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Sesamin ameliorates doxorubicin-induced cardiotoxicity: Involvement of Sirt1 and Mn-SOD pathway

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HIGHLIGHTS

- Sesamin can ameliorate DOX-induced oxidative stress and mitochondrial damage.
- Sesamin can up-regulate the activity and protein expression of Mn-SOD.
- Sesamin also can up-regulate the protein expression of Sirt1.
- The protective mechanism of sesamin is mainly through activation of Sirt1.

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ABSTRACT

Oxidative stress caused by doxorubicin (DOX) is believed to be a major underlying molecular mechanism of DOX-induced cardiotoxicity. Sesamin (Ses), an active component extracted from sesame seeds, exhibits antioxidative and anti-inflammatory effects. In the present study, possible protective mechanisms of Ses on DOX-induced cardiotoxicity were investigated in rats and cultured H9C2 cells. We demonstrated that Ses exhibits a significant protective effect on cardiac tissue in animal and cell models of DOX-induced cardiac injury. Moreover, Ses can ameliorate DOX-induced oxidative stress and mitochondrial damage. Further studies suggested that Ses is able to up-regulate the protein expression of Mn-SOD in normal rats and to restore the decreased expression of Mn-SOD in DOX-induced cardiac injury rats. Exposure to Ses or DOX alone slightly increased the protein expression of Sirt1; however, a more remarkable increase in Sirt1 protein level was detected in the Ses + DOX group. Treatment with a pan-sirtuin inhibitor (nicotinamide) or a Sirt1-specific inhibitor (EX-527) partially antagonised the effect of Ses on DOX-induced mitochondrial damage and completely abolished the effect of Ses on Mn-SOD expression. These findings indicate that the protective mechanisms of Ses on DOX-induced cardiotoxicity are involved in the alleviation of oxidative stress injury and Mn-SOD dysfunction, partially via the activation of Sirt1.

1. Introduction

Doxorubicin (DOX) is an effective anthracycline antibiotic that is used to treat a wide range of cancers; however, the toxic side effects on healthy tissues, specifically the heart, make DOX dose limiting. DOX-induced cardiotoxicity is thought to be a complex multifactorial process, which includes oxidative stress (Doroshow, 1983; Olson and Mushlin, 1990). The mitochondrial electron transport chain is the main source of reactive oxygen species (ROS) in cardiac myocytes. DOX-induced oxidative stress in cardiomyocyte mitochondria is associated with cell death and is responsible for cardiac damage (Kotamraju et al., 2000; Sarvazyan, 1996). Manganese SOD (Mn-SOD, SOD2) is an important antioxidative enzyme and mainly regulates ROS metabolism in the mitochondria. Mitochondrial oxidative stress associated with Mn-SOD dysfunction contributes to DOX-induced heart injury (Danz et al., 2009). The cardiotoxicity of DOX is less severe in transgenic mice expressing high levels of Mn-SOD than in nontransgenic mice. Therefore, conditions that lead to Mn-SOD dysfunction could increase ROS production and hence induce tissue damage associated with DOX cardiotoxicity. On the other hand, an increase in Mn-SOD levels

Abbreviations: Ses, sesamin; DOX, doxorubicin; Mn-SOD, Manganese SOD; Sirt1, Sirtuin 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; MDA, malondialdehyde.

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may provide a prognostic advantage for preventing DOX-induced cardiotoxicity.

Sirt1, a NAD-dependent class III histonedeacetylase, is an important regulator of cell survival and lifespan (Brachmann et al., 1995). Sirt1 catalyses the deacetylation of numerous proteins and generates nicotinamide (Nic) as a by-product; Nic is a negative regulator of Sirt1 activity (Li et al., 2010; Komers et al., 2007). Sirt1 has been shown to increase cell resistance and survival from stress through a number of pathways (Wang et al., 2009; Brunet et al., 2004). Moderate overexpression of Sirt1 protects the heart from oxidative stress through upregulation of antioxidants such as Mn-SOD (Alcendor et al., 2007). Sirt1 has been shown to be activated by oxidative stress and by resveratrol or tetrahydroxystilbene glucoside treatment (Li et al., 2010; Yun et al., 2012).

Sesamin (Ses), one of the major lignans in sesame seeds, has received a great deal of interest. Previous studies have revealed that Ses has a wide range of pharmacological functions, including antioxidative, antihypertensive and antihyperlipemic properties in different murine models (Hou et al., 2003; Wu et al., 2012; Li et al., 2012; Hsieh et al., 2011). The beneficial effects are thought to be due to its antioxidative properties. Nevertheless, the possible protective effect of Ses against DOX-induced cardiotoxicity and the underlying mechanisms remain unclear. Therefore, we investigated this possibility via in vivo and in vitro experiments in the present study. The underlying mechanisms were also studied by investigating the involvement of possible Mn-SOD and Sirt1-related pathways.

2. Materials and methods

2.1. Chemicals

Ses was purchased from Inner Mongolia Kailu Irrigation Pharmaceutical Co., LTD. (Inner Mongolia, China). DOX, Nic and EX-527 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carboxy-HDCFDA and Rh123 were purchased from Molecular Probes (Eugene, OR, USA). Mn-SOD, β-actin and Sirt1 antibodies were purchased from Santa Cruz Biotechnological Co. (Santa Cruz, CA, USA).

2.2. Animals and treatments

Adult male Sprague-Dawley rats (200 ± 20 g, $n = 40$) were provided by the Experimental Animal Center of Hebei Medical University (Shijiazhuang, China). All animals were treated in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health. The animals were housed under standard laboratory conditions (12 h light:12 h dark and 24 ± 3 °C), food and water were provided ad libitum. The rats were randomly divided into four groups: control, sesamin (Ses), doxorubicin (DOX), and DOX + Ses. The control group received carboxymethylcellulose (CMC; 0.5%) orally for 10 consecutive days. The Ses group received Ses alone suspended in 0.5% CMC (20 mg/kg; orally once daily for 10 consecutive days). The DOX group received CMC orally for 10 consecutive days and a single dose of DOX (20 mg/kg, i.p.) on day 7. The DOX + Ses group received both Ses and DOX at the previously mentioned doses; Ses was administered for 10 consecutive days, and DOX was administered once on day 7. The dosing volume was 0.5 ml/100 g body weight. The selected dose was based on our preliminary study.

2.3. Cell culture and treatment

Rat cardiac H9C2 cells (Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin in tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO_2. The cells were fed every 2–3 days and were subcultured once they reached 70–80% confluence. H9C2 cells were then treated with Ses at concentrations of 0, 20, 40, 80, or 160 μM or vehicle for 24 h and then exposed to 2 μM DOX for 24 h. In separate experiments, cells were preincubated with Nic (20 mM) or EX-527 (1 μM), 30 min before the addition of 40 μM Ses. The same volumes of corresponding solvents were added to the controls.

2.4. Electrocardiography (ECG)

Twenty-four hours after the last Ses or CMC treatment (day 11), the rats were anesthetised with thiopentone (35 mg/kg; i.p.) and subjected to ECG recordings using a Biopac ECG recorder (Bioscience, Washington, USA). Needle electrodes were inserted under the skin for the limb lead at position II. Blood samples were collected from the ophthalmic artery in the orbital rim prior to sacrifice. The blood was centrifuged at $3000 \times g$ for 15 min to separate the sera, which were stored at −80 °C for biochemical analyses. The abdomen of each rat was opened and the hearts were rapidly dissected out and washed in ice-cold isotonic saline. Part of the heart was kept at −80 °C and part of the heart was placed in a fixative solution (4% paraformaldehyde).

2.5. Serum biochemical assays

Creatine phosphokinase (CPK), creatine kinaseisoenzyme-MB (CK-MB) activities were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain) and a CHEMIX-180 automatic biochemistry analyser (Sysmex).

2.6. Histopathological examination of the heart tissue

Paraformaldehyde fixed heart tissue samples that were embedded in paraffin wax were serially sectioned (7 μm thickness) and stained with hematoxylin and eosin for the assessment of histopathological changes.

2.7. Determination of lipid peroxides (measured as MDA)

The fresh heart tissue was rinsed and then homogenised in buffer [10 mM Tris–HCl, 137 mM NaCl, 1 mM Na_2EDTA, and 0.5 mM dithiotreitol (DTT)], and 250 mM sucrose at pH 7.4 using a homogeniser (T 18 basic Ultra-Turrax®; Mandel Scientific Company Inc., Guelph, Canada). The homogenate was centrifuged at $1000 \times g$ for 15 min at 4 °C. The supernatants were removed and total protein concentration was measured using a protein assay kit. The supernatants were used for biochemical assay and western blot analysis. The malondialdehyde (MDA) content in heart tissue and sera was used as index of lipid superoxide level. Measurements were conducted using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) with a spectrophotometer (Perkin-Elmer, Norwalk, Conn., USA).

2.8. Cell viability assay

Cell viability was determined using the MTT assay. Briefly, cells were seeded in a 96-well plate at a density of 1×10^5/mL. After the drug treatment, 20 μL MTT (5 mg/mL) was added to each well, and the cells were cultured for another 4 h at 37 °C. The medium was then removed, and 150 μL dimethyl sulfoxide (DMSO) was added to each well. The absorbance of each well was recorded at 490 nm.

2.9. Detection of intracellular reactive oxygen species (ROS)

H9C2 cells were treated with DOX and Ses for specified time periods, and DCFH-DA (10 μM) was then added to the cells in the dark at 37 °C. The cells were then digested with trypsin, re-suspended in 1 × PBS buffer, transferred to flow cytometer tubes and examined using the flow cytometer (Becton Dickinson, USA) at an emission wavelength of 525 nm and an excitation wavelength of 488 nm. Lastly, ROS generation was quantified using the median fluorescence intensity of 10,000 cells.

2.10. Measurement of mitochondrial membrane potential ($\Delta\Psi_m$)

H9C2 cells were treated with DOX and Ses for specified time periods; Rh123 (5 μM) was then added to the cells in the dark at 37 °C. The cells were then digested with trypsin, re-suspended in 1 × PBS buffer, transferred to flow cytometer tubes and examined using the flow cytometer (Becton Dickinson, USA) at an emission wavelength of 525 nm and an excitation wavelength of 488 nm. Lastly, $\Delta\Psi_m$ was quantified using the median fluorescence intensity of 10,000 cells.

2.11. Mn-SOD activity

The Mn-SOD activity was measured by inhibiting extracellular and cytosolic Cu/Zn SOD activity using an SOD assay kit (WST-1, Beyotime Institute of Biotechnology, China). Mn-SOD activity was calculated in terms of protein content (U/mg).

2.12. Western blotting examination of Mn-SOD and Sirt1 expression

An equal amount of protein (50 μg) from each sample was resolved by SDS–PAGE, transferred to PVDF membranes and blocked with 5% nonfat dry milk in TBST (50 mM Tris–HCl, 150 mM NaCl, 0.1% Tween, pH 7.4) for 1 h at room temperature. Membranes were then incubated with the polyclonal IgG for Mn-SOD and Sirt1 (dilution 1:200) over night at 4 °C, washed with TBST three times for 10 min each, and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000) for 1 h at 37 °C. After washing, blots were detected using an enhanced chemiluminescence plus system (ZhongShan Bioengineering Institute, Beijing, China). Western blot signals were quantified based on densitometric measurements. The data were normalised to the ratios of β-actin (dilution 1:800) detected on the same blot to control for possible variations in protein loading.