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Cardioprotective effects of nanoceria in a murine model of cardiac remodeling

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ABSTRACT

Isoproterenol (ISO), a synthetic β_1 adrenergic agonist is a well-known agent to be associated with severe cardiotoxicity manifested as marked myocardial necrosis and fibrosis. Oxidative stress plays a crucial role in mediating ISO induced cardiotoxicity. In present study, we have investigated the possible protective effect of nanoceria (NC) in ISO induced cardiac injury. We have given long duration exposure (a total of 10 days) of low dose ISO (20 mg/kg/day) to investigate the protective effects of NC in chronic cardiac injury model. ISO (20 mg/kg/day for 10 days) produced cardiac injury as evident by increased plasma LDH and CK-MB, AST, ALT, cardiac hypertrophy, severe myocardial fibrosis (MF) and significantly higher levels of cytokines, IL-6, TGF-β and TNF-α. Interestingly, the treatment with NC (0.2 and 2 mg/kg) abrogated cardiotoxicity symptoms and provided protection from ISO induced cardiac injury. The results from present study demonstrated strong evidences of cardioprotective effects of NC as shown by reduction in the levels of LDH ($p < 0.05$ at 2 mg/kg) and CK-MB ($p < 0.05$ at 2 mg/kg). In addition, NC reduced oxidative stress parameters MDA ($p < 0.05$ at 2 mg/kg) and enhanced GSH levels which is physiological antioxidant ($p < 0.01$ at both doses). Further, NC exhibited promising anti-inflammatory activity and curbed the levels of cytokines ($p < 0.05$ at 0.2 mg/kg and $p < 0.001$ for IL-1β and $p < 0.001$ at both doses for IL-6). In addition, NC also reduced the levels of pro-fibrotic cytokine, TGF-β ($p < 0.05$ at 2 mg/kg) and helped in reduction of collagen deposition in heart thereby, preventing the myocardial remodeling. Our results strongly suggested that NC might be of potential use as a cardioprotective agent.

1. Introduction

Cardiovascular diseases (CVDs) are leading cause of mortality with global prevalence. According to a statistical report from American Heart Association (AHA, 2017), 92.1 million American adults are suffering from CVDs [1]. Further, by 2030, 43.9% of US population is projected to have some form of CVD. The pathological remodeling of heart as evident by change in shape, size and function is primarily responsible for cardiac abnormalities observed in CVDs. Myocardial fibrosis (MF), an important aspect of cardiac remodeling is a common pathological hallmark of almost all types of CVDs irrespective of the etiology. MF has become a strong predictor of therapeutic outcome in clinics and the severity of MF has been well linked with prognosis in CVDs. MF results from abnormal deposition of extracellular matrix (ECM) proteins in cardiac interstitium causing its stiffening and ultimately leading to compromised functioning and even failure of heart in severe cases. Although, the mechanism behind progression of MF is yet to be elucidated due to its complex nature; there are strong evidences pointing towards central role of oxidative stress in it [2]. Oxidative stress results from uncontrolled production of reactive oxygen species (ROS) such as hydroxyl radicals and superoxide ions, which bring about damage to vital cellular components and activate cellular signaling pathways responsible for fibrosis. Transforming growth factor-β (TGF-β) has been recognized as a central pro-fibrotic cytokine which plays a major role in deposition of excessive ECM in various organs including heart [3]. There are strong evidences which demonstrate that cardiac oxidative stress is also linked to induced expression of TGF-β which ultimately contributes to development of cardiac fibrosis [4,5]. Further, antioxidants have been demonstrated to show protective effects in fibrosis by combating the oxidative stress. Despite the critical role of fibrosis in CVDs, there is still scarcity of potential anti-fibrotic agents which could be of use in different pathological conditions.

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Isoproterenol (ISO), chemically known as 3,4-dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol hydrochloride, is a synthetic sympathomimetic β-adrenergic agonist which is well-known to produce cardiotoxicity following its administration. The cardiac abnormalities observed in ISO induced cardiac injury are primarily due to remodeled cardiac tissue which in turn is responsible for the compromised cardiac function. The animal model of ISO induced cardiac injury is associated with severe cardiac remodeling and shows striking resemblance with cardiac dysfunction seen in humans. ISO induced cardiotoxicity model is a well-established model of MF and has been used widely for screening of cardioprotective agents. ISO is known to cause profound increase in oxidative stress by generating highly cytotoxic quinone and other free radicals through auto-oxidation [6]. These generated free radicals cause peroxidation of membrane phospholipids and change the membrane permeability, consequently contributing to loss of structure, function and integrity of myocardial cells ultimately progressing towards MF. The irreversible damage caused by ISO to myocardial cells is also accompanied by increased plasma levels of cardiac injury markers such as lactate dehydrogenase (LDH), creatinine kinase muscle/brain (CK-MB) subtype, aspartate transaminase (AST), alanine transaminase (ALT), induced expression of TGF-β, pathological alterations in structure of myocardial cells along with MF and necrosis. The necrosis of myocardial cells compromises the efficiency of cardiac tissue and activates inflammatory pathways. In addition to this, ISO also alters the levels of triglycerides (TG) and cholesterol (CHL) changing metabolic status of the body which may consequently lead to cardiovascular complications. Different antioxidants have been tried due to critical involvement of ROS in pathogenesis of MF, but response to these agents has not been found to be satisfactory. Therefore, more effective and promising therapies are need of the hour to fill the gap created by lack of anti-fibrotic therapeutics.

Nanomaterials have emerged as promising agents due to their superior physicochemical properties. The nanoparticle form of cerium oxide, also known as nanoceria (NC) possesses greater potential as an antioxidant and anti-inflammatory agent in comparison to bulk cerium oxide metal. The inherent property of NC to switch between different oxidative states (Ce^{+3} and Ce^{+4}) makes it a promising antioxidant with autocatalytic properties [7]. Due to these promising properties, NC has been found to be a promising material in conditions involving oxidative stress such as neurodegeneration, diabetes, pancreatitis and radiation-induced damage [8,9]. Moreover, owing to its redox regenerative potential, NC may prove to be an extraordinary rare earth based nanoparticle for curbing oxidative stress mediated disorders. Further, NC increases the levels of SOD and catalase by acting as their mimetic, further strengthening its case against ROS driven disorders [10,11]. Although the cardioprotective effects of NC have been already proven against doxorubicin induced cardiotoxicity and monocrotaline induced pulmonary hypertension, its effect on ISO induced cardiac remodeling are largely unknown [12,13]. The present study was designed due to our keen interest in investigating the possible protective effects of NC in a murine model of cardiac injury. We performed various enzymatic assays to assess the cardioprotective potential of NC. Electrocardiography, estimation of oxidative stress markers and inflammatory cytokines were estimated to assess the pharmacological benefits. Further, the histological damage was measured by H&E staining. The effect on collagen levels was estimated by measurement of hydroxyproline and picrosirius red staining.

2. Material and methods

2.1. Drugs and chemicals

Isoproterenol hydrochloride, nanoceria, reduced glutathione (GSH), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA), hydrogen peroxide, sodium dodecyl sulphate (SDS), and glacial acetic acid were purchased from Sigma Aldrich, USA. ELISA kits for TGF-β1, IL-1β and IL-6 were procured from eBioscience, USA. Biochemical kits for LDH, CK-MB, AST, ALT, TG and CHL were purchased from Accurex biomedical Pvt. Ltd., India. All other chemicals were purchased from commercial suppliers unless otherwise stated.

2.2. Physicochemical characterization of NC

Hydrodynamic diameter and zeta potential of NC were measured by using zeta sizer Nano-ZS (Malvern Instruments, UK) after suspending the nanoparticle powder in ultrapure water and probe sonicating it (Sonics VCX 750, USA). NC powder was analyzed for crystallinity using X-ray diffraction (XRD) on Ultima-IV X-ray diffractometer (M/s. Rigaku Corporation, Japan) with a 2 h scan range of 10–80 and scan speed of 2 min^{-1} at 40 kV and 30 mA. Fourier transform infrared (FTIR) experiments were performed using Perkin Elmer FTIR, (USA) in attenuated total reflection (ATR) mode. For recording the spectra 5 mg of sample was placed on the sample holder at a scanning range of 400–4000 cm^{-1} for a total of 16 scans and the resolution was set at 4 cm^{-1}. Diffuse reflectance method was used with TGS detectors. The surface morphology of NC was studied using scanning electron microscopy (SEM) with Quanta 250 FEG (FEI, USA) using a 3.0 kV accelerating voltage and 10.0 mm working distance, at magnification of 60,000X.

2.3. Experimental animals

The study was carried out in male Swiss albino mice of weight range 25–30 g. The animal protocol was approved by Institutional Animal Ethics Committee (Protocol approval no. NIP/7/2015/RT/144). The animals were housed in temperature and humidity-controlled environment during the whole experimental period maintaining temperature and relative humidity at 24 ± 2 °C and 55 ± 15%, respectively [14]. Animals were allowed access to water and food *ad libitum* and 12 h light/dark photo period was provided. The experiments were started after acclimatization of animals for at least 3–4 days. To evaluate various parameters, animals were sacrificed as per the study plan. All the experiments on animals were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the safe use and care of experimental animals.

2.4. Experimental procedure and induction of cardiac injury

Animals were divided into different random groups with 6–8 animals per group. Normal control animals received isotonic saline for 28 days. Chronic cardiac injury was induced by administration of low dose of ISO (20 mg/kg/day, s.c.) for 10 consecutive days starting from day 1 to day 10. ISO was dissolved in isotonic saline and the individual dose was calculated based on the body weight of animals. Two dose levels were selected for NC treatment *i.e.* low dose (0.2 mg/kg) and high dose group (2 mg/kg) for 28 days starting from day 1 to day 28 by i.p. route. Further, NC was administered followed by ISO maintaining a minimum of 6-hour gap between ISO and NC administration. NC control (*per se*) group received high dose (2 mg/kg) of NC daily for 28 days by i.p. route. Weighed quantity of NC was suspended in normal saline and probe sonicated (Sonics VCX 750, USA) to prepare a uniform suspension followed by administration to animals.

2.5. Biochemical analysis for injury markers

At the end of experimental period (29th day), blood samples from all the animals were collected by cardiac puncture. The plasma was separated from blood by centrifugation at 2000*g* at 4 °C and stored at −80 °C for further estimation of plasma parameters. Biochemical parameters were evaluated in plasma using commercially available kits as per manufacturer's protocol (Accurex biomedical Pvt. Ltd., India). The respective absorbance values were recorded using