RESEARCH ARTICLE

Pretreatment Effect of Running Exercise on HSP_{70} and DOX-Induced Cardiotoxicity

Vahid Shirinbayan[&], Valiollah Dabidi Roshan*

Abstract

Objective: The purpose of this study was to determine pretreatment effects of moderate-term endurance training before the various dosages (10 and $20_{mg.kg}^{-1}$) of DOX on a heat shock protein (HSP_{70kda}) and cardiotoxicity in heart tissue. **Methods**: Forty-eight male rats were randomly assigned to nontraining (NT) and training (T) groups and three subgroups; $DOX_{10mg.kg}^{-1}$ and $DOX_{20mg.kg}^{-1}$ and saline treatment. The training program included treadmill running between 25-39 min/day and 15-17 m/min, 5 days/wk for 3 wk. **Result**: DOX administration, in particularly with $20_{mg.kg}^{-1}$, caused up-regulation of oxidants and cardiac damage (MDA, CK, CPK-MB and CK/CPK-MB) and down-regulation of cardioprotection (HSP_{70}, SOD) markers, as compared to NT+saline group. Pretreatment effect of treadmill running endurance exercise in the presence of DOX with $10_{mg.kg}^{-1}$ caused a significant increase in HSP_{70}, SOD and a significant decrease in MDA and insignificant decrease in CK, CPK-MB and CK/CPK-MB, in comparison T+DOX_{10} with NT+DOX_{10} group. However, there was no significant difference between T+$DOX_{10mg.kg}^{-1}$ and T+$DOX_{20mg.kg}^{-1}$ in the aforesaid markers. **Conclusion**: Dox-induced cardiotoxicity is related to oxidative stress. Our study suggests that pretreatment with endurance exercise may be considered as a potentially useful strategy to improve myocardial tolerance against single dose DOX-induced oxidative damage.

Keywords: Doxorubicin - cancer - cardiotoxicity - endurance exercise

Introduction

Cancer is the subject of intense research around the world and many questions about how the disease works remain unanswered. In recent years, chemotherapeutic drugs are widely used for the treatment of cancer (Rios et al., 2012). One of the mostly used chemotherapeutic drugs is the highly effective anthracycline Doxorubicin (DOX). However, its clinical use is limited by the dose-related and cumulative cardiotoxicity and consequent dysfunction (Kavazis et al., 2010; Visw et al., 2012). It is believed that oxidative stress and the formation of free radicals, which also involves a reaction of DOX with iron, play a crucial role in the mechanism of DOX toxicity (Rade et al., 2008; Jovanka et al., 2009). DOX is significantly toxic to most tissues and organs, but its cardio and hepatotoxicity are limiting factors in the cancer therapy with this agent (Rašković et al., 2011). It`s particularly toxic to heart tissue and constitutes a major cause of morbidity and mortality in cancer patients (Ammaret al., 2011). In fact, the weakness of the heart to oxidative damage may be in part explained by the fact that heart demonstrates a slow turnover and relatively lower levels of antioxidant enzyme activity when compared to most other tissues such as liver (Ascensao et al., 2005; Babaei et al., 2008; Fei et al., 2011). Many researchers have tried to find ways to reduce the adverse effects associated with DOX therapy (Rašković et al., 2011). Several strategies for detecting and preventing cardiotoxicity have been developed, some of which are more effective than others. Including limiting cumulative dose, altering anthracycline administration, using anthracycline analogues, adding cardioprotectants to the regimen and employing nutritional supplements (Wouters et al., 2005). Prior endurance exercise as a nonpharmacological strategy may be possible that promotes defense against DOX cardiotoxicity (Hydock et al., 2008; 2011; Wonders et al., 2008). Although the mechanism(s) by which exercise training protects cardiomyocytes against these myocardial insults remains unknown (Kavazis et al., 2010). It has been speculated that further stress induced by acute exercise could be potentially detrimental (Douglas., 2008). Some side-effects of physical training are ascribed to reactive oxygen species production. Consumption of large amounts of oxygen in endurance athletes increases the production of the reactive oxygen species (ROS) and leads to the oxidative stress (Bulduk et al., 2011; Feairheller et al., 2011; Jakovljević et al., 2011; Kruk., 2011). In the other hand, some researchers have reported that internal mechanisms were activated by regular exercise to remove oxidatively modified proteins and/or that antioxidant systems were better able to remove reactive oxygen

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species from the circulation (Trapp et al., 2010). To our knowledge, there are few studies dealing with the pretreatment effect of moderate-term endurance training on Dox-induced cardiotoxicity and oxidative stress in heart tissue.

Heat shock proteins (HSPs) are highly conserved proteins that are expressed both constitutively and under stressful conditions. In particular, those in the 70 kDa family are released from various cell types including cancer cells (Ogawa et al., 2011). HSPs playing an essential role in assistance to maintain and regain cellular homeostasis (Bayer et al., 2006). Although, researchers have stated moderate intensity exercise training is effective in preceding the increase of HSP_{70} (Zong-Yan et al., 2011). The preventive and pretreatment role of regular endurance exercise as an antioxidant factor on HSP_{70} in heart has not been sufficiently studied. Also, while most recent studies have focused on the treatment effect of endurance exercise in induce DOX cardiotoxicity (Chicco et al., 2006; Hydock et al., 2008; Kavazis et al., 2010; Ascensão et al., 2011). We are the first to investigate the cardioprotective effects of prior (pretreatment) treadmill running endurance exercise on HSP_{70} and doxorubicin-induced cardiotoxicity with various dosages. The hypothesis proposed was that if DOX cardiotoxicity are related to free radical formation and oxidative stress, an enhancement in antioxidant/oxidation ratio after regular endurance exercise may protect against DOX-induced toxicity in the heart. Therefore, the purpose of this study was to determine pretreatment effects of endurance exercise on heat shock protein (HSP_{70kda}) - as a cardioprotection protein-, superoxide dismutase (SOD)- as an enzymatic antioxidant-, cardiac isoenzyme of creatine phosphokinase (CPK-MB), total creatine kinase (CK) and malondialdehyde (MDA)- as biomarkers related to cellular oxidative damage-, after a multi-dose administration of DOX.

Materials and Methods

Experimental Design and Laboratory Environment; All experiments were approved by department of physiology, university of Mazandaran and Use Committees before their initiation and followed guidelines established by the Council of the American Physiological Society for the use of animals in research.

Forty-eight wistar male rats (10 week old, 257±28 g body wt at the beginning of the experiments) were purchased from the laboratory of animal bearing and multiplying at the Pasture institute of Iran and housed in collective cages (4 per cage) in a room at normal atmosphere (21-22°C, 50-60% humidity) in a 12:12-h light-dark cycle. Animals were allowed free access to standard laboratory food (daily regimen of 10 g per 100 gr body weight for each rat) and water (ad libitum). The animals were randomly divided into two groups: training (T) and nontraining (NT). Animals were habituated to treadmill running for one week (once a day for 10 min/session at 10 m/min, 0% grade).

Exercise Training Program and Subjects Classification; Subjects in the training group were assigned to perform a regular aerobic treadmill running which consisted of
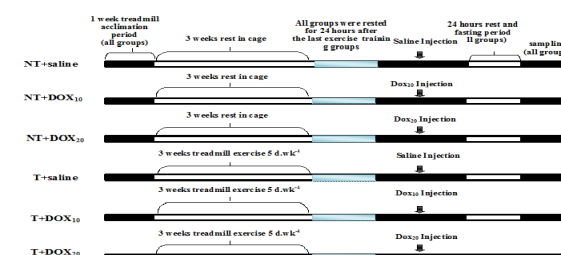
5 minutes of warm-up running at five minutes before the start of training and then 25-39 min/session and 15-17 m/min with zero slope, 5 days/week for 3 week (Table 1 shown the over the course of training intensity during the protocol). This protocol pattern is Dabidi et al. (2011). All groups were rested for 24 hours after the last session exercise of training groups and then anew randomization of them into subgroups as: nontraining+Saline(NT+saline); nontraining+$DOX_{10mg.kg}^{-1}$(NT+DOX_{10}); nontraining+$DOX_{20mg.kg}^{-1}$(NT+DOX_{20}); treadmill running exercise+saline(T+saline); treadmill running exercise+DOX_{10}(T+DOX_{10}) and treadmill running exercise+DOX_{20}(T+DOX_{20}) performed. There were eight rats in each group (Figure 1 shows the process of doing research protocol).

DOX treatment: DOX was obtained from EBEWE Pharma Ges.m.b.H.Nfg.KG (A-4866 unterach, Austria) as a vial of ph. Eur. In order to bring the drug concentration of 10 and 20 mg.kg^{-1}, it was dissolved in 0.9% saline for administration. The dose 20 mg.kg^{-1} of DOX is human clinical doses that are pharmacologically scaled for use in rats (Ogawa et al., 2011). Saline was used as the vehicle and the placebo treatment and was used to form saline solution (0.9% NaCl ip).

Heart Tissue collection and preparation; Rats in all groups were anesthetized with ketamine and xylazine after 10-12 hours overnight fasting and placed in the supine position. The abdominal cavity was opened to expose the left ventricle and a 2 ml blood sample was collected in a tube. Then hearts were rapidly excised, rinsed, carefully dried, weighed and it was placed into Petri dishes containing cold isolation medium (0.1 mol/L K2HPO4, 0.15 mol/L NaCl, pH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC. Heart samples homogenized in a homogenization buffer (0.05 M Tris, 0.03 M L-serine, and 0.06 M boric acid, pH 7.6; 100 mg of tissue/ml of buffer) 27. 5 ml/g of tissue with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma Aldrich, St. Louis, USA) 100 ul/1 ml, and 10 mMtris base (Sigma-Aldrich, St.

Table 1. Exercise Training Protocol in the Current Study

Training sessions and variables	Weeks of endurance exercise		
(m.min^{-1}/min)	1	2	3
1. Speed/Time	15/25	16/30	17/35
2. Speed/Time	15/26	16/31	17/36
3. Speed/Time	15/27	16/32	17/37
4. Speed/Time	15/28	16/33	17/38
5. Speed/Time	15/29	16/34	17/39

Figure 1. Research Protocol in the Present Study

Louis, U.S.A), pH 7.4 and centrifuged at 1500 g at 4ºC for 15 min. Heart supernatant was diluted 1:30. Plasma was diluted 1:10 homogenized in doubly distilled water.

Homogenates were centrifuged (2 min at 2,000 g, 4°C) to eliminate cellular debris, and the resulting supernatant was stored at liquid nitrogen (-80°C) for later determination of HSP$_{70}$, SOD, MDA (cardiac ventricle), and the blood sample was first centrifuged by a refrigerated centrifuge at 3,000 rpm for 15 minutes within 30 minutes of collection and serum was separated then stored at -80 C before assay biochemical estimations of total CK and CPK-MB.

Biochemical analysis: Heat shock protein (HSP$_{70}$) in heart was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cusabio biotech co., LTD). In summary, 100 μl of standard, Blank, or Sample added per well. The liquid removed of each well and 100 μl of Biotin-antibody working solution added to each well. Then, aspirated each well and washed and repeated these step for three times. In addition, 100 μl of HRP-avidin working solution added to each well and the aspiration and washing repeated five times as step 4. Moreover, 90 μl of TMB Substrate added to each well. Also, 50 μl of stop solution added to each well when the first four wells containing the highest concentration of standards develop obvious blue color. Finally, the optical density of each well was determined within 30 minutes; a microplate reader set to 450 nm was used. Furthermore, the levels of SOD activity and MDA content in supernatants were evaluated with the superoxide dismutase assay kit (Cayman chemical campany) using the method described by Dabidi et al. (2011). Moreover, thiobarbituric acid reactive substances (TBARS) using the method of Dabidi et al. (2011), respectively. Also, total CK and CPK-MB in serum were measured using an CK-NAC, DGKC/IFCC method by creatine kinase kit(darman kave-Iran) and photometric method by CPK-MB kit (pars azmoon-Iran) Respectively.

Statistical analysis

All data have been expressed as mean±SD. Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Data of the biomarkers related to the cardioprotective system and cardotoxicity were normally distributed after log-transformation. A one-way analysis of variance (Statistics software, StatSoft, Inc., Tulsa, OK) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various biomarkers between groups. Differences were considered statistically significant at p-value<0.05.
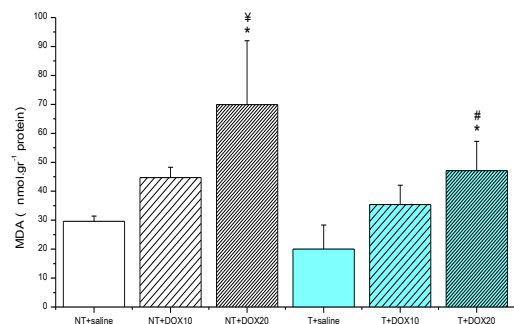
Results

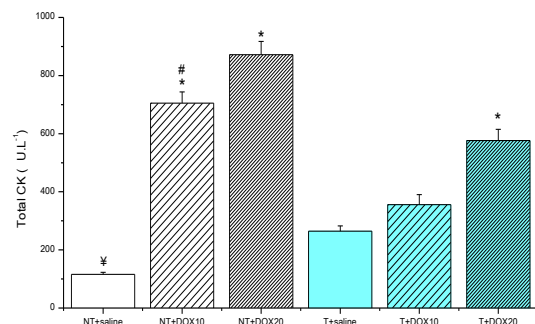*The effect of DOX and pretreatment with endurance exercise on biomarkers related to cardiotoxicity*

Figures of 2, 3, 4 and 5 shows changes in biomarkers related to cardiotoxicity, in the rats exposed to DOX-induced oxidative stress. While, administration of DOX with dosage $10_{mg.kg}{}^{-1}$ led to insignificantly increase in MDA level (51%), administration of DOX with dosage $20_{mg.kg}{}^{-1}$ caused a significantly increase in MDA level

(136.1%), as compared to NT+saline group (Figure 2). Furthermore, the administration of DOX in $10_{mg.kg}{}^{-1}$ and $20_{mg.kg}{}^{-1}$ caused a significant increase in total CK (510.4% and 653.6%, respectively) and CPK-MB (472.7%, 482.9%, respectively), in comparison with NT+saline group (Figures 3, 4). However, the administration of DOX in dose $10_{mg.kg}{}^{-1}$ and $20_{mg.kg}{}^{-1}$ led to insignificantly increase in total CK/CPK-MB ratio (155.6%, 272.7%, respectively), as compared to NT+saline group (Figure 5). Although, there was no significant difference between NT+DOX$_{10}$ and NT+DOX$_{20}$ treatments in total CK and CPK-MB levels and total CK/CPK-MB ratio, there was a significant difference between NT+DOX$_{10}$ and NT+DOX$_{20}$ treatments in MDA levels.

In the other hand, 3 week of endurance exercise led to an insignificantly decrease (67.6 %,) in MDA, in comparison with NT+saline group. In addition, pretreatment of endurance exercise led to an insignificantly increase in total CK, CPK-MB and total CK/CPK-MB ratio, in comparison with NT+saline group(128.4%, 70.6% and 69%, respectively). However, DOX20 injection caused a further increase in the MDA, total CK, CPK-MB and total CK/CPK-MB ratio, in comparison with NT+DOX$_{10}$ group (56.3%, 23.6%, 1.7% and 46.4%, respectively). In addition, pretreatment effect of treadmill running exercise

Figure 2. Malondialdehyde (MDA) Level After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups. *significantly different with the NT+saline group (P<0.05), #significantly different with similar dose of DOX in nontraining (NT) groups (P<0.05), ¥significantly different with similar activity status (NT or T) and $10_{mg.kg}{}^{-1}$ dose of DOX (P<0.05)
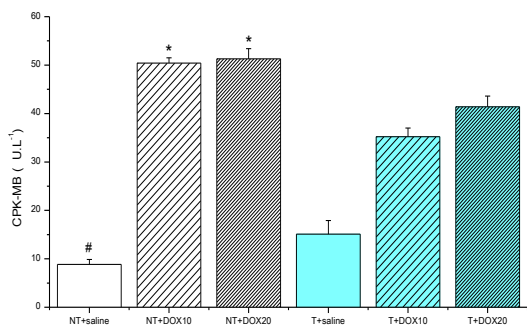
Figure 3. Total Creatine Kinase (CK) Level After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups. *significantly different with the NT+saline group (P<0.05), #significantly different with similar dose of DOX in nontraining (NT) groups (P<0.05), ¥significantly different with similar activity status (NT or T) and $10_{mg.kg}{}^{-1}$ dose of DOX (P<0.05).

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in the presence of the $10_{mg.kg}^{-1}$ dose of DOX caused an significant decrease in total CK (98.2%,), an insignificant decrease in MDA and CPK-MB (26.2% and 43.1%, respectively) and an insignificantly increase in total CK/CPK-MB ratio (17.3%), as compare to NT+DOX$_{10}$ group. Also, as compared with the T+DOX$_{20}$ group and with NT+DOX$_{20}$ group , it was found that pretreatment effect of treadmill running exercise caused a significant decrease in MDA (48.4%) and insignificant decrease in total CK, CPK-MB and total CK/CPK-MB ratio levels (51.3%, 23.9% and 17.8%, respectively). Moreover, result shown that in the presence of treadmill running aerobic exercise, the administration of dose $20_{mg.kg}^{-1}$ led to an insignificantly increase in MDA, total CK, CPK-MB and total CK/CPK-MB ratio, in T+DOX$_{20}$ group to comparison with T+DOX$_{10}$ group(33%, 61.9%, 17.6% and 5.4%, respectively).
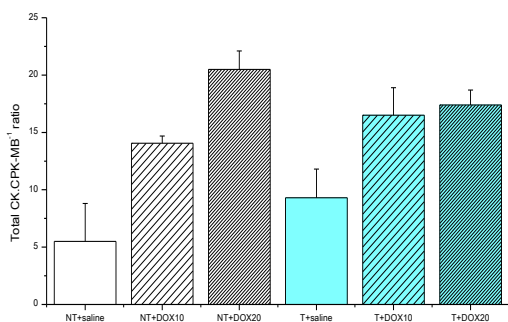
The effect of DOX and pretreatment with endurance exercise on biomarkers related to cardioprotection

Changes in biomarkers related to biomarkers related to cardioprotection, in the rats exposed to DOX-induced cardiotoxicity showed in Figures 6 and 7. DOX administration ($20_{mg.kg}^{-1}$) lead to a significantly increase in HSP$_{70}$ levels (30.4%), as compared to NT+saline group (Figure 6). After administration of DOX, both 10 and $20_{mg.kg}^{-1}$, a significantly decrease in SOD levels (9.4% and 22.5%, respectively), were detected, as compared to NT+saline group (Figure 7).

Three weeks of the treadmill running endurance

Figure 4. Cardiac Isoenzyme of Creatine Phosphokinase (CPK-MB) Level After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups.*significantly different with the NT+saline group (P<0.05), #significantly different with similar dose of DOX in nontraining (NT) groups (P<0.05)
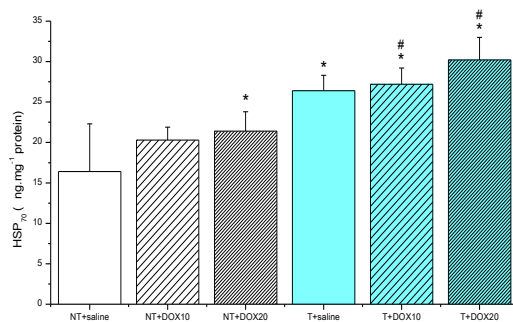

Figure 5. Amounts of Total CK/CPK-MB Ratio After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups
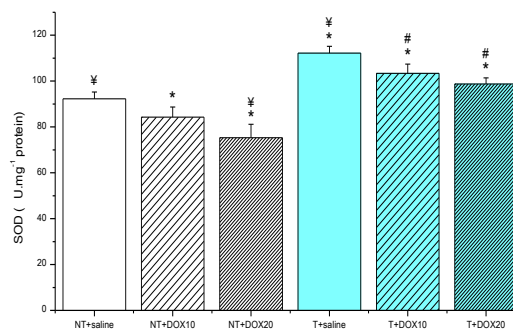
training led to a significantly increase of heart HSP$_{70}$ levels and an insignificantly increase in SOD levels (60.9% and 21.5%, respectively), in comparison with NT+saline group. However, after 3 weeks of treadmill running endurance training and DOX treatment with 10 mg.kg^{-1}, a significantly increase in SOD (11.9%), and an insignificantly increase in HSP$_{70}$ (5.4%), were detected in comparison with NT+DOX$_{10}$ group. In contrast, 3 weeks of treadmill running endurance training and DOX treatment with 20 mg.kg^{-1} resulted in a significantly increase in HSP$_{70}$ and SOD (41.1%, 31%, respectively); in T+DOX$_{20}$ group as comparison to NT+DOX$_{20}$ group. Also, pretreatment effect of treadmill running endurance exercise in the presence of DOX treatment with 10 mg.kg^{-1} caused a significant increase in HSP$_{70}$ and SOD in T+DOX$_{10}$ to comparison with NT+DOX$_{10}$ group (33.9% and 22.6%, respectively). However, there was no significant difference between T+DOX$_{10}$ and NT+DOX$_{20}$ groups in HSP$_{70}$ and SOD levels.

Discussion

Although, previous studies have focused on role physical activity as a nonpharmacological strategy in various cancers, we are the first to investigate the pretreatment effect of moderate-term endurance

Figure 6. Heat Shock Protein (HSP$_{70}$) Level After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups.* significantly different with the NT+saline group (P<0.05), # significantly different with similar dose of DOX in nontraining (NT) groups (P<0.05)

Figure 7. Superoxide Dismutase (SOD) Level After 3 Weeks of Aerobic Training and DOX Administration. Data are presented as the Mean±SD for 8 Rats in all groups.* significantly different with the NT+saline group (P<0.05), # significantly different with similar dose of DOX in nontraining (NT) groups (P<0.05), ¥ significantly different with similar activity status (NT or T) and $10_{mg.kg}^{-1}$ dose of DOX (P<0.05)

training before the various dosages (10 and $20_{mg.kg}{}^{-1}$) of Doxorubicin (DOX) on markers of related to cardiotoxicity and heat shock proteins (HSP$_{70kda}$) in heart tissue. Our study demonstrated, although, $10_{mg.kg}{}^{-1}$ of DOX induced myocardial damage in rats, which was characterized by increased heart MDA and serum total CK, CPK-MB and total CK/CPK-MB ratio, but a significantly increase in MDA, total CK, CPK-MB were detected following $20_{mg.kg}{}^{-1}$ of DOX. While, DOX treatment, both 10 and $20_{mg.kg}{}^{-1}$, induced a significantly decrease SOD in cardiac tissue, a significantly increase in HSP$_{70}$ was detected after administration $20_{mg.kg}{}^{-1}$ of DOX, only. The results indicate that there is a potent relationship between oxidative stress and DOX-induced cardiotoxicity.

Doxorubicin/Adriamycin (Dox) is an effective chemotherapeutic agent for treatment of many kinds of cancers. However, its clinical usefulness is still restricted due to its specific toxicities to cardiac tissues (Kavazis et al., 2010; Visw et al., 2012). The clinical utility of DOX is marred by an increased risk of myocardial injury, which is mainly caused by reactive oxygen species from DOX disposition (Menna et al., 2010; Fei et al., 2011). In other word, the possible mechanisms proposed for cardiotoxic effects of Dox include free radical induced myocardial injury, lipid peroxidation and cellular toxicity (Visw et al., 2012; Fei et al., 2011). Because of the relatively lower levels of the antioxidant defences in the cardiomyocytes, heart is more susceptible to oxidative damage than other tissues (Ascensao et al., 2005; Babaei et al., 2008; Fei et al., 2011). In this regard, the present study revealed severe biochemical changes as well as oxidative damage in the cardiac tissue after treatment with $10_{mg.kg}{}^{-1}$ and $20_{mg.kg}{}^{-1}$ of Dox. The mechanism of cardiotoxicity induced by Dox is not clearly known from the present study, although large body of evidence indicates toward the formation of oxygen free radicals, which can damage cells by lipid peroxidation (Fei et al., 2011; Visw et al., 2012). However, in rat treated with Dox of $20_{mg.kg}{}^{-1}$, we found significant increase in heart tissue MDA levels suggesting increased lipid peroxidation. In addition, cardiac tissue damage may be due to increased oxidative stress and depletion of antioxidants similarly in rats reported earlier. In our study, Dox treated rats (both $10_{mg.kg}{}^{-1}$ and $20_{mg.kg}{}^{-1}$) showed an increase in serum total CK, CPK-MB and total CK/CPK-MB, with decrease in level of SOD, which confirms the oxidative stress and cardiac damage.

On the other hand, regular treadmill running endurance exercise prevented the Dox-induced changes in HSP$_{70}$, SOD, MDA levels and enzymes (total CK, CPK-MB and total CK/CPK-MB levels). In the present study, a significant increase in the HSP$_{70}$ and SOD activity and a decrease in lipid peroxidation in heart tissue of T+DOX treated groups, suggests the protective and pretreatment effect of endurance exercise in DOX-induced cardiotoxicity. The current study provided additional support to understand how regular physical exercise, particularly treadmill running training, could contribute to augmentation of cardiac muscle resistance against free radical-induced cardiotoxicity induced by DOX administration. Two lines of evidence can be emphasized from the present study. First and considering cardiac stress markers, namely total CK, CPK-MB, total CK/CPK-MB, treadmill running endurance exercise decreased the rise of cardiac disturbances induced by an acute dose of DOX administration, in particularly with dosage of $20_{mg.kg}{}^{-1}$. Second, and according to changes observed in cardioprotection system responses (SOD and HSP$_{70}$) in both nontrained and trained rats hearts treated with 10 and $20_{mg.kg}{}^{-1}$ of DOX, it is likely that these systems might be considering as essential cellular defense against free radical-based cardiotoxicity caused by DOX, providing enhanced tolerance to trained myocardium at least in the first 48 h after the end of training period. In this study, DOX treatment with 10 and $20_{mg.kg}{}^{-1}$ caused a lower rise in myocardial total CK, CPK-MB and total CK/CPK-MB release to the serum in the T+DOX group compared to NT+DOX groups. These results are in accordance with many other reports in which DOX induced increased heart lipid peroxidation by-products either throughout 24-48 hours after DOX administration8. Although regular treadmill running endurance exercise did not significantly decrease the rise in cardiac MDA level in DOX treated rats (NT+DOX groups vs. T+DOX groups), a declining trend was apparent in T+DOX groups suggesting that regular exercise may have a protective effect against ROS induced oxidative stress by DOX administration.

The other important finding in the present study was that after DOX administration ($20_{mg.kg}{}^{-1}$), a significant increase in the HSP$_{70}$ activity in heart tissue of T+DOX treated groups were found. The mechanistic link between doxorubicin-induced oxidative stress and cardiac muscle up-regulation of HSP$_{70}$ remains unclear. Moreover, although current data demonstrate that exercise training protects the heart against Dox-induced damage (Ascensao et al., 2005, Chicco et al., 2006); the mechanism(s) by which exercise training protects cardiomyocytes remain unclear. There were three possible pathways to explain the protective effects of regular endurance exercise against DOX-induced cardiotoxicity. At present, the principal mechanism of Dox-induced cardiotoxicity is believed to be increased oxidant production by the mitochondria (Ascensao et al., 2005; Chicco et al., 2005; 2006; Kavazis et al., 2010). Our data indicate that Dox administration, in particularly with $20_{mg.kg}{}^{-1}$, increased ROS production in cardiac tissue. In addition, an interesting finding in the present study that may provide further insight into the effects of Dox on the myocardium was a slightly increase in HSP$_{70}$ protein content in the heart of the Dox-treated rats, as compared to NT+saline group. In contrast, regular endurance exercise lead to significant increase in the HSP$_{70}$ and SOD activity and decrease in lipid peroxidation in heart tissue of T+DOX treated groups. Induction of HSP$_{70}$ is known to occur in myocardial tissue following exercise training and is associated with the exercise-induced preservation of cardiac function during states of oxidative stress (Kavazis et al., 2009; 2010). Primary functions of HSPs include: nontraining of protein folding, prevention of denaturation and aggregation of intracellular proteins during stress, acceleration of the breakdown of damaged proteins, and serving as a molecular chaperone (Powers et al., 2001; Ascensao et al., 2005; 2011; Kavazis et al., 2010). Other putative effects of HSP$_{70}$ include

protection against apoptosis, protection against oxidative damage, maintenance of cellular calcium handling, and preservation of mitochondrial integrity in cardiac tissue exposed to a variety of oxidative stressors (Ascensao et al., 2005; 2011; Powers et al., 2011). Regardless of its role related to cardioprotection, HSPs overexpression can be undoubtedly interpreted as an acute sign of cellular stress (Ascensao et al., 2005). Hence, given the vast protective properties of HSP_{70}, we hypothesized that exercise training-induced increases in myocardial HSP_{70} levels play a required role in exercise training-induced cardioprotection against Dox-mediated cardiac injury (Chicco et al., 2006).

In summary, we conclude that the cardiotoxicity induced by Dox is related with oxidative stress. In addition, the present investigation provides new insights into the biochemical mechanisms by which pretreatment of endurance exercise through its potent antioxidant properties, protects cardiac muscle tissue against the toxicity induced by DOX. Moreover, endurance training-induced HSP_{70} up-regulation could also be considered as another possible strategy involved in the cardiomyocyte protection against DOX. Finally, our study suggests that pretreatment of endurance exercise may be considered as a potentially useful candidate for improve myocardial tolerance against single dose of DOX-induced oxidative damage.

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