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Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis

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ABSTRACT

Doxorubicin (DOX) is one of the most effective chemotherapeutic drugs; however, its incidence of cardiotoxicity compromises its therapeutic index. Chrysin, a natural flavone, possesses multiple biological activities, such as antioxidant, anti-inflammatory and anti-cancer. The present study was designed to investigate whether chrysin could protect against DOX-induced acute cardiotoxicity; and if so, unravel the molecular mechanisms of this protective effect. Chrysin was administered to male albino rats once daily for 12 consecutive days at doses of 25 and 50 mg/kg orally. DOX (15 mg/kg; i.p.) was administered on day 12. Chrysin pretreatment significantly protected against DOX-induced myocardial damage which was characterized by conduction abnormalities, increased serum creatine kinase isoenzyme-MB (CK-MB), and lactate dehydrogenase (LDH) and myofibrillar disarrangement. As indicators of oxidative stress, DOX caused significant glutathione depletion, lipid peroxidation and reduction in activities of antioxidant enzymes; catalase (CAT) and superoxide dismutase (SOD). Chrysin pretreatment significantly attenuated DOX-induced oxidative injury. Additionally, DOX provoked inflammatory responses by increasing the expressions of nuclear factor kappa-B (NF-κB), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and the levels of tumor necrosis factor-alpha (TNF-α) and nitric oxide while chrysin pretreatment significantly inhibited these inflammatory responses. Furthermore, DOX induced apoptotic tissue damage by increasing Bax and cytochrome c expressions and caspase-3 activity while decreasing the expression of Bcl-2. Chrysin pretreatment significantly ameliorated these apoptotic actions of DOX. Collectively, these findings indicate that chrysin possesses a potent protective effect against DOX-induced acute cardiotoxicity via suppressing oxidative stress, inflammation and apoptotic tissue damage.

1. Introduction

Doxorubicin (DOX), an anthracycline antibiotic, has long been one of the most effective chemotherapeutic agents for treatment of a variety of human neoplasms, including leukemias, lymphomas, and solid tumors (Sant et al., 2009). However, its clinical utility is markedly hampered by high incidence of a dose-dependent cardiotoxicity; irreversible degenerative cardiomyopathy and congestive heart failure (Smith et al., 2010). With the increasing use of this anthracycline antibiotic, an acute cardiotoxicity has been recognized as a severe complication of DOX chemotherapy (Hayek et al., 2005).

The pathogenesis of DOX-induced cardiotoxicity is not entirely clear, but a solid body of evidence indicates that oxidative stress, inflammation and apoptosis are involved (Minotti et al., 2004). Nonetheless, oxidative stress remains the cornerstone. DOX has been shown to induce generation of reactive oxygen species which are involved in the interplay of a number of processes, including redox cycling of the quinone moiety of DOX, disturbance of iron metabolism and DOX metabolites in the heart (Chen et al., 2007). Reactive oxygen species ultimately lead to oxidative damage of cellular and mitochondrial membranes and cellular macromolecules (Goormaghtigh et al., 1990).

Moreover, there is growing evidence that DOX also elicits inflammatory effects in the vasculature and the myocardium by increasing the expression of nuclear factor kappa-B (NF-κB), a key regulator of genes that are involved in the immune responses and inflammatory responses (Hou et al., 2005). Subsequently, DOX induces the production of several proinflammatory mediators such

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as tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2) and nitric oxide (Abd El-Aziz et al., 2012).

Besides, accumulating evidence indicates that cellular apoptosis or programmed cell death plays a critical role in the pathogenesis of DOX-induced cardiotoxicity (Reeve et al., 2007). As oxidative stress evoked by DOX triggers the intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes (Kluza et al., 2004). This phenomenon results in mitochondrial dysfunction and myofibrillar degeneration (Arola et al., 2000). As DOX continues to be a mainstay in chemotherapy, so the search for a safe and effective remedy to reverse DOX-induced cardiotoxicity remains a critical issue in both cardiology and oncology.

Nowadays, much of attention has been given to the usage of phytochemicals as a protective strategy against DOX-induced cardiotoxicity (Xiao et al., 2012). Flavonoids are natural polyphenolic phytochemicals that are beneficial in preventing and treating many diseases such as cancer, cardiovascular diseases, neurodegenerative diseases as well as diabetes (Khan et al., 2012a). Chrysin (5,7-dihydroxyflavone) belongs to this category which is found in bee propolis, honey and various plants (Barbaric et al., 2011). It has multiple biological properties such as antioxidant, anti-inflammatory, antiapoptotic and anti-cancer (Sultana et al., 2012). Recently, chrysin has been proven to protect against DOX-induced hepatotoxicity and nephrotoxicity (Rashid et al., 2012). Accordingly, this study aimed to investigate the potential protective effect of chrysin against DOX-induced cardiotoxicity in rats and to elucidate the underlying molecular mechanisms in terms of oxidative stress, inflammatory and apoptotic mediators.

2. Materials and methods

2.1. Drugs and chemicals

DOX was purchased as Adriablastine (50 mg doxorubicin hydrochloride, Pharmacia & Upjohn, Milan, Italy). Chrysin, reduced glutathione (GSH), Ellman's reagent [3,3'-dithiobis(6-nitrobenzoic acid)], bovine serum albumin, dimethylsulfoxide (DMSO) and thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). N-butanol, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and trichloroacetic acid were purchased from El-Nasr Chemical Co. (Egypt). All other chemicals were of the highest purity grade commercially available.

2.2. Animals

The study was conducted according to ethical guidelines (Ain Shams University, Egypt). Male albino rats (150–250 g) were obtained from Nile Co. for Pharmaceutical and Chemical Industries, Egypt. Rats were housed in an air-conditioned atmosphere, at a temperature of 25 °C with alternatively 12 h light and dark cycles and allowed free access to food and water. Animals were acclimated for 2 weeks before experimentation. They were kept on a standard diet and water ad libitum. Standard diet pellets (El-Nasr, Abu Zaabal, Egypt) contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture.

2.3. Experimental design

Rats were randomly assigned to five groups (ten animals per group) and treated as follows; the first group (control group) received 2.5 ml/kg of mixture of DMSO and corn oil (1:9) which was used as a vehicle for chrysin through oral gavage once daily for 12 consecutive days,. The second group received mixture of DMSO and corn oil (1:9) through oral gavage once daily for 12 consecutive days and a single intraperitoneal injection of DOX (15 mg/kg) on the 12th day. The third and fourth groups were pretreated with an oral dose of chrysin 25 mg/kg and 50 mg/kg respectively for 12 consecutive days followed by a single intraperitoneal injection of DOX (15 mg/kg) on the 12th day after 1 h of the last treatment of chrysin in both groups. Chrysin doses were determined based on previous studies reporting its antioxidant and antiapoptotic properties while protecting against cisplatin-induced nephrotoxicity and jejunum and colon toxicities (Khan et al., 2012a, 2012b; Sultana et al., 2012). The last group received chrysin only at an oral dose of 50 mg/kg for 12 consecutive days. Forty-eight hours after DOX injection, rats were anesthetized with ketamine (75 mg/kg; i.p.) and subjected to ECG recording. After-that, blood samples were collected from the retro-orbital plexus and allowed to clot. Serum was separated by centrifugation at 3000*g* for 10 min and used for biochemical analyses. Rats were sacrificed and heart tissues were dissected out and washed with ice-cold saline. The body and heart weights were measured. Hearts were homogenized in saline then the homogenate was used for assessment of different biochemical parameters. In addition, heart specimens from different groups were fixed in 10% buffered formalin for histopathological and immunohistochemical examination.

2.4. Electrocardiography (ECG)

ECG was recorded at the beginning of the experiment to ensure the normal ECG pattern of the rats. At the end of the experiment, ECG was recorded in ketamine anesthetized rats 48 h after DOX injection using Bioscience ECG recorder (Bioscience, Washington, USA). Anesthetized rats were placed in the supine position on a board and needle electrodes were inserted beneath the skin for the limb lead at position II (right forelimb to left hind limb). Every recording lasted for at least 5 min. ECG recording speed was 50 mm/s and the voltage was 1 mV/cm. Noise was minimized by a digital filter. Analysis of ECG waves was done to calculate heart rate (beats/min), QRS duration (ms), QT interval (ms), which was corrected for heart rate using the Bazett formula [QTc=QT/(square root of RR interval)], and PR interval (ms). For each parameter, measurements were done at three non-consecutive, randomly chosen points in every 5 min recording. The results are reported as mean of the three randomly selected segments.

2.5. Assessment of cardiotoxicity indices

Creatine kinase isoenzyme-MB (CK-MB) and lactate dehydrogenase (LDH) activities were determined according to standard methods using available commercial kits (Spectrum diagnostics, Cairo, Egypt). Heart index was calculated according to the formula: (heart weight/body weight) × 100.

2.6. Assessment of oxidative stress markers

To determine GSH, 0.5 ml homogenate was added to a tube with 0.5 ml of 10% trichloroacetic acid. The tubes were shaken gently and intermittently for 15 min, followed by centrifugation at 1000*g* for 10 min. An aliquot of the resulting supernatant (0.2 ml) was added to a tube containing 1.7 ml phosphate buffer and 0.1 ml Ellman's reagent then the absorbance was read at 412 nm within 5 min (Ellman, 1959). The results were expressed as μM of GSH/g of wet tissue. Lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances measured as malondialdehyde (MDA), according to the method of Mihara and Uchiyama (1978). Briefly, the reaction mixture (0.5 ml homogenate+2.5 ml 20% trichloroacetic acid+1.0 ml 0.6% thiobarbituric acid) was heated for 20 min in a boiling water bath followed by