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Acute Exercise Protects Against Doxorubicin Cardiotoxicity

Karen Y. Wonders, PhD, David S. Hydock, PhD, Carole M. Schneider, PhD, and Reid Hayward, PhD

Numerous methods have been used to minimize the cardiotoxic effects of the chemotherapeutic agent doxorubicin (DOX), and most have had limited success. Chronic endurance exercise has been shown to protect against DOX cardiotoxicity, but little is known regarding the effects of acute exercise on DOX-induced cardiac dysfunction. *Purpose.* The purpose of this study was to determine the effects of a single bout of acute endurance exercise on the cardiac dysfunction associated with DOX treatment. *Methods.* Male Sprague-Dawley rats either performed an acute exercise bout on a motorized treadmill for 60 minutes at a maximal speed of 25 m/min with a 5% grade (EX) or remained sedentary (SED) 24 hours before receiving either a 15-mg/kg DOX bolus dose or saline (SAL). Cardiac function was then analyzed 5 days post injection using a Langendorff isolated perfused heart model. In addition, myocardial lipid peroxidation was analyzed as an indicator of oxidative stress. *Results.* Doxorubicin treatment alone (SED+DOX) promoted a significant decline in end-systolic

pressure (−35%), left ventricular developed pressure (−59%), and the maximal rate of left ventricular pressure development (−43%) as well as a 45% increase in lipid peroxidation products when compared with SED+SAL ($P < .05$). Acute exercise 24 hours before DOX treatment, however, had a cardioprotective effect, as end-systolic pressure, left ventricular developed pressure, and the maximal rate of left ventricular pressure development were significantly higher in EX+DOX compared with SED+DOX ($P < .05$) and EX+DOX had similar levels of lipid peroxidation products as SED+SAL. *Conclusions.* An acute exercise bout performed 24 hours before DOX treatment protected against cardiac dysfunction, and this exercise-induced cardioprotection may partly be explained by a reduction in the generation of reactive oxygen species.

Keywords: cancer, cardiac function, chemotherapy, physical activity, preconditioning.

Doxorubicin (DOX) is a highly effective anthracycline antibiotic used in the treatment of breast, bladder, lung, and thyroid carcinomas as well as Hodgkin's disease and non-Hodgkin's lymphomas. Unfortunately, the occurrence of a dose-related cardiomyopathy limits the clinical use of DOX. DOX treatment is associated with acute cardiotoxicity that develops within minutes of intravenous administration and is marked by vasodilatation, hypotension, tachycardia, and cardiac arrhythmias. Although these symptoms are reversible, this early-onset cardiotoxicity has been shown to be predictive of future heart failure.^{1,2} DOX treatment also significantly compromises both systolic and diastolic cardiac function in several animal models, which may be manifested within days^{3,4} of DOX exposure and can be maintained for

weeks or months following treatment.⁵ This chronic DOX cardiotoxicity often presents as dilated cardiomyopathy and is characterized by hypotension, cardiac dilation, and decreased left ventricular ejection fraction.

Efforts have been made to explore alternative therapies to reduce DOX-induced cardiac dysfunction, such as the use of slow infusions,⁶⁻⁸ antioxidants (probenecol,^{9,10} ubiquinone,¹¹ ambroxiron,¹² α -lipoic acid,¹³ *P*-coumaric acid,¹⁴ and melatonin¹⁵), iron chelators,^{16,17} and drug-encapsulated liposomes.¹⁸ However, these strategies provide limited improvements in cardiac function and in some instances may have additional negative side effects for cancer patients.

The benefits of chronic aerobic exercise training on the cardiovascular system are well established. Regular aerobic exercise has been shown to reduce the risk of heart disease, control hypertension, and protect the heart against injury caused by oxidative stress and apoptosis. In addition, as little as 1 or 2 exercise bouts have been shown to preserve cardiac function and protect the heart against oxidative stress.^{19,20} Although a growing body of evidence indicates that regular aerobic exercise before DOX treatment can alleviate its negative cardiac side effects,^{3,21-24} it has yet to be determined whether acute exercise can protect the heart against the cardiac dysfunction that accompanies DOX treatment.

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To our knowledge, only 2 studies have been conducted that specifically address the effects of acute exercise on DOX-induced cardiotoxicity. Ji and Mitchell²⁵ reported that DOX administration after a single bout of acute exhaustive exercise reduced the exercise-induced increase in cardiac mitochondrial respiration. In an early report, Combs et al²⁶ hypothesized that an acute bout of exhaustive exercise after DOX treatment (18-23 mg/kg) would exacerbate the drug-induced cardiotoxicity by decreasing survival rate. Results indicated that the survival rate was actually increased in those animals that had been subjected to exhaustive exercise after DOX treatment. However, neither study attempted to assess cardiac function in the context of acute exercise. Therefore, the purpose of this study was to determine whether a single acute bout of exercise performed before DOX administration would protect against DOX-induced cardiac dysfunction.

Materials and Methods

Animal Care

Male Sprague-Dawley rats were obtained from Harlan Sprague Dawley, Inc (Indianapolis, Ind). All animals were housed 2 per cage in a temperature-controlled facility, were provided standard rat chow and water ad libitum, and were adapted to a 12/12-hour light/dark cycle. All protocols were approved by the University of Northern Colorado Institutional Animal Care and Use Committee and were in compliance with Animal Welfare Act guidelines.

Acute Exercise Protocol

Animals were randomly assigned to 4 experimental groups: exercise + DOX (EX+DOX), sedentary + DOX (SED+DOX), EX + saline (EX+SAL), and SED+SAL. Animals in the exercise groups were habituated to treadmill exercise over a 2-week period. In the first week of acclimation, rats ran 10 min/d, 3 d/wk at a speed of 10 m/min and 0% grade. During the second week, animals ran 10 min/d, 3 d/wk at a speed of 20 m/min and 0% grade. This acclimation protocol has been shown to have minimal effect on mitochondrial enzymes. Twenty-four hours after the acclimation protocol, animals completed a single acute bout of exercise. The 60-minute acute exercise bout was divided into 3 phases. During phase 1 (minutes 0-5) animals ran at 15 m/min and a 0% grade. During phase 2 (minutes 5-10) animals ran at 23 m/min and a 0% grade. During phase 3 (minutes 10-60) animals ran at 25 m/min and a 5% grade. Animals in both sedentary groups were placed on a non-moving treadmill belt for 60 minutes.

Administration of DOX

Twenty-four hours following the completion of the acute exercise bout or sedentary period, animals in the EX+DOX

and SED+DOX groups were treated with a 15-mg/kg intraperitoneal bolus of DOX (Bedford Labs, Bedford, Ohio). Animals in the EX+SAL and SED+SAL groups received a single 0.5-mL intraperitoneal injection of 0.9% sterile saline.

Isolated Heart Preparation

Five days following SAL or DOX treatment, cardiac function was determined in isolated hearts using a constant-pressure Langendorff perfusion preparation. All rats were anesthetized with 50 mg/kg sodium pentobarbital (Sigma Chemical, St. Louis, Mo) administered intraperitoneally along with 500 U of sodium heparin serving as an anticoagulant. The heart was rapidly excised, and the ascending aorta was cannulated for retrograde perfusion with a Krebs bicarbonate buffer consisting of 120 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl, 25 mM NaHCO₃, 17 mM glucose, and 0.5 mM EDTA. Buffer was saturated with 95% O₂/5% CO₂ and maintained at 37°C in a water-jacked reservoir suspended above the isolated heart, providing constant pressure of 80 mm Hg. To assess left ventricular function, a microtip catheter pressure transducer (SPR-671; 1.4F catheter; Millar Instruments Inc, Houston, Tex) was inserted into the left ventricular (LV) cavity via the mitral valve for determination of LV pressures. Following a 15-minute equilibration period, hearts were paced at 300 beats/min via the atria using a stimulus isolator in conjunction with a PowerLab/8e system (ADInstruments, Colorado Springs, Colo). After an additional 5-minute stabilization period, baseline values of LV developed pressure (LVDP), its maximal and minimal first derivatives (dp/dt_{max} and dp/dt_{min} , respectively), end-systolic pressure (ESP), and end-diastolic pressure (EDP) were recorded using the PowerLab data acquisition system and software. Immediately following the isolated perfused heart protocol, the heart was removed and the left ventricle isolated. Left ventricular tissue was frozen in liquid nitrogen and stored for subsequent biochemical analyses.

Tissue Preparation

Approximately 100 mg of frozen left ventricular tissue was pulverized with a porcelain mortar and pestle and transferred into ice-cold 20 mM Tris-HCl (ACS Reagent grade; Sigma-Aldrich, St. Louis, Mo), pH 7.4, at a dilution of 1:5 wt/vol. Samples were then homogenized and sonicated. Homogenates were centrifuged at 3000g for 10 minutes at 4°C, and the supernatant was obtained for biochemical analysis. Total protein concentration was determined using the method of Bradford with bovine serum albumin (Pierce, Rockford, Ill) as the reference standard.

Myocardial Lipid Peroxidation

To examine the effect of DOX and acute exercise on myocardial lipid peroxidation, malondialdehyde and

4-hydroxy-alkenals (MDA+4-HAE) were analyzed in left ventricular tissue using a commercially available assay kit (OXIS International, Inc, Portland, Ore). The MDA+4-HAE assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA+4-HAE at 45°C. To perform the assay, 200 μ L of sample was added to 10 μ L of probucol and 640 μ L of N-methyl-2-phenylindole in acetonitrile and briefly vortexed. Next, 150 μ L of concentrated HCl was added, vortexed, and incubated at 45°C for 60 minutes. Samples were then centrifuged for 10 minutes at 10 000g to remove any interference from turbidity. The supernatant was then transferred to a cuvette and the absorbance was measured using a Genesys 20 spectrophotometer (ThermoSpectronic, Rochester, NY) at 586 nm. Total MDA+4-HAE was estimated using linear regression based on values obtained with an MDA standard curve to yield the final concentration of MDA+4-HAE (μ M). All samples were assayed in duplicate, and any samples varying more than 5% were reassayed.

Statistical Analysis

Data from the 4 treatment groups were evaluated for differences between groups by 2-way analysis of variance. In the presence of significant *F* values, Tukey's post hoc test was performed to identify significant group differences ($P < .05$).

Results

General Observations

At the time of injection, there were no significant differences in mean body mass of rats among the experimental groups (Table 1). Five days following treatment, animals exposed to DOX lost an average of approximately 15% of their body mass irrespective of their exercise status. In addition, whereas heart mass/body mass ratio was significantly higher in all groups in comparison to SED+SAL ($P < .05$), only the SED+DOX group possessed a mean absolute heart mass significantly lower than that of the SED+SAL group ($P < .05$).

Cardiac Function

Figures 1 and 2 summarize cardiac function assessment. There were no significant differences in any cardiac function variable between saline-treated groups, with the exception of dP/dt_{\min} (-1732 ± 62 vs -2136 ± 86 ; $P < .05$). DOX treatment resulted in a considerable degree of cardiac dysfunction as evidenced by a 162% increase in EDP, a 35% decline in ESP, a 59% decline in LVDP, and a 43% decline in dP/dt_{\max} ($P < .05$ for all variables). Acute exercise before DOX treatment (EX+DOX) completely preserved ESP, and although LVDP and dP/dt_{\max} were significantly lower than

Table 1. Animal Characteristics^a

	SED+SAL	EX+SAL	SED+DOX	EX+DOX
Injection mass, g	302 \pm 3	306 \pm 8	307 \pm 7	324 \pm 8
Sacrifice mass, g	314 \pm 3	320 \pm 9	259 \pm 10*	278 \pm 9*
Δ body mass, g	12 \pm 2	14 \pm 2	-47 \pm 12*	-45 \pm 6*
Heart mass, mg	1128 \pm 24	1160 \pm 35	965 \pm 43*	1070 \pm 56
HM/BM, mg/g	3.5 \pm 0.07	3.9 \pm 0.03*	3.7 \pm 0.04*	3.8 \pm 0.06*

NOTES: SED = sedentary; SAL = saline; EX = exercise; DOX = doxorubicin; HM/BM = heart mass/body mass ratio.

^aValues are mean \pm SE.

*Significantly less than SED+SAL and EX+SAL ($P < .05$).

SED+SAL, mean values for these variables were significantly higher in comparison to SED+DOX ($P < .05$). Despite the fact that acute exercise did not influence the effects of DOX on EDP, dP/dt_{\min} was significantly higher in EX+DOX compared with SED+DOX ($P < 0.5$).

Myocardial Lipid Peroxidation

To provide an index of oxidative stress, MDA+4-HAE was quantified in left ventricular samples (Figure 3). Sedentary animals exposed to DOX showed a 45% increase in lipid peroxidation 5 days after treatment. A single bout of acute exercise 24 hours before DOX treatment completely eliminated the DOX-induced increase in MDA+4-HAE at this time interval, suggesting that the exercise significantly reduced left ventricular lipid peroxidation.

Discussion

This study tested the hypothesis that an acute bout of exercise before treatment with DOX would protect the heart from DOX-mediated oxidative stress and the subsequent manifestation of cardiac dysfunction. This hypothesis was based on observations that (1) DOX treatment is associated with severe myocardial oxidative stress and (2) acute exercise preserves cardiac function in models of cardiac oxidative stress. Our results indicate that DOX treatment caused severe cardiac dysfunction 5 days after its administration, and this dysfunction was significantly attenuated by prior acute exercise. ESP was completely preserved in those animals subjected to an acute exercise bout 24 hours before DOX exposure, whereas both LVDP and dP/dt_{\max} were significantly higher in exercised animals compared with their sedentary counterparts. In addition, although the rate of pressure decline (dP/dt_{\min}) was significantly improved in EX+DOX animals, acute exercise afforded no benefit for EDP.

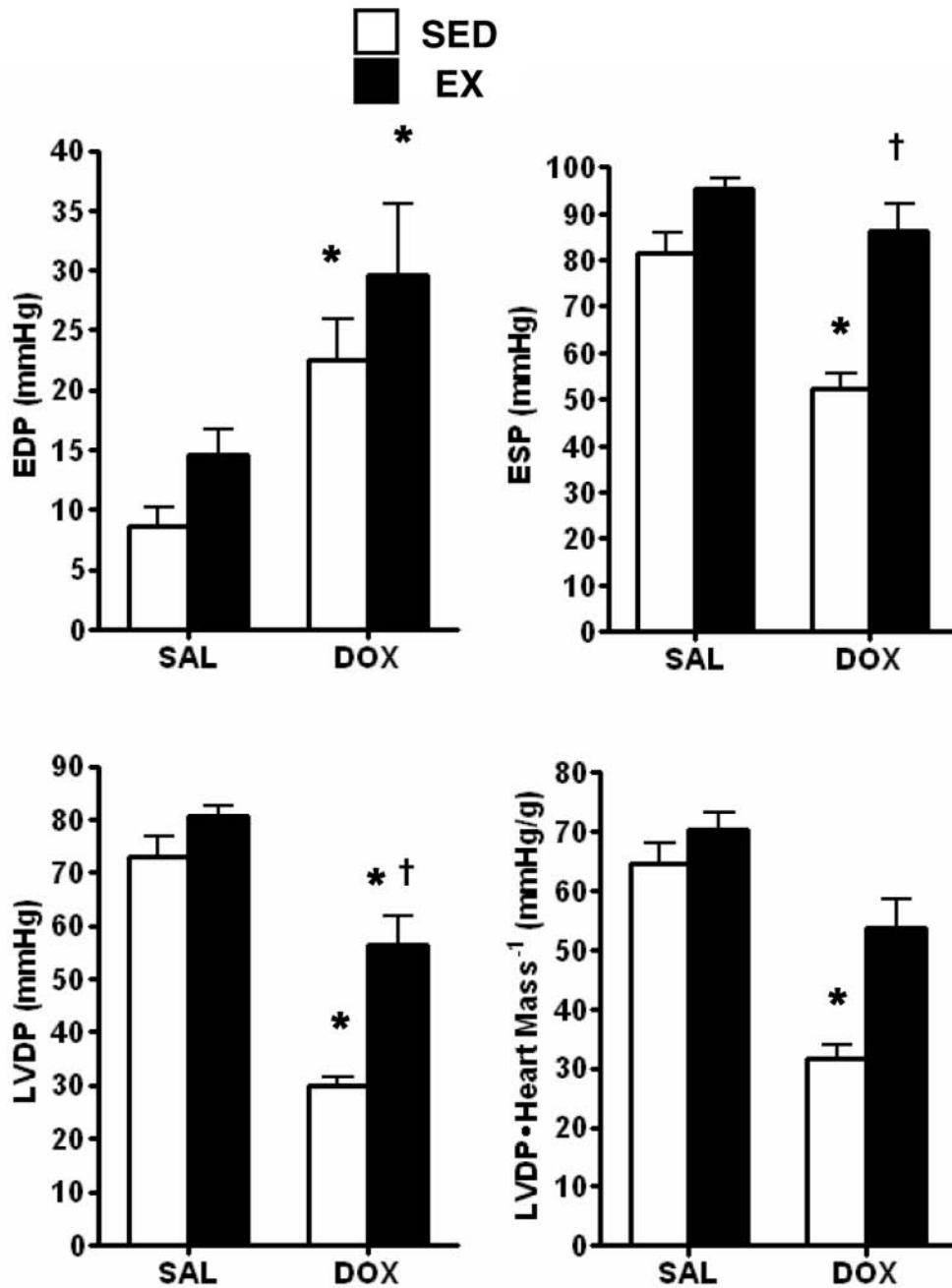


Figure 1. Left ventricular pressures in sedentary (SED) and acutely exercised (EX) animals 5 days following doxorubicin (DOX) treatment. EDP, left ventricular end-diastolic pressure; ESP, left ventricular end-systolic pressure; LVDP, left ventricular developed pressure; LVDP × heart mass, ratio of left ventricular developed pressure to heart mass in mm Hg per gram of heart mass; SAL, saline. Values are mean ± SE. **P* < .05 vs SED+SAL; †*P* < .05 vs SED+DOX.

Animals in the SED+DOX group demonstrated all of the classic signs of DOX cardiotoxicity in this model. Specifically, these animals had significant reductions in body mass, heart mass, ESP, LVDP, and dP/dt_{max} as well as increases in dP/dt_{min} and EDP. The cardioprotective effects of acute exercise against DOX-induced cardiac dysfunction

5 days after treatment were remarkably similar to the protective effects afforded by chronic endurance exercise. Our laboratory has shown that 8 weeks of voluntary wheel running attenuated the in vitro cardiac dysfunction associated with DOX perfusion in the isolated heart,²³ and 12 weeks of treadmill running protected against DOX

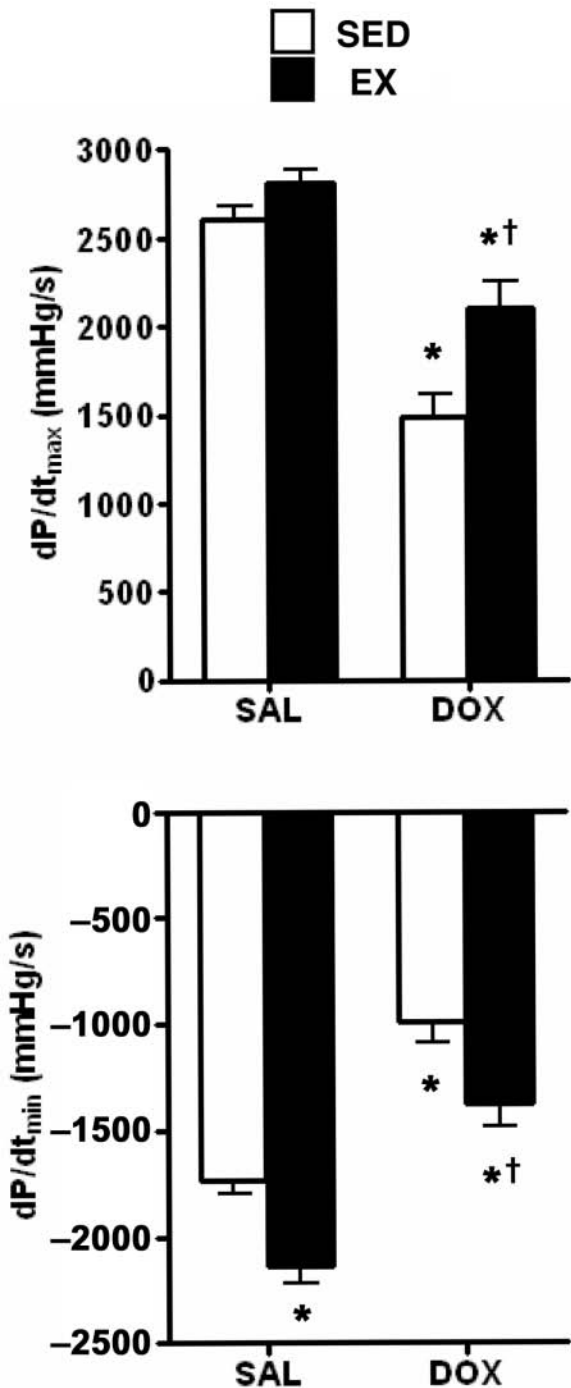


Figure 2. Maximal first derivatives (dP/dt_{max} , top) and minimal first derivatives (dP/dt_{min} , bottom) of left ventricular pressure in sedentary (SED) and acutely exercised (EX) animals 5 days following doxorubicin (DOX) treatment. Values are mean \pm SE. SAL, saline. * $P < .05$ vs SED+SAL; † $P < .05$ vs SED+DOX.

cardiotoxicity when DOX was administered *in vivo*.²² In both studies, the most dramatic improvements were observed with indicators of systolic function (ie, ESP, LVDP, dP/dt_{max}), whereas little or no protection was provided

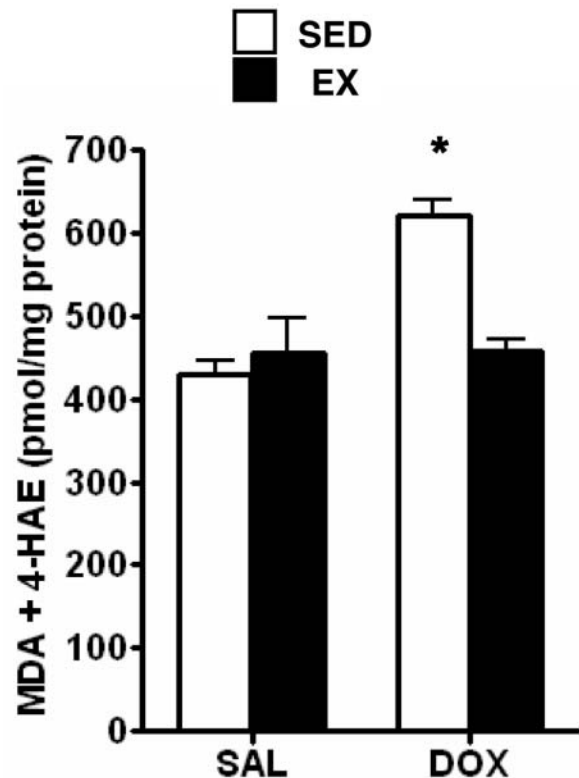


Figure 3. Lipid peroxidation products in left ventricular homogenates. Data are mean \pm SE. * $P < .05$ vs SED \times SAL. MDA, malondialdehyde; 4-HAE, 4-hydroxy-alkenal; EX, exercise; SED, sedentary; SAL, saline; DOX, doxorubicin.

against increases in EDP. The same pattern of protection appears to be provided by acute exercise in this model.

Although we report that acute endurance exercise protected against DOX-induced cardiac dysfunction via a reduction in cardiac oxidative stress, it is possible that the 2 oxidative stressors (ie, DOX and exercise) exacerbated cardiac oxidative stress in the days following treatment. Our laboratory and others²⁷⁻²⁹ have reported that acute endurance exercise increases cardiac oxidative stress. Previously, we found that strenuous acute endurance exercise resulted in a decrease in cardiac function and an increase in oxidative stress that persisted for up to 48 hours after the bout of exercise.³⁰ In that study, indicators of oxidative stress returned to baseline levels by 72 hours post exercise. In the present study, MDA was not measured until 5 days after DOX treatment and 6 days after the acute exercise bout. Therefore, although there may have been increases in oxidative stress during the 72 hours post DOX treatment, exercise preconditioning clearly attenuated the cardiac oxidative burden. This is supported by the fact that 5 days after DOX treatment, sedentary animals exhibited significant increases in MDA whereas exercised animals showed levels of MDA that were not different from

sedentary controls. In addition, variations in cardiac oxidative stress among these studies may be explained by variations in exercise modality (swimming vs running), exercise duration (60 minutes vs exhaustive), or running protocol (continuous vs progressive).

The deleterious effects of DOX can be attenuated by antioxidative agents, which supports the premise that DOX-induced cardiotoxicity may be related to the production of free radicals.^{9,31} Transgenic mice overexpressing cardiac-specific metallothionein, a potent antioxidant, are highly resistant to DOX cardiotoxicity as evidenced by an attenuation of cardiac morphological alterations and the prevention of sarcoplasmic and mitochondrial structural changes observed in wild-type mice treated with DOX.³² Likewise, mice overexpressing the antioxidant enzyme catalase show a significant reduction in DOX-related cardiac dysfunction as measured by ventricular contractile force.³³

Adaptations in the content and/or activity of enzymatic and nonenzymatic antioxidant defenses following exercise have been highly variable and inconsistent. Some studies have reported no change^{34,35} or decreases^{36,37} in myocardial antioxidants following endurance exercise training. However, long-term exercise training has been shown to increase the antioxidant status of the heart by increasing cardiac glutathione levels and up-regulating cardiac copper/zinc-superoxide dismutase, manganese superoxide dismutase, catalase, and glutathione peroxidase activities and protein expression.³⁸⁻⁴³ Additionally, a number of studies have been conducted that describe the cardioprotective effects of short-term exercise against ischemia-reperfusion injury, a model that is associated with myocardial oxidative stress. These studies indicate that 5 bouts,^{44,45} 3 bouts,^{43,44,46} 2 bouts,²⁰ or even 1 bout^{19,47,48} of exercise can improve postischemic cardiac function. Improved function in this model has been associated with an enhancement in antioxidant defenses,^{44,48} an increased expression of heat shock protein,^{46,48} and an up-regulation in the inducible form of nitric oxide synthase.⁴⁷ Although such myocardial adaptations may contribute, collectively or independently, to exercise-induced cardioprotection, several studies have shown that increases in antioxidants, heat shock protein, or nitric oxide synthase are not necessarily a requisite for cardioprotection afforded by acute exercise.^{19,20,49} Nevertheless, a single bout of acute exercise did attenuate increases in myocardial MDA, and this was associated with a preservation of cardiac function. Thus, an increase in antioxidant defenses is a viable mean by which acute exercise could protect against DOX cardiotoxicity.

Conclusion

These are the first data indicating that as little as 1 bout of exercise can alleviate the cardiac dysfunction associated with DOX exposure. The protective effects of exercise were

manifested as a preservation of peak systolic pressure, developed pressure, the peak rate of pressure development, and the peak rate of pressure decline in the left ventricle. Preservation of cardiac function in exercised animals was associated with the prevention of DOX-induced increases in MDA, suggesting that attenuation of oxidative stress is a possible explanation. However, the attenuation of oxidative stress leaves a great deal of speculation as to the exact mechanism, because the prevention of oxidative stress could have a number of effects on calcium handling, protein expression and function, DNA damage and repair, apoptosis, mitochondrial function, and cardiomyocyte bioenergetics. Further studies are warranted to investigate these or other potential mechanisms that lead to the protective effects of acute exercise before treatment with DOX. Although we have previously shown that long-term exercise training can attenuate the acute cardiac dysfunction associated with DOX, relatively few patients participate in long-term exercise training before undergoing DOX treatment. Here we show that a single bout of exercise can attenuate DOX-mediated cardiac dysfunction, suggesting that this may be a more clinically relevant means to alleviate DOX-induced cardiotoxicity in those individuals who have not been physically active before DOX treatment.

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