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Protective role of nebivolol in cadmium-induced hepatotoxicity via downregulation of oxidative stress, apoptosis and inflammatory pathways

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ABSTRACT

Cadmium (Cd) intoxication in human occurs through inhalation of cigarette smoke and ingestion of contaminated water and food. We investigated the role of nebivolol (NEB) in Cd induced hepatotoxicity. In our study; NEB was given as (10 mg/kg/d) orally to rats for 6 weeks, in the presence or absence of hepatotoxicity induced by oral administration of Cd (7 mg/kg/d) for 6 weeks. Levels of serum liver enzyme biomarkers; alanine transaminase (ALT), aspartate transaminase (AST) and serum total antioxidant capacity (TAC) were measured. In addition; mean arterial pressure and total cholesterol levels were measured. Hepatic superoxide dismutase (SOD) and malondialdehyde (MDA) were detected. Hepatic histopathological features, inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) immunoexpressions were evaluated. Tumor necrosis factor alpha (TNF-α) and B-cell lymphoma-2 (Bcl-2) mRNA gene expressions were detected using real time-PCR (rt-PCR). Our results showed marked increase in all measured parameters except SOD, TAC, eNOS immunoexpression and Bcl2 mRNA gene expression which decreased in Cd induced hepatotoxicity group. NEB showed marvelous protective effect against Cd induced changes. NEB decreased liver enzymes (ALT and AST), mean arterial pressure, total cholesterol levels, MDA, iNOS immunoexpression and TNF-α gene expression but significantly increased SOD, TAC, eNOS immunoexpression and Bcl-2 gene expression. Moreover; NEB markedly improved the histopathological changes induced by Cd. These findings prove the antioxidant, anti-apoptotic and anti-inflammatory properties of NEB and its protective role in Cd induced hepatotoxicity.

1. Introduction

Cadmium (Cd) is a deleterious pollutant that threats both animals and human health. It is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) (1993). The major sources of Cd intoxication are cigarette smoking and ingestion of contaminated food or water. Cd toxicity affects liver, lung, kidney and testis (Skipper et al., 2016).

After absorption, Cd is transported throughout the body and bound to a sulfhydryl group-containing protein like metallothionein. About 30% of Cd deposits in the liver and 30% in the kidneys, the rest distributes throughout the body. Its clearance half-life is twenty-five years (Bernhoft, 2013).

Furthermore, Cd is a non-redox metal that indirectly causes oxidative stress by depleting cellular GSH. It competes with essential metals such as zinc (Zn), selenium (Se), cupper (Cu) and calcium (Ca) interfering with various cellular processes, enzyme activities, DNA repair systems, redox state of the cell and signal transduction (Arroyo et al., 2012).

Cd toxicity depends on generation of reactive oxygen species (ROS). Cd causes oxidant/antioxidant imbalance and it increases the generation of ROS. These agents include superoxide anion and hydrogen peroxide (Nair et al., 2013). That leads to a high level of hydroperoxides and impairment of lipid metabolism (Bernhoft, 2013).Moreover; Cd inhibits nitric oxide (NO) production in endothelial cells via inhibiting endothelial nitric oxide synthase (eNOS) phosphorylation. This leads to endothelial dysfunction and hypertension but induces inflammation, apoptosis and oxidative stress by inducible nitric oxide synthase (iNOS) stimulation. This effect increases NO production and peroxynitrite ($ONOO^-$) formation following binding with free radicals (Kukongviriyapan et al., 2014).

In addition; Cd causes up-regulation of different markers of inflammation and apoptosis such as tumor necrosis factor alpha (TNF-α) and interleukins 6, 8,1B. These mechanisms are responsible for Cd induced inflammation, apoptosis and tumor development (Lee and Lim, 2011).

Nebivolol is a β-adrenergic receptor blocking drug. It has been

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shown to cause vasodilatation in animals and humans. This effect is mediated by inducing eNOS. NO released by endothelial cells has been shown to be a key participant in numerous biological processes including maintenance of vascular integrity and vascular relaxation. Moreover; It was reported that nebivolol had additional anti-oxidative; iNOS inhibitor and anti-apoptotic effects (Rofaeil et al., 2017).So that we aimed in our study to investigate the role of eNOS stimulator, iNOS inhibitor, B-blocker, antioxidant, anti-inflammatory, anti-apoptotic and antihypertensive agent (nebivolol) in Cd induced hepatotoxicity in rats.

2. Materials and methods

2.1. Chemicals

NEB was from Marcyrl pharmaceutical Co. Egypt. We purchased Cd chloride from Sigma Aldrich co., USA. The polyclonal rabbit/antirat iNOS and eNOS anti-bodies and biotinylated goat antirabbit secondary antibodywere purchased from Thermo Fisher Scientific Inc./Lab Vision (Fermont, CA, USA). Alanine transaminase (ALT) and aspartate transaminase (AST), total cholesterol kits were from spectrum diagnostic co., Egypt.Total antioxidant capacity (TAC) kit was purchased from Biodiagnostic, Egypt.

2.2. Animals and experimental design

Adult male Wistar albino rats weighing about 250–300 g were purchased from the Animal Research Centre, Giza, Egypt. Rats were left in standard housing conditions (3 rats/cage) and were left to acclimatize for one week. Animals were supplied with laboratory chow and tap water. This study was conducted with adherence to the ethical standards and was approved by committee of faculty of medicine, Minia University, Egypt in accordance with European (EU) directive 2010/63/EU.

Rats were randomly divided into 4 groups (n = 6 each):

Group I was received vehicle oral distilled water for 6 weeks.

Group II was administered NEB (10 mg/kg/d orally) for 6 weeks (Morsy and Heeba, 2016).

Group III was treated with Cd (7 mg/kg/day) for 6 weeks (Winiarska-Mieczan, 2015).

Group IV was administered NEB (10 mg/kg/d orally) (Morsy and Heeba, 2016) and Cd (7 mg/kg/day) for 6 weeks (Winiarska-Mieczan, 2015).

2.3. Measurement of blood pressure

At the end of experiment and before scarification; arterial blood pressure was measured in rats by a manometer (LETICA, Panlab S.L., Barcelona, Spain) using the tail-cuff method. The rats were kept at 38 °C for 15 min to make the pulsation of the tail artery detectable. Measuring arterial blood pressure was carried out for five times for each animal, and values were based on the mean of several successive measurements (Miguel et al., 2005).

2.4. Collection of samples and storage

After 6 weeks of Cd administration, each rat was weighed then sacrificed. Venous blood was collected from the jugular vein and centrifuged at 5000 rpm for 15 min (JanetzkiT30 centrifuge, Germany).

After sacrifice, liver was excised and weighed.Part of the liver was taken then fixed in 10% formalin and embedded in paraffin for histopathological and immunohistochemical examinations. The remained part of the liver was snap frozen in liquid nitrogen and kept at −80 °C. Liver tissue homogenates was prepared for biochemical analysis (Glas-Col homogenizer, USA). Liver homogenate was centrifuged at 3000 rpm for 20 min and the supernatant was kept at −80 °C till used.

2.5. Measurements

2.5.1. Evaluation of AST, ALT liver enzymes and total cholesterol

ALT, AST and total cholesterol were measured in serum samples by enzymatic colorimetric methods using commercial kits.

2.5.2. Assessment of TAC and superoxide dismutase (SOD) levels

The assessment of SOD levels depends on the ability of the enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye and results were expressed as unit/g tissue (Nishikimi et al., 1972).

Measuring TAC depends on that the antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual amount is determined colorimetrically and results were expressed as mmol/l (Koracevic et al., 2001).

2.5.3. Assessment of malondialdehyde (MDA) levels

Hepatic lipid peroxidation was determined as thiobarbituric acid reacting substance and is expressed as equivalents of MDA, using 1, 1, 3, 3-tetramethoxypropane as standard. Results were expressed as nmol/g tissue (Mihara and Uchiyama, 1983).

2.5.4. Histopathological examination

After sacrifice; the liver was dissected, fixed in 10% formaldehyde for 24 h. Sections were processed, embedded in paraffin wax and five µm serial sections were prepared and stained with hematoxylin and eosin. Assessment of the slides was performed in a blinded fashion under light microscopy using an Olympus microscope, Japan. Sections were evaluated by random selection of 10 fields from each rat.

Hepatic injury was classified as grade 0: minimal or no evidence of injury; grade 1:mild injury characterized by cytoplasmic vacuolization and focal nuclear pyknosis; grade 2: moderate injury exhibiting cytoplasmic vacuolization, but no frank necrosis, sinusoidal dilatation and congestion with blurring of intercellular borders; grade 3: moderate to severe injury with areas of coagulative necrosis, cytoplasmic hypereosinophilia, extensive sinusoidal dilatation and congestion; grade 4:severe injury consisting of severe confluent coagulative necrosis and hemorrhage into hepatic cords leading to a loss of tissue architecture (Gunduz et al., 2015).

2.5.5. Immunohistochemistry

Five µm thick sections were placed on positively charged slides. Sections were de-paraffinized with xylene and rehydrated in graded ethyl alcohol. Endogenous peroxidase activity was inactivated by treatment with 3% hydrogen peroxide for 30 min then washed in phosphate-buffered saline (PBS) solution. For antigen retrieval, sections were boiled in citrate buffer (pH 6.0) for 20 min in microwave.

Sections were incubated overnight in a humidity chamber using iNOS and eNOS primary antibodies, and then applying the biotinylated secondary antibody (Lab Vision Laboratories) for 30 min. Sections were washed in PBS and incubated with the streptavidin-biotin complex reagent (Lab Vision Laboratories) for 30 min. Finally, 3, 3-diaminobenzidinetetra hydrochloride (DAB) (Lab Vision Laboratories) was applied for 5 min, then washed in distilled water, counterstained with Mayer's haematoxylin, dehydrated, cleared in xylene, mounted and covered slipped.

Screening of sections was done under light microscope magnification X200. To assess positive staining for iNOS, sections were evaluated for cytoplasmic staining according to the following criteria; when < 5% of cells were positive classified as negative, < 50% of cells positive considered as low intensity and > 50% positive was classified as high intensity (Hassan et al., 2013).Cytoplasmic immunoexpression of eNOS was classified by the intensity and distribution patterns of the staining reaction using a semiquantitative score; graded as 0 = no staining, 1 = slight positive, 2 = moderate positive, 3 = intense positive (Verstegen et al., 2002).