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Exercise during pregnancy decreases doxorubicin-induced cardiotoxic effects on neonatal hearts

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ABSTRACT

Cancer treatment with Doxorubicin (DOX) is limited due its dose-dependent cardiotoxicity, mainly related to the oxidative stress production. In experimental models of DOX treatment exercise can be used as a beneficial adjuvant therapy. This work aimed to investigate the effects of exercise during pregnancy on DOX-induced cardiotoxicity in cardiomyocytes of progeny, examining the possible intergenerational cardioprotective effects of maternal exercise. For this purpose pregnant rats were divided in control and exercise groups and pre-treated during gestational days. Hearts of newborns were used to obtain a culture of cardiomyocytes to be treated with DOX for analyses of cell viability, apoptosis and necrosis; ROS production; DNA damage; SOD and CAT activities; and Sirt6 protein expression. The results showed that exercise during pregnancy induced an increase in the viability of neonatal cardiomyocytes and a decrease in DOX-induced apoptotic and necrotic death which were correlated to the decrease in ROS production and an increase in antioxidant defenses. Exercise also protected neonatal cardiomyocytes from DOX-induced DNA damage, demonstrating a reduction in the oxidative DNA breaks. Likewise, exercise induced an increase in expression of Sirt6 in neonatal cardiomyocytes. Therefore, these results demonstrate for the first time that exercise performed by mothers protects the neonatal heart against DOX-induced toxicity. Our data demonstrate the intergenerational effect of exercise in cardiomyocytes of progeny, where the modulation of oxidative stress through antioxidant enzymes, and DNA integrity via Sirt6, were induced due to exercise in mothers, increasing the resistance of the neonatal heart against DOX toxicity.

1. Introduction

The chemotherapeutic drug doxorubicin (DOX) is a highly effective anthracycline antitumor antibiotic, commonly used to treat many types of cancer (Datta et al., 2015). In affected cells, the cytotoxic action of DOX involves DNA intercalation and the formation of additional bonds between nitrogen bases of DNA strands, compromising the replication and transcription process. However, the use of DOX in the treatment of human tumors is limited due to its strong cardiotoxicity leading to a dose-dependent cardiomyopathy and heart failure (Smith et al., 2016; Volkova and Russell, 2011).

Multiple mechanisms are involved in the DOX-associated cardiotoxicity. The most commonly accepted mechanism is the iron-mediated formation of reactive oxygen species (ROS), which promotes myocardial oxidative stress (Cascales et al., 2012; Rochette et al., 2015). This ROS generation can cause damage to lipid membranes and nucleic acids, which together with reduced levels of antioxidants and sulfhydryl groups leads to the initiation of apoptosis in cells (Alexieva et al., 2014; Chao et al., 2011; Octavia et al., 2012). In addition, the mitochondria plays an essential role in the pathogenesis of DOX-induced cardiotoxicity due its function in the maintenance of myocardial tissue homeostasis. It has been demonstrated that DOX causes an impairment of mitochondrial

Abbreviations: DOX, doxorubicin; Sirt6, sirtuin6; HDAC, histone deacetylase; FPG, formamidopyrimidine DNA glycosylase; EndoIII, endonuclease III; PARP, poly-ADP-ribose polymerase; NAD^+, nicotinamide adenine dinucleotide; SOD, superoxide dismutase; ROS, reactive oxygen species; CAT, catalase; Annexin V-PE, annexin V-phycoerythrin; 7-AAD, 7-amino-actinomycin; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; FBS, fetal bovine serum; LMP, low melting-point; HMP, high melting-point; H_2DCF-DA, 2′-7′-dihydrodichorofluorescein diacetate; DCF, 2′-7′-dichorofluorescein; TB, trypan blue; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

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respiration (Carvalho et al., 2010; Lipshultz et al., 2016) and inhibition of the oxidative phosphorylation, which involves a metabolic remodeling between the aerobic fatty acid oxidation and the anaerobic glycolysis (Carvalho et al., 2010). Conversely, the stimulation of basal mitochondrial respiration is able to decrease DOX-induced apoptotic signaling in cardiomyoblasts (Deus et al., 2015). This indicates that the protection of mitochondrial function can be beneficial for counteracting the DOX-induced cardiotoxic effects (Pereira et al., 2011) that ultimately leads to cardiomyocyte cell death and consequent cardiovascular dysfunction.

Notwithstanding this, the prevention of DOX-induced cardiomyopathy is still unsolved and the only truly effective method to prevent its cardiotoxicity is a dose-limiting approach that may compromise its chemotherapeutic properties (Gratia et al., 2012). To reduce the cardiac side effects of DOX-treatment, researchers have investigated adjuvant therapies using animal models. Among a number of non-pharmacological strategies, physical exercise of different types and features has been studied. Both acute and chronic models of exercise trigger a preconditioning effect on DOX-treated rats that protects cardiac tissue and especially mitochondria against the drug-induced negative remodeling (Ascensão et al., 2012, 2011a, 2011b). Moreover, studies suggest that exercise acts against the damaging consequences of *in vivo* and *in vitro* DOX treatment on rodent hearts either by preventing or attenuating the toxicity, and these effects are mainly related to enhancement in the antioxidant defenses and the decrease of the ROS production and apoptosis (Ascensão et al., 2005a, 2005b; Marques-Aleixo et al., 2015). Regardless of the valuable action of exercise, the improvement of aerobic capacity in patients undergoing adjuvant therapy is small, due in part to low adherence to programs of exercise (Jones et al., 2011).

Exercise has preventive/therapeutic benefits on many pathological and physiological situations. During pregnancy, in the absence of obstetric complications, moderate exercise could be beneficial to the maternal–fetal unit and infant through improved physiological, metabolic and psychological parameters, along with reduced risk of morbidity and mortality (Marques et al., 2014; Melzer et al., 2010; Prather et al., 2012). Experimental models have demonstrated the beneficial effects of maternal exercise during pregnancy to offspring, such as the improvement of mitochondrial function and biogenesis in the brain of the offspring (Park et al., 2013), an increase in object recognition memory (Robinson and Bucci, 2014), and a decrease in the risk of mammary tumorigenesis (Camarillo et al., 2014). Moreover, it has also been shown that exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring (Laker et al., 2014), demonstrating the protective intergenerational effects of exercise by mothers.

In recent decades interest in a family of proteins named as sirtuins has increased because of their role as regulators involved in numerous cellular signaling pathways. Sirtuins are a family of seven members (Sirt1 to Sirt7) of nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylases implicated in the regulation of multiple pathophysiological processes, including oxidative stress, DNA damage repair, cell metabolism, apoptosis, tumorigenesis, neurodegeneration, and aging (Barreiro and Gea, 2015; Lawless et al., 2010; Morris, 2013). Of particular interest is the histone deacetylase Sirt6 as a scaffold protein in DNA damage repair. This histone deacetylase is a chromatin-bound protein that is rapidly recruited to sites of double strand breaks following DNA damage, acting on critical steps required for proper recruitment of downstream DNA damage repair factors and efficient repair (Cai et al., 2012; Toiber et al., 2013).

Deacetylation is the main function of sirtuins, however some of them have deacylase, adenosine diphosphate-ribosylase, demalonylase, glutarylase, and desuccinylase properties (Morris, 2013). Sirtuins can be activated upon exercise or caloric restriction, and control critical cellular processes in the nucleus, mitochondria and cytoplasm, such as the maintenance of metabolic homeostasis, reduction of cellular damage and inflammation, all of which protect against a range of age-related diseases, including cardiovascular pathologies. In cardiovascular diseases, sirtuins have gained interest by their protective effects. Particularly, Sirt6 is seen to play an important role in cardiovascular disease including cardiac hypertrophy, heart failure and myocardial hypoxic damage (Cai et al., 2012; Maksin-Matveev et al., 2015; Sundaresan et al., 2012). Moreover, previous experimental research demonstrated that exercise is able to change Sirt6 and Sirt1 levels in skeletal muscle of aged rats (Huang et al., 2016; Koltai et al., 2010a).

In view of the evidence about the protective effects of exercise on DOX-induced cardiotoxicity and the beneficial effects to offspring of exercise by mothers, this study aimed to investigate the effects of exercise during pregnancy on DOX-induced cardiotoxicity in the hearts of progeny, examining the possible intergenerational cardioprotective effects of maternal exercise.

2. Methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum (SFB) and penicillin/streptomycin were obtained from Gibco-BRL (Grand Island, NY, USA). Low melting-point agarose (LMP), normal melting-point agarose (NMP), 2′-7′-dihydrodichorofluorescein diacetate (H_2DCF-DA), 2′-7′-dichorofluorescein (DCF), trypan blue (TB), gelatin, bovine serum albumin (BSA), sodium dodecyl sulfate (SDS) and pancreatin were purchased from Sigma (St. Louis, MO, USA). Primary antibodies anti-sirt6 (ab62739) and anti-actin (C-2) (sc-8432) were purchased from Abcam (UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Annexin V-Phycoerythrin (PE) and 7-Amino-Actinomycin (7-AAD) were purchased from BD Biosciences (San Diego, CA). Formamidopyrimidine DNA-glycosylase (FPG) and endonuclease III (EndoIII) were obtained from BioLabs (New England, USA). All other reagents were of analytical grade and purchased from local commercial suppliers.

2.2. Ethical approval and animals

The research followed the ethical rules established by the Brazilian Guidelines for the Care and Use of Animals for Scientific and Didactic Purposes (DOU 27/5/13, MCTI, p.7). All procedures outlined in this study were approved by the Research Ethics Committee of the Federal University of Health Sciences of Porto Alegre (CEUA number 182/13).

Female (n = 16) and male (n = 8) albino Wistar rats (aged 4 weeks and weighing 70–100 g) from the Center for the Reproduction of Laboratory Animals of the UFCSPA, were kept under a day/night cycle (lights on 7:00 am to 7:00 pm), room temperature 23 °C ± 1, and 50% ± 5 relative humidity. Throughout the experiment the animals received a standard pellet diet (Nuvital CR1®, Paraná, Brazil) and tap water *ad libitum*.

2.3. Adaptation to treadmill exercise

Before mating, the females were acclimatized to the treadmill exercise (Motorized Treadmill for Rats, ANSprojects®). During 3 days, 10 min/day, animals underwent the recognition of a motorized horizontal treadmill (5 min, stationary) and running in a low intensity (5 min, 8 m/min, at 0° of inclination) (Kim et al., 2007). Subsequently, during 5 days, 10 min/day, females were