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Role of oxidative stress, inflammation, nitric oxide and transforming growth factor-beta in the protective effect of diosgenin in monocrotaline-induced pulmonary hypertension in rats

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ABSTRACT

Pulmonary hypertension is a progressive disease of various origins that is associated with right ventricular dysfunction. In the present study, the protective effect of diosgenin was investigated in monocrotaline-induced pulmonary hypertension in rats. Pulmonary hypertension was induced by a single subcutaneous injection of monocrotaline (60 mg/kg). Diosgenin (100 mg/kg) was given by oral administration once daily for 3 weeks. At the end of the experiment, mean arterial blood pressure, electrocardiography and echocardiography were recorded. Rats were then sacrificed and serum was separated for determination of total nitrate/nitrite level. Right ventricles and lungs were isolated for estimation of oxidative stress markers, tumor necrosis factor-alpha, total nitrate/nitrite and transforming growth factor-beta contents. Myeloperoxidase and caspase-3 activities in addition to endothelial and inducible nitric oxide synthase protein expression were also determined. Moreover, histological analysis of pulmonary arteries and cardiomyocyte cross-sectional area was performed. Diosgenin treatment provided a significant improvement toward preserving hemodynamic changes and alleviating oxidative stress, inflammatory and apoptotic markers induced by monocrotaline in rats. Furthermore, diosgenin therapy prevented monocrotaline-induced changes in nitric oxide production, endothelial and inducible nitric oxide synthase protein expression as well as histological analysis. These findings support the beneficial effect of diosgenin in pulmonary hypertension induced by monocrotaline in rats.

1. Introduction

Pulmonary hypertension (PH) is a life-threatening disease which is characterized by an extensive narrowing of the pulmonary vascular bed with a progressive increase in pulmonary vascular resistance (Umar et al., 2010). When untreated, the disease ultimately results in elevation of pulmonary arterial pressure and right ventricular (RV) hypertrophy with subsequent RV failure and death (Shao et al., 2011). Monocrotaline (MCT)-induced PH is an experimental model that largely mimics human PH regarding hemodynamic disorders, histological changes and high mortality (Henriques-Coelho et al., 2004).

MCT is a pyrrolizidine alkaloid which selectively injures the pulmonary vascular endothelium and induces pulmonary vasculitis (Hessel et al., 2006). MCT-treated rats demonstrated an early endothelial cell injury, followed by progressive pulmonary arterial structural changes which resulted in the development of PH and RV hypertrophy progressing to failure within weeks (Lipke et al., 1993; Aziz et al., 1997).

Oxidative stress plays a pivotal role in the pathogenesis and/or the development of PH by MCT. Increased oxidative stress mediates MCT-induced apoptosis and endothelial dysfunction in the pulmonary vascular endothelial cells (Grobe et al., 2006). Moreover, impaired nitric oxide (NO) synthesis or bioactivity is the main pathological change that is significantly implicated in PH (Ozturk and Uma, 2010). In the presence of oxidative stress, NO reacts with superoxide anion generating peroxynitrite which is a highly toxic molecule leading to more progressive endothelial dysfunction during the development of PH (Oishi et al., 2006). As a result of the lack of available NO and increased oxidative stress, inflammatory and proliferative cascades proceed with further progression of the disease (Bhargava et al., 1999).

Many patients with PH remain symptomatic despite therapy. Current treatments can reduce the severity of hemodynamic disorder; however, gradual deterioration and progression of the disease often necessitate a lung transplant (Umar et al., 2010). The imbalance between NO and oxidative stress plays an important role in the process of many cardiovascular and pulmonary

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diseases. Administration of antioxidants might be beneficial in the treatment of PH.

Estrogen deficiency (i.e. ovariectomy) exacerbates pulmonary hypertension and treatment with estradiol attenuates the disease (Rabinovitch et al., 1981; Resta et al., 2001). Estradiol has shown significant protection against RV hypertrophy and pulmonary arterial medial hypertrophy in pulmonary hypertension in rats (Tofovic et al., 2006). Diosgenin is a plant-derived sapogenin and is a precursor of steroid hormones (Adlercreutz et al., 1991; Au et al., 2004). Diosgenin (as a phytosterogen) is known to possess anti-hyperlipidemic, anti-inflammatory and antioxidant properties (Raju and Mehta, 2009). The beneficial role of diosgenin has been studied in several models of metabolic diseases, inflammation, blood and cerebral disorders, cardiovascular diseases, and cancer (Patel et al., 2012). Moreover, diosgenin has shown to ameliorate palmitate-induced endothelial dysfunction and insulin resistance through improvement of endothelial insulin signaling and enhancement of NO production (Liu et al., 2012). The relaxant response elicited by diosgenin on vascular smooth muscle cells probably occurs due to the activation of cGMP–NO–L-Arginine pathway (Dias et al., 2007). This implicates the possibility of its application in the treatment of many cardiovascular diseases including pulmonary hypertension. Therefore, the goal of the present study was to explore the protective effects of diosgenin on MCT-induced PH through examination of its effects on associated hemodynamic, biochemical and histological alterations.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 180–210 g were obtained from the animal facility of Faculty of Pharmacy, Cairo University. Rats were housed under controlled temperature ($25 \pm 2^{\circ}C$) and constant light cycle (12 h light/dark) and allowed free access to a standard rodent chow diet and water. The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

2.2. Chemicals

MCT and diosgenin were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other used chemicals were of analytical grade.

2.3. Experimental design

Rats were randomly divided into three groups, 11 animals each. Group I served as a normal group. Group II received a single subcutaneous injection of MCT (60 mg/kg). MCT was dissolved in 0.1 M HCl and adjusted to pH 7.4 with 0.1 M NaOH (Pullamsetti et al., 2005). Groups III received MCT as in group II followed by daily oral administration of diosgenin (100 mg/kg) for 21 days. Diosgenin was freshly prepared daily in saline. Dose of diosgenin was selected based on its effectiveness and safety as a protective agent in previous experimental studies (Ma et al., 2002; Hamrita et al., 2012).

2.4. Mean arterial blood pressure, electrocardiographic and echocardiographic measurements

After 3 weeks, animals were weighed. Heart rate (HR) and blood pressure (BP) were measured by the non-invasive tail cuff method using PowerLab data acquisition systems (ADInstruments, Australia). Rats were then anesthetized with thiopental (50 mg/kg, i.p.) and kept warmed with a heating lamp to prevent the incidence of hypothermia. Subcutaneous peripheral limb electrodes were inserted for electrocardiographic recording (HPM 7100, Fukuda Denshi, Tokyo, Japan) to determine QRS amplitude and duration. Spontaneously breathing rats were screened for any RV abnormalities by echocardiography using a Sonosite SonoHeart Elite echo machine (Bothell, USA) with 8-MHz ultrasound probe. Right ventricular anterior wall thickness (RVAWT) was measured in the two-dimensional short-axis parasternal view below the tricuspid valve or in the long-axis parasternal view by M-mode. Right ventricular end-diastolic diameter (RVEDD) was measured in M-Mode of long-axis parasternal view as the distance between interventricular septum and RV anterior wall at the time of left ventricular end diastole. Each parameter was averaged over three cardiac cycles. At the end of the experiment, blood was collected from the retro-orbital sinus using non heparinized capillary tubes for serum separation. Animal was euthanized and lung, RV and left ventricle with septum (LVS) were rapidly excised, washed with ice-cold saline, dried and weighed. For each group, two sets of experiments were conducted; one for biochemical examination and the other ($n=3$) for histological examination.

2.5. Biochemical measurements

Parts of lung and RV were homogenized in ice-cold KCl (1.15%) and NaCl (0.9%), respectively, using a homogenizer (HeidolphDiax 900, Germany) to prepare 10% homogenate. The resultant homogenates were used for determination of the following parameters.

2.5.1. Reduced glutathione

Reduced glutathione (GSH) content was determined using Ellman's reagent according to the method described by Beutler et al. (1963) and expressed as nmol/100 mg protein.

2.5.2. Lipid peroxidation products

Lipid peroxidation products were estimated by determination of the level of thiobarbituric acid reactive substances (TBARS) that were measured according to the assay of Buege and Aust (1978) and expressed as nmol/mg protein.

2.5.3. Myeloperoxidase activity

Myeloperoxidase (MPO) activity was determined kinetically at 460 nm by measuring rate of H_2O_2-dependent oxidation of o-dianisidine that is catalyzed by MPO (Bradley et al., 1982) and expressed as mU/mg protein. One unit of MPO activity is defined as the amount of enzyme that degrades 1 μmol peroxide per min at 25 °C.

2.5.4. Tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF-α) content was assessed using rat TNF-α ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/mg protein.

2.5.5. Serum and tissue total nitrate/nitrite (NO_x)

NO_x was determined spectrophotometrically at 540 nm using Griess reagent after reduction of nitrate to nitrite by vanadium trichloride (Miranda et al., 2001) and expressed in serum as μmol/l and in lung and RV tissues as μmol/g protein.