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Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats

Asmaa I. Matouk, Ashraf Taye, Gehan H. Heeba, Mohamed A. El-Moselhy*

Department of Pharmacology & Toxicology, Faculty of Pharmacy, Minia University, Minia, Egypt

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ABSTRACT

The present study aimed to examine whether the co-administration of quercetin (QRN) and losartan (LOS) can produce additional protective effects against chronic DOX cardiotoxicity. Cardiotoxicity in rats was induced by intraperitoneal injection of doxorubicin (DOX) in a cumulative dose of 15 mg/kg for two weeks. Results revealed that DOX administration exhibited elevated serum levels of TNF-α, creatine kinase (CK-MB), lactate dehydrogenase (LDH) in addition to increased myocardial lipid peroxide (MDA) and nitric oxide (NO) alongside attenuating cardiac antioxidant defense system of superoxide dismutase (SOD) and catalase (CAT) activities. DOX produced leukocyte infiltration and myocardial lesions. Pretreatment with QRN (10 mg/kg, orally) solely or in combination with LOS (0.7 mg/kg, orally) for 6 weeks markedly ameliorated all these biochemical characteristics, and substantially reduced the myocardium peroxidative damage. The protective effects obtained by LOS were more pronounced by its combination with QRN. Our results suggest that quercetin potentially augmented the cardioprotective effect of losartan against chronic DOX cardiotoxicity *via* its antioxidant and anti-inflammatory properties.

1. Introduction

Doxorubicin (DOX) is a member of anthracycline antibiotics which is extensively used as an effective and broad spectrum anti-cancer agent. However, the clinical use of DOX is limited by its acute and chronic cardiotoxicity. Acute effects can occur immediately after treatment and characterized by transient arrhythmias, reversible hypotension and pericarditis (Upadhyay et al., 2010), while chronic cardiotoxicity can manifest years to decades after treatment. It is irreversible and dose dependent cardiotoxicity that is characterized by progressive left ventricular dysfunction and may lead to congestive heart failure (Corna et al., 2004).

In spite of that generation of reactive oxygen species (ROS) represents the main mechanism that underlines the pathophysiology of DOX-induced cardiomyopathy (Fouad and Yacoubi, 2011), other mediators as angiotensin (Ang II) has been found to have a role in the development of DOX-induced cardiomyopathy (Toko et al., 2002). Ang II plays a significant role in the pathophysiology of hypertensive and ischemic heart diseases and the suppression of its effects can ameliorate the remodeling process of the heart and prolongs long-term survival in animal models and humans with cardiac hypertrophy and failure (Kawabata et al., 2001; Wang et al., 2009). It is noteworthy that Ang II is able to stimulate the production of cytokines like, tumor necrosis factor-α (TNF-α) *via* acting on Ang II type 1 receptor (AT_1R), which is present on monocytes, macrophages and vascular smooth muscle cells (Hahn et al., 1994).

A strong association between oxidative stress and cardiac inflammatory response including cytokine release after DOX

* *Corresponding author at*: Department of Pharmacology & Toxicology, Faculty of Pharmacy, Minia University, 61511 Minia, Egypt. Tel.: +20 116825727; fax: +20 862369075.
E-mail address: Ashraf_taye70@yahoo.com (A. Taye).
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treatment has been reported (Bien et al., 2007). Previous studies have shown that TNF-α is involved in development of cardiovascular disease (Duvnjak and Duvnjak, 2009).

Many drugs are effective in preventing cardiovascular diseases, but their use is often limited by their side effects. Losartan (LOS) is the first of the new class of Ang II receptor blockers that selectively and completely blocks AT_1R (Awan and Mason, 1996). Numerous studies have demonstrated reversal of left ventricular hypertrophy, reduced fibrosis and improvement in cardiac function following LOS treatment (Schieffer et al., 1994).

Recently, a pay attention has been given to food products derived from medicinal plants that have been found to have certain preventive actions in the treatment of cardiovascular diseases. Quercetin (QRN; 3,3′,4′,5,7-pentahydroxy flavone), the most abundant of the flavonoid molecules is present in a variety of foods including vegetables, fruits and wine (Hertog et al., 1996). There has been an upsurge of interest to explore the cardioprotective potential of natural products (Yogeeta et al., 2006).

An extensive amount of *in vitro* and *in vivo* animal research has focused on the antioxidant potential of QRN (Glasser et al., 2002; Annapurna et al., 2009; Kalender et al., 2011). Animal evidence suggests QRN's antioxidant effects afford protection of the brain, heart, and other tissues against ischemia-reperfusion injury, toxic compounds, and other factors that can induce oxidative stress. The antioxidant properties of QRN might be due to its ability to chelate transition metal ions, such as Fe^{2+} and Cu^{2+} and scavenge free radicals (Sestili et al., 1998). Several *in vitro* and *in vivo* studies have evaluated quercetin combined with DOX for breast cancer and leukemia treatments, revealing synergistic effects (Shen et al., 2008; Du et al., 2010; Staedler et al., 2011). A quite recent study in human showed that QRN can increase the chemosensitivity of breast cancer cells to doxorubicin (Li et al., 2013). Moreover, the combined treatment of QRN and DOX may be beneficial against human liver cancer, since QRN can reduce the hepatotoxicity of DOX in normal liver cells (Wang et al., 2012). However, there is an increasing interest concerning whether the concurrent administration QRN and LOS could produce additional protective effects against chronic DOX cardiotoxicity.

The present study was conducted to examine effects of the single and combined administration of QRN and LOS on the chronic DOX cardiotoxicity and to investigate the possible preliminary mechanisms underling these effects.

2. Materials and methods

DOX hydrochloride vial 10 mg was purchased from Pharmacia Italia, S.P.A., Italy, Quercetin powder 25 g was purchased from Sigma–Aldrich, St. Louis, MO, USA. Other chemicals were purchased from Al-Gomhorria and El-Motaheda, Cairo, TNF-α (Koma Biotech Inc., Korea).

2.1. Experimental design

Adult male Wister rats weighing 190–220 g were used. Animals were left to acclimatize for a period of two weeks. Throughout the period of the experiment they were kept under constant environmental conditions of a 12 h dark/12 h light cycle and a temperature (21 °C). They were allowed free access to standard laboratory food and water. Experiments were conducted in accordance with the guidelines for animal care of the United States Naval Medical Research Center, Unit No. 3, Abbaseya, Cairo, Egypt. The adopted guidelines are in accordance with "Principles of Laboratory Animals Care" (NIH publication No. 85-23, revised 1985). The study protocol was approved by members of "The Research Ethics Committee" and by the Pharmacology and Toxicology Department, Faculty of Pharmacy, Minia University, Egypt.

Rats were randomly divided into five groups, as following:

Group 1: Control normal group (8 rats); rats received the same volume of the solvent.
Group 2: DOX-treated group (15 rats); rats were injected with DOX (2.5 mg/kg, i.p.) in six equal injections at 48 h intervals for two consecutive weeks to achieve accumulative dose of 15 mg/kg. The large number of animals was used because of the reported high mortality rate upon sole administration of DOX (Siveski-Iliskovic et al., 1994).
Group 3: DOX + QRN-treated group (10 rats); QRN suspension was orally administered (10 mg/kg/day) to rats (Haleagrahara et al., 2009). Administration of QRN was started at the same day of DOX administration and continued for further 4 weeks after stopping of DOX (6 weeks total period).
Group 4: DOX + LOS-treated group (10 rats); rats were orally given 0.7 mg/kg LOS (Lovric-Bencic et al., 2004). Administration of LOS was started at the same day of DOX administration and continued for further 4 weeks after stopping of DOX (6 weeks total period).
Group 5: DOX + QRN + LOS-treated group (10 rats); the combined administration of QRN and LOS was started at the same day of DOX administration and continued for further 4 weeks after stopping of DOX (6 weeks total period).

2.2. Serum and tissue sampling

Twenty-four hours following the last DOX injection, rates were sacrificed by decapitation. A blood sample of each animal was collected into a dry centrifuge tube. Serum and heart tissues were collected and stored at −80 °C till the time of analysis. Serum was separated by centrifugation at 8000 × *g* for 10 min and used to determine creatine kinase (CK-MB), lactate dehydrogenase (LDH) and TNF-α serum levels. For determination of the other cardiac biochemical parameters and histopathological changes, heart tissues were removed, dissected, washed by ice-cold saline and blotted between filter papers. The remainder of heart tissue of each rat was weighed and homogenized in ice-cold saline for 1 min, forming 10% (w/v) homogenate. Total protein concentration was also determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Chemicals).

2.3. Determination of serum TNF-α, CK-MB and LDH levels

Serum TNF-α level was assessed in this study using enzyme-linked immunosorbent assay (ELISA) using a microplate reader (Spectra III Classic, Tecan, Salzburg, Austria) as