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Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity

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ABSTRACT

Doxorubicin (DOXO) is commonly used to treat a wide range of malignant tumors, but its clinical use is limited by acute and chronic cardiotoxicity. The precise mechanism underlying DOXO-induced cardiotoxicity is still not completely elucidated, but cardiac inflammation seems to be involved. Effects of DOXO on proinflammatory cytokines, inflammatory cell infiltration, and necrosis have been proven only when a functional impairment has already occurred, so this study aimed to investigate the acute effect of DOXO administration in mouse heart. The results of our study demonstrated alterations in cardiac function parameters assessed by ultrasound within 24 h after a single injection of DOXO, with a cumulative effect along the increase of the dose and the number of DOXO administrations. At the same time, DOXO causes a significant production of proinflammatory cytokines (such as TNF-α and IL-6) with a concomitant reduction of IL-10, a well-known antiinflammatory cytokine. Furthermore, overexpression of inducible nitric oxide synthase (iNOS) in heart tissue and increased levels of serum nitrite in DOXO-treated mice were detected. Notably, DOXO administration significantly increased nitrotyrosine expression in mouse heart. Our data support the hypothesis that these early events, could be responsible for the later onset of more severe deleterious remodeling leading to DOXO induced cardiomyopathy.

1. Introduction

Doxorubicin (DOXO) is one of the most widely used anticancer drug (Riad et al., 2009), but its clinical application is hampered by cardiotoxicity. DOXO induces progressive myocardial stress, cardiomyopathy and heart failure that can be fatal (Wong et al., 2013).

Intensive investigations on DOXO-induced cardiotoxicity have continued for decades. Different lines of evidences have provided putative mechanisms, nevertheless the precise mechanism underlying DOXO-induced cardiotoxicity is still not completely elucidated (Octavia et al., 2012). Traditionally, reactive oxygen species (ROS) have received more attention, although basic research has proven other factors (such as sarcomeric structure disruption, iron metabolism, energetic alterations and inflammation) to be important as well (Salazar-Mendiguchía et al., 2014). Indeed, numerous studies showed that cardiac production of proinflammatory cytokines, inflammatory cell infiltration, and necrosis are increased in hearts of chronic DOXO treated mouse (Ikegami et al., 2007; Li et al., 2006; Riad et al., 2009).

To note, it is believed that the activation of the innate immune system with the ensuing proinflammatory cytokines release are at the basis of the pathogenesis of DOXO-induced cardiotoxicity (Hadi et al., 2012). In support to this hypothesis, it has been demonstrated that DOXO induces release of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, *via* the activation of nuclear factor kappa-B (NF-κB), in the heart of rats treated with DOXO over a 2-weeks' timeframe (Abd El-Aziz et al., 2012). A relationship between TNF-α release and ROS increase in the failing hearts of patients with dilatative cardiomyopathy was found (Tsutamoto et al., 2001), and this could give an indication of the possible timeline that links inflammation and oxidative stress. Inflammatory cytokines have been shown to participate in several heart diseases in that they affect heart rate to a negative inotropic effect and induce deleterious left ventricular remodeling (Zhu et al., 2009). In this scenario, higher expression of inducible nitric oxide synthase (iNOS) in cardiomyocytes is responsible of high levels of cytosolic nitric oxide (NO), which lead to proinflammatory mediators release by the innate immune cells (Takimoto et al., 2002).

The most of studies report cardiotoxic effects of DOXO after chronic administration, however, a weakness in these studies is that structural cardiac damage is usually detected only when a functional impairment has already occurred, which leaves little room for early, preventive

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strategies (Cardinale and Sandri, 2010). Currently, the most commonly used methods to detect anthracycline-induced cardiotoxicity are the evaluation of functional parameters including the left ventricular ejection fraction and fractional shortening by echocardiography and radionucleotide imaging. Unfortunately, impairment in these parameters is often detected only after considerable cell loss has taken place (Mercuro et al., 2007). So, in recent years the biomarkers-enzymes, hormones, markers of cardiac stress and malfunction, as well as myocyte injury of inflammation, appear to have growing clinical importance and have become the subject of intense inquiry (Kinova et al., 2008). Anthracycline cardiotoxicity does not develop abruptly, however very little is known on the acute effects of DOXO administration. Some *in vitro* and *in vivo* models (Robert 2007; Polegato et al., 2015) indicate alterations in cardiac function after the first dose, but few data refer to the underlying molecular changes.

Inflammation seems to be much more serious in the acute DOXO-induced cardiotoxicity model (Zhu et al., 2011), so this study aimed to investigate the acute effect of DOXO administration on heart functionality, looking at the release of inflammatory cytokines in mice. Our results indicate that DOXO administration induces echocardiographic alterations of the main cardiac functional parameters, which are correlated to an increase in inflammatory cytokines production and iNOS expression in the heart of mice already after a single administration and after short-time treatment.

2. Materials and methods

2.1. Materials

DOXO was purchased from Baxter manufacturing S.p.a. (Officina di Sesto Fiorentino, Florence, Italy). Where not indicated otherwise, the products were purchased from Sigma (Italy).

2.2. Animals

Female C57BL/6j mice (weighting 20–22 g) were obtained from Charles River (Lecco, Italy) at 6 weeks of age. All animal experiments were conducted under protocols that followed the Italian and European Community Council for Animal Care (DL. no. 26/2014). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Salerno.

2.3. Experimental protocols

C57BL/6j mice were administered intraperitoneal DOXO (2 or 10 mg/kg). The doses used in this study were chosen according to what reported in literature (Imondi et al., 1996) and they correspond

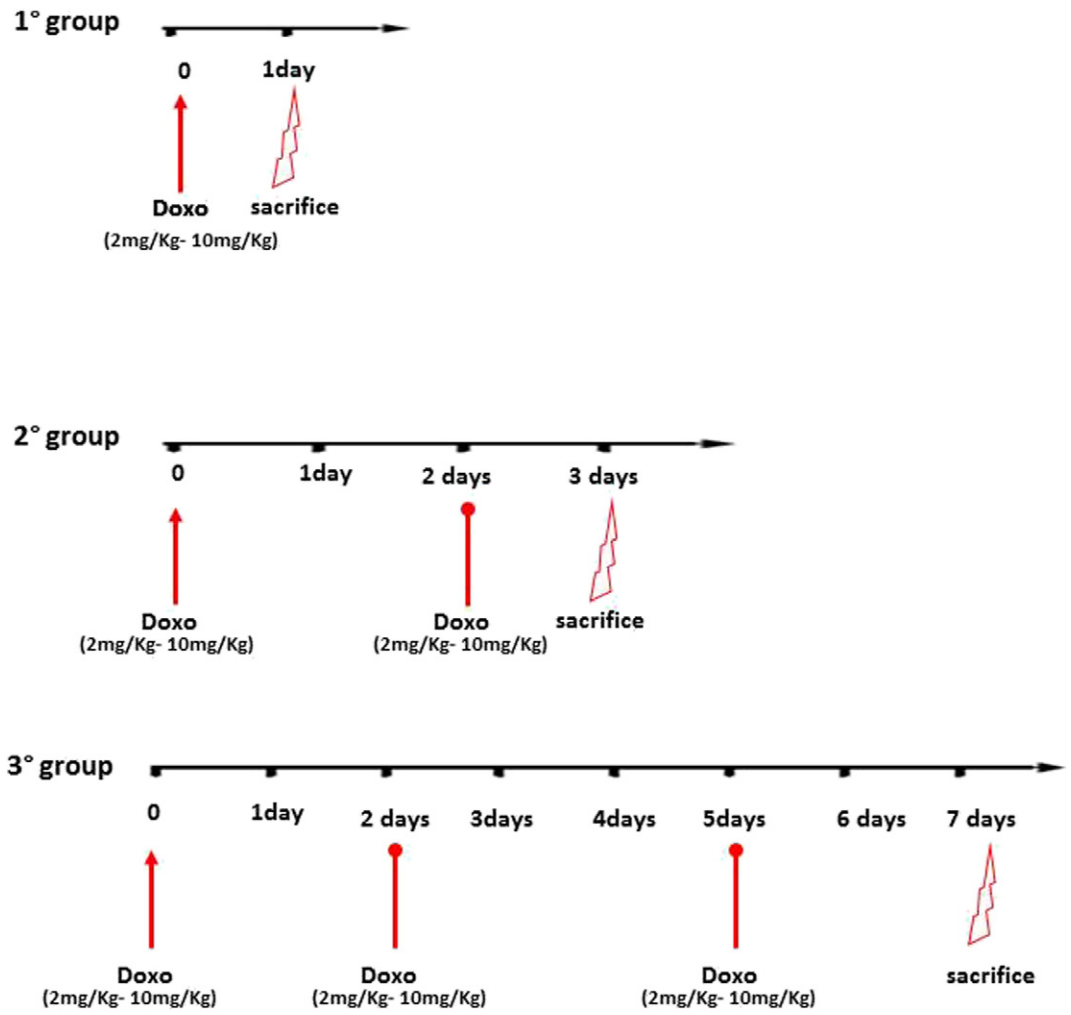

Fig. 1. C57BL/6j female mice were randomly divided into three groups. The 1st group received a single administration of DOXO (2 mg/kg and 10 mg/kg; i.p.) and was sacrificed after 24 h; the 2nd group received two administrations of DOXO (2 mg/kg and 10 mg/kg; i.p.) once every other day and was sacrificed 3 days after the first injection; the 3rd group received three administration of DOXO (2 mg/kg and 10 mg/kg; i.p.) once every other day and was sacrificed 7 days after the first injection. Control group received saline solution. At the end of experimental time, mice were sacrificed and the heart was removed and frozen for biochemical analysis.