 73 (2015) 80–86

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original Article

Cerium oxide nanoparticles alleviate oxidative stress and decreases Nrf-2/HO-1 in D-GALN/LPS induced hepatotoxicity

Reem M. Hashem [a], Laila A. Rashd [b], Khalid S. Hashem [c], Hatem M. Soliman [a,*]

[a] Department of Biochemistry, Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt
[b] Department of Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt
[c] Department of Biochemistry, Faculty of Veterinary Medicine, Beni Suef University, Beni Suef, Egypt

ARTICLE INFO

Article history:
Received 8 May 2015
Accepted 22 May 2015

Keywords:
Cerium oxide nanoparticles
D-galactoseamine
Hepatotoxicity
HO-1
Lipopolysaccharide
Nrf-2

ABSTRACT

Translocation of the master regulator of antioxidant-response element-driven antioxidant gene, nuclear factor erythroid 2 (Nrf-2) from the cytoplasm into the nucleus and triggering the transcription of hemoxygenase-1 (HO-1) to counteract the oxidative stress is a key feature in D-galactoseamine and lipopolysaccharide (D-GALN/LPS) induced hepatotoxicity. We mainly aimed to study the effect of cerium oxide (CeO_2) nanoparticles on Nrf-2/HO-1 pathway whereas; it has previously shown to have an antioxidant effect in liver models. Administration of CeO_2 nanoparticles significantly decreased the translocation of the cytoplasmic Nrf-2 with a concomitant decrement in the gene expression of HO-1 as it reveals a powerful antioxidative effect as indicated by the significant increase in the levels of glutathione (GSH), glutathione peroxidase (GPX1), glutathione reductase (GR), superoxide dismutase (SOD) and catalase. In synchronization, a substantial decrement in the levels of inducible nitric oxide synthase (iNOS), TBARS and percentage of DNA fragmentation was established. These results were confirmed by histopathology examination which showed a severe degeneration, haemorrhages, widened sinusoids and focal leukocyte infiltration in D-GALN/LPS treatment and these features were alleviated with CeO_2 administration. In conclusion, CeO_2 is a potential antioxidant that can effectively decrease the translocation of the cytoplasmic Nrf-2 into the nucleus and decrease HO-1 in D-GALN/LPS induced hepatotoxicity.

1. Introduction

Nanoparticles have a recent increase interest in the intersection of nanotechnology and biotechnology as nanoparticles have a prophylactic and therapeutic role in biological toxicity [1].

In this study, we have demonstrated the potential uses of a novel environmental nanotechnology in the therapeutic use in biological systems and we aimed to investigate the mechanistic effect of cerium oxide nanoparticles as an antioxidant substance. Most recently, cerium oxide (CeO_2) nanoparticles have been established as a free radical scavenger [2] to provide protection against oxidative stress from chemical or biological insults that promote the production of the free radicals. CeO_2 nanoparticles chemistry supports its potential role as a safe and effective biological antioxidant or free radical scavenger [3]. The CeO_2 nanoparticles promote intracellular cell longevity and decrease toxic xenobiotics by virtue of their antioxidant effects [4], this effect is due to the reduction of the accumulated reactive oxygen species (ROS) and inhibition of the apoptotic response and death of the cells [5].

The severe liver injury induced by D-GALN/LPS is a well-established model of oxidative stress-mediated liver injury [6–8]. The production of ROS can initiate a wide range of toxic oxidative reactions [9]. Protective genes, whose products could reduce oxidative stress, contain a common promoter element called the antioxidant-response element (ARE). The nuclear factor E2-related factor 2 (Nrf-2), the master regulator of ARE-driven antioxidant gene is responsible for activating transcription in response to oxidative stress [10]. Nrf-2 trans-activates the expression of a group of cytoprotective phase II antioxidant enzymes, such as heme oxygenase-1 (HO-1), glutathione reductase (GR), glutathione peroxidase (GPX1) and superoxide dismutase (SOD). HO-1 expression is ubiquitous; it plays a central role in iron haemostasis and antioxidant defence in living cells against heme, metalloporphyrins and transition metals [11]. There is increasing evidence that induction of HO-1 in liver has a potential therapeutic target for various liver diseases [12].

* Corresponding author. Department of Biochemistry, Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt. Tel.: +201022724292.
E-mail address: hatemmsoliman@gmail.com (H.M. Soliman).

http://dx.doi.org/10.1016/j.biopha.2015.05.006
0753-3322/

Various pro-inflammatory cytokines including nitric oxide (NO) were secreted from Kupffer cells (liver macrophage) upon stimulation with LPS. Induction of NO has a potential damage effect on hepatocytes [13,14]. LPS has been demonstrated to cause iNOS expression in Kupffer cells and hepatocytes [15].

Neither the role of Nrf-2/HO-1 pathway nor the effect of the CeO_2 nanoparticles on this pathway in D-GALN/LPS-induced hepatotoxicity has been established yet. The present study is mainly aimed to establish the effect of CeO_2 nanoparticles on Nrf-2/HO-1 pathway and their protective effect against oxidative stress in D-GALN /LPS-induced hepatotoxicity in rat model.

2. Materials and methods

2.1. Chemicals

D-galactoseamine (D-GALN) and lipopolysaccharide (LPS) were purchased from Sigma Chemical Co. (St. Louis, MO). CeO_2 nanoparticles (25 nm particle size, 10 wt% in H_2O) were obtained from Sigma-Aldrich (St Louis, MO, USA). ALT, AST were measured by using Diamond measuring kits (Diamond Co. Egypt). Anti-Nrf-2 were purchased from Santa Cruz Biotechnology (CA, USA).

GSH assay kit, superoxide dismutase (SOD), catalase (CAT), and TBARS were purchased from Bio diagnostic Company for chemicals Egypt, Glutathione reductase (GR) were purchased from Sigma-Aldrich Company for chemicals, percentage of DNA fragmentation assay kits were purchased from Fisher Scientific Company (LLC, San Diego, CA, USA).

2.2. Experimental animals

Eighty male Albino Westar rats weighing average 200–250 grams were used in this study. They were obtained from the Animal House of the Research Institute of Ophthalmology (Giza, Egypt). They were kept under suitable conditions for one week for adaptation. They were maintained in stainless steel cages in a well-ventilated animal house at normal temperature (22 ± 5 °C) under 12:12 h light dark cycle. They were fed with standard diet and given water ad libitum. All studies were conducted in accordance with the Animal Care and Use Committee of the Biochemistry Department, Faculty of Pharmacy, Beni-Sueif University.

2.3. Experimental design

2.3.1. Experimental groups

The rats were randomly divided into four equal groups (20 rats each). Control group: rats received a single dose of sterile physiological saline (0.5 mL IP), D-GALN/LPS group was simultaneously given a single dose of D-galactoseamine (130 mg/kg IP) and lipopolysaccharide (100 nag/kg IP) [16].

D-GALN/LPS + CeO_2 group rats received CeO_2 (0.01 μg/kg; 0.5 mL in PBS IP) [17] on days 1, 3, 5 and 7, then a single dose of D-GalN/LPS on the seventh day. CeO_2 group rats received CeO_2 (0.01 μg/kg; 0.5 mL in PBS IP) on days 1, 3, 5, and 7.

After 24 hours from last injection, blood samples were collected and then rats were euthanized by carbon dioxide asphyxiation for tissue sampling.

2.3.2. Blood sample

Blood was collected from the medial canthus blood capillaries of the eye in dry centrifuge tubes. The tubes were placed in an inclined position for 5 minutes allowed to coagulate, and then placed in an incubator at 37 °C for 10 minutes. Centrifugation at $1000 \times g$ for 20 minutes was performed and clear sera were separated and kept in the deep freezer (–80 °C) till use according to the instruction of assay kits of each measure parameter.

2.3.3. Tissue homogenates

After scarification of rats, liver was collected and rinsed with physiological saline for removing any clotted blood or blood cells. The liver was divided into three parts. The first part weighted 0.5 g of liver and was homogenized in 5 mL physiological saline by using homogenizer (Ortoalresa, Spain). The homogenates were centrifuged at $1000 \times g$ for 15 minutes. The supernatants were collected in eppindorf tubes that were kept in the deep freezer (at –80 °C) for further biochemical investigations or according to the instructions of the biochemical assay kits. The second part weighted 0.5 g and kept in formalin 10% for histological investigations. The third part was kept for molecular biological investigations as described below.

2.4. Serum biochemical parameters

Liver function test were measured by using the ALT and AST-assay test kit of Diamond measuring kits according to the method described by Gella et al. [18].

2.4.1. Detection of GPX1, iNOS and HO-1 gene expression by real-time-polymerase chain reaction (RT-PCR)

Total RNA was isolated from liver tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer's instruction. The concentration of RNA was measured using a UV spectrophotometer.

2.4.1.1. cDNA synthesis. Five-microgram RNA was reverse transcribed using oligonucleotide (dT)18 primer (final concentration, 0.2 mM) and was denatured at 70 °C for 2 minutes. Denatured RNA was placed on ice and reverse transcription mixture containing 50 mM KCl, 50 mM Tris HCl (pH 8.3), 0.5 mM of deoxyribonucleotide triphosphate (dNTP), 3 mM MgCl2, 1 U/mL RNase inhibitor, and 200 units of moloney murine leukemia virus reverse transcriptase. The reaction tube was placed at 42 °C for 1 hour, followed by heating to 92 °C to stop the reaction.

2.4.1.2. Real-time quantitative polymerase chain reaction (PCR). For real-time quantitative PCR, 5 μL of first-strand cDNA was used in a total volume of 25 μL, containing 12.5 μL 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer as shown in Table 1. PCR reactions consisting of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles), were performed on step one plus real-time PCR system (Applied Biosystems). Data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta-actin genes, all these steps were described by Kenneth and Thomas [19].

2.4.2. Detection of Nrf-2 by western blot technique

Expression of Nrf-2 in the liver was determined by western blot briefly liver tissue (50 mg) was homogenized using a polytron homogenizer in 1.5 mL cold lysis buffer (50 mmol/L Tris-HCL, pH 8.0, 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS and 0.5 mmol/L phenylmethylsulfonylfluoride). The homogenate was centrifuged for 20 min at 4 °C and the supernatant was collected. Samples were stored at –80 °C until use. After boiling at 95 °C for 5 min, samples (50 μg/lane) were subjected to 7% SDS-PAGE gel and then transferred to nitrocellulose membranes