Original Research

Rehabilitative exercise in a rat model of doxorubicin cardiotoxicity

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Abstract

The use of exercise to minimize doxorubicin (DOX)-induced cardiotoxicity is gaining attention. However, very few clinically relevant reports exist investigating the effects of exercise performed during and following DOX treatments. The purpose of this study, therefore, was to examine the effects of voluntary wheel running during and following DOX treatment using two models of late-onset DOX cardiotoxicity in the rat. Female Sprague-Dawley rats received either DOX or saline injections using one of two separate treatment regimens. These regimens involved either daily or weekly DOX injections with cumulative doses for both protocols totaling 15 mg/kg. Daily DOX injections were 1 mg/kg and lasted for 15 consecutive days while weekly DOX injections were 2.5 mg/kg and lasted for six consecutive weeks with control animals receiving matched saline injection regimens. Immediately following the initial DOX/saline injection, animals were randomly housed in cages with voluntary running wheels or standard rat cages throughout DOX/saline treatments and continued until reaching 10 weeks. Cardiac function was then assessed using echocardiography and an isolated working heart model, and myosin heavy chain (MHC) isoform distribution was assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When compared wth controls, daily DOX treatment resulted in reduced running wheel distances at weeks 2–10 ($P < 0.05$), and weekly DOX treatment resulted in reduced running wheel distances at weeks 2, 6 and 10 ($P < 0.05$). Nonetheless, wheel running during and following daily and weekly DOX dosing protected against DOX-induced cardiotoxicity by preserving maximal mitral and aortic blood flow velocities, left ventricular developed pressure and MHC isoform expression. In conclusion, the overall reduced volume of activity during and following daily and weekly DOX treatments attenuated DOX-induced cardiac dysfunction suggesting that low-volume endurance training may be an effective rehabilitative approach in minimizing DOX cardiotoxicity in cancer patients.

Keywords: adriamycin, anthracycline, heart failure, physical activity

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Introduction

The chemotherapeutic agent doxorubicin (DOX, trade name Adriamycin®) is associated with a dose-dependent cardiotoxicity which can manifest as early-onset (acute) cardiotoxicity or late-onset (chronic) cardiotoxicity. Early-onset DOX cardiotoxicity can present itself within minutes to hours of drug administration in the form of hypotenstion or arrhythmias[1] or within days of drug administration in the form of depressed left ventricular fractional shortening (FS) and developed pressure.[2,3] Although early-onset DOX cardiotoxicity is a concern, the late-onset form of DOX cardiotoxicity, which may set in weeks, months or years following drug administration, is typically more severe than the acute form. Late-onset DOX-cardiotoxicity may be characterized by symptoms of dilated cardiomyopathy or heart failure,[4,5] and these abnormalities may be present long after early-onset cardiotoxicity symptoms have subsided or develop without evidence of early-onset cardiotoxicity.[6,7] This chronic form of cardiac dysfunction has been attributed to, among other things, a shift in myosin heavy chain (MHC) isoform distribution toward increased β-MHC expression.[8–10]

Because DOX is an effective anticancer drug, interventions aimed at minimizing its cardiotoxicity have received much attention. These interventions include, among others, treatment with antioxidants such as superoxide dismutase,[11] polyphenols such as resveratrol,[12] flavinoids

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such as kaempferol[13] and hydrogen sulfide.[14] However, these interventions have had limited success since DOX cardiotoxicity is multifaceted (for review see Ref.[15]), and each of these aforementioned interventions target only a small portion of the known cardiotoxic mechanisms. Targeting multiple mechanisms of DOX cardiotoxicity, therefore, would be of value, and one such intervention gaining popularity involves the use of exercise to ameliorate DOX-induced cardiotoxicity. It has consistently been shown that prior endurance training protects against early-onset DOX-induced cardiotoxicity analyzed 24 h,[16] 5 days[2,17] and 10 days[3] post bolus drug administration.

Although these exercise preconditioning reports demonstrate that endurance exercise plays a role in attenuating DOX cardiotoxicity, two very important clinical issues have yet to be addressed: (1) cancer patients may not be endurance trained prior to DOX treatment and (2) DOX treatments typically involve small doses administered over time to reach a cumulative dose (as opposed to bolus administration). The first issue is a concern because although prior endurance exercise protects against DOX-induced cardiac dysfunction, cancer patients receiving DOX as part of their chemotherapy regimens often experience severe fatigue,[18,19] and it is unlikely that cancer patients receiving DOX would be able to exercise at the high volumes typically used in exercise preconditioning studies. Therefore, cancer rehabilitation programs typically prescribe relatively low-dose endurance exercise,[20–22] and although our laboratory has previously shown that short duration treadmill running[23] and voluntary wheel running[24] during DOX treatment effectively attenuated cardiotoxicity, it is unknown as to what effects endurance exercise during and following treatment has on chronic DOX cardiotoxicity which may manifest after the cessation of treatment. The second clinical issue of incremental dosing is also of importance since the majority of DOX cardiotoxicity studies focus on acute toxicities following a bolus DOX dose. This approach, although important in understanding toxicity mechanisms, does not translate well clinically as patients may have treatments spread out over the course of months,[25,26] and the effects of incremental DOX dosing on cardiac function differ from that of bolus DOX dosing.[5]

These two clinical issues are addressed in the current study by introducing sedentary rats to voluntary running wheels during incremental DOX dosing administered either daily or weekly until a specific cumulative dose was achieved (15 mg/kg), and rats were allowed continued access to voluntary running wheels at the completion of treatments until reaching the predetermined endpoint (10 weeks). This design not only allowed for the assessment of chronic cardiac dysfunction as a result of two different incremental dosing schemes, but it also allowed rats to run at will in response to DOX treatment. It was hypothesized that incremental DOX treatment would result in a reduction in voluntary wheel running activity, but this reduced exercise volume would still provide protection against late-onset DOX cardiac dysfunction by preserving MHC distribution.

Materials and methods

Animals, animal care, DOX treatments and activity treatments

All procedures were approved by the Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act guidelines. Female Sprague-Dawley rats (~200 g) were purchased from Harlan (Indianapolis, IN, USA) and were housed in an environmentally controlled facility on a 12:12 light:dark cycle and were provided chow and water *ad libitum*. Initially, rats were randomly assigned to two separate experimental subgroups designed to incorporate two different schemes for incremental DOX dosing. Animals assigned to the first subgroup received either daily 1 mg/kg intraperitoneal DOX injections administered over the course of 15 consecutive days (DOX1, $n = 24$) or daily intraperitoneal injections of 0.9% saline over the course of 15 consecutive days as a control (SAL1, $n = 16$). Immediately following the first injection (day 1), DOX1 and SAL1 animals were housed individually in cages outfitted with voluntary running wheels (WR + DOX1, $n = 9$; WR + SAL1, $n = 8$) or housed with one cage mate in a standard cage to act as a sedentary treatment (SED + DOX1, $n = 15$; SED + SAL1, $n = 8$). WR animals were allowed 24 h access to the running wheels, and the only time animals did not have running wheel access was during times of DOX or SAL injections. Activity treatments (WR or SED) for this subgroup continued for 10 weeks at which time cardiac function was assessed.

Animals assigned to the second subgroup were included in a separate set of experiments and received either weekly 2.5 mg/kg intraperitoneal DOX injections administered over the course of six consecutive weeks (DOX2, $n = 20$) or weekly intraperitoneal injections of 0.9% saline over the course of six consecutive weeks as a control (SAL2, $n = 15$). Immediately following the first injection (day 1 of week 1), DOX2 and SAL2 animals were housed individually in cages outfitted with voluntary running wheels (WR + DOX2, $n = 10$; WR + SAL2, $n = 8$) or housed with one cage mate in a standard cage to act as a sedentary treatment (SED + DOX2, $n = 10$; SED + SAL2, $n = 7$). WR animals were allowed 24 h access to the running wheels, and the only time animals did not have running wheel access was during times of DOX or SAL injections. Activity treatments (WR or SED) for this subgroup continued for 10 weeks at which time cardiac function was assessed.

Echocardiography

Cardiac function was assessed *in vivo* in the first and second subgroup (DOX1/SAL1 and DOX2/SAL2) at the completion of week 10 using transthoracic echocardiography (Toshiba Nemio 30; 10 MHz pediatric transducer) as described previously by our laboratory.[3,5] Left ventricular (LV) M-mode images were obtained from sedated rats (ketamine 40 mg/kg intraperitoneally) to determine septal and posterior absolute wall thicknesses at end systole (SWs and PWs, respectively) and diastole (SWd and PWd, respectively) and LV end-systolic and end-diastolic diameter (LVDs and LVDd, respectively) using a leading

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edge-to-leading edge technique as described by the American Society of Echocardiography.[27] In addition, the above-mentioned variables were used to calculate relative wall thickness (RWT, [PWd + SWd]/LVDd), left ventricular mass (LV mass, 1.04 [(LVDd + PWd + SWd)^3 − LVDd^3]) and FS ([LVDd − LVDs]/LVDd).

Pulsed wave Doppler images were then acquired to obtain profiles of mitral and aortic valve blood flow. These blood flow profiles were analyzed using SimPACS software (Toshiba America Medical Systems Inc., Tustin, CA, USA) to determine maximal mitral blood flow velocity (MV_{max}) and maximal aortic blood flow velocity (AV_{max}). Mitral valve time velocity integrals (derived from SimPACS software) were used to determine mean mitral blood flow velocity (MV_{mean}) and aortic valve time velocity integrals were used to determine mean aortic blood flow velocity (AV_{mean}). For all echocardiography measures, data from three consecutive cardiac cycles, when possible, were obtained and averaged for each animal.

Isolated working heart

Following echocardiography, *ex vivo* cardiac function was analyzed using an isolated working heart model as described previously.[3,28] Hearts were excised from anesthetized (sodium pentobarbital 50 mg/kg intraperitoneally delivered with 500 U heparin) rats, and the aorta was cannulated and perfused in a retrograde manner with warm (37°C), oxygenated (95% O_2/5% CO_2) Krebs–Hanseleit buffer (120 mmol/L NaCl, 5.9 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L MgCl, 25 mmol/L $NaHCO_3$, 17 mmol/L glucose and 0.5 mmol/L EDTA, pH 7.4). Once coronary blood was flushed and non-cardiac tissue was removed, the pulmonary vein was cannulated, and flow was redirected from the aorta to the left atrium to initiate the working heart mode under a standardized preload and afterload (10 and 100 cm H_2O, respectively). A micotip pressure transducer (Scisence Inc., London, ON, Canada) was inserted into the LV cavity for acquisition of end systolic pressure (LVESP), end diastolic pressure (LVEDP), developed pressure (LVDP, calculated as LVESP − LVEDP), maximal rate of pressure development ($+dP/dt_{max}$) and maximal rate of pressure decline ($-dP/dt_{max}$). During data collection, hearts were paced at 240 bpm using a stimulus isolator (ADInstruments, Colorado Springs, CO, USA). Following the isolated working heart preparation, hearts were dissected, and the LV was isolated, flash frozen in liquid nitrogen and stored at −80°C until biochemical analysis.

Myosin heavy chain

MHC isoform distribution was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously.[3,28] LV tissue was homogenized in 10 volumes of buffer (250 mmol/L sucrose, 100 mmol/L KCl, 5 mmol/L EDTA and 20 mmol/L Tris-Base, pH 6.8), centrifuged at 1000*g* for 10 min, and the pellet washed (175 mmol/L KCl, 0.5% Triton X-100, 2 mmol/L EDTA and 20 mmol/L Tris-Base, pH 6.8) and centrifuged twice, and the resulting pellet was re-suspended (150 mmol/L KCl and 20 mmol/L Tris-Base, pH 7.0). Total protein was determined using the Bradford method.[29] A total of 9.75 μg of protein from each sample was separated on an 8% polyacrylamide separating gel with a 4% polyacrylamide stacking gel and run at 100 V (Sure-Lock electrophoresis unit, Invitrogen Corporation, Carlsbad, CA, USA) until tracking dye reached the bottom of the gel. Gels were stained with Coomassie blue, and α- and β-isoforms were analyzed using densitometry.

Statistical analysis

All results are expressed as mean ± SEM. A 2 × 2 (activity × drug) analysis of variance (ANOVA) was performed to identify activity (WR) and drug (DOX) main effects and determine if interactions existed in the first experimental subgroup (SED + SAL1, SED + DOX1, WR + SAL1, WR + DOX1), and if significant main effects or an interaction was observed, Bonferroni *post hoc* testing was performed to detect differences within drug treatment groups (SED + SAL1 versus WR + SAL1 and SED + DOX1 versus WR + DOX1). Likewise, a 2 × 2 (activity × drug) ANOVA was performed to identify activity (WR) and drug (DOX) main effects and determine if interactions exist in the second experimental subgroup (SED + SAL2, SED + DOX2, WR + SAL2, WR + DOX2), and if significant main effects or an interaction was observed, Bonferroni *post hoc* testing was performed to detect differences within drug treatment groups (SED + SAL2 versus WR + SAL2 and SED + DOX2 versus WR + DOX2). Additionally, wheel running distances within the first experimental subgroup (WR + SAL1 versus WR + DOX1) were analyzed using a two-way (group × week) repeated measures ANOVA with Bonferroni *post hoc* testing to determine between group weekly distance differences. Wheel running distances within the second experimental subgroup (WR + SAL2 versus WR + DOX2) were also analyzed using a two-way (group × week) repeated measures ANOVA with Bonferroni *post hoc* testing to determine between group weekly distance differences. For all procedures, significance was set at $\alpha = 0.05$.

Results

Wheel running distances

Weekly volumes of wheel running during the 10-week experimental period are illustrated in Figure 1. With both the daily (WR + DOX1, WR + SAL1) and weekly (WR + DOX2, WR + SAL2) injection regimens, significant group and week wheel running differences were observed ($P < 0.05$); however, no significant group × week interactions were detected. *Post hoc* testing revealed significant between group running distance differences in the daily injection groups (WR + DOX1 versus WR + SAL1) at weeks 2–10 ($P < 0.05$, Figure 1a) and significant between group running distance differences in the weekly injection groups (WR + DOX2 versus WR + SAL2) at weeks 2, 6 and 10 ($P < 0.05$, Figure 1b).

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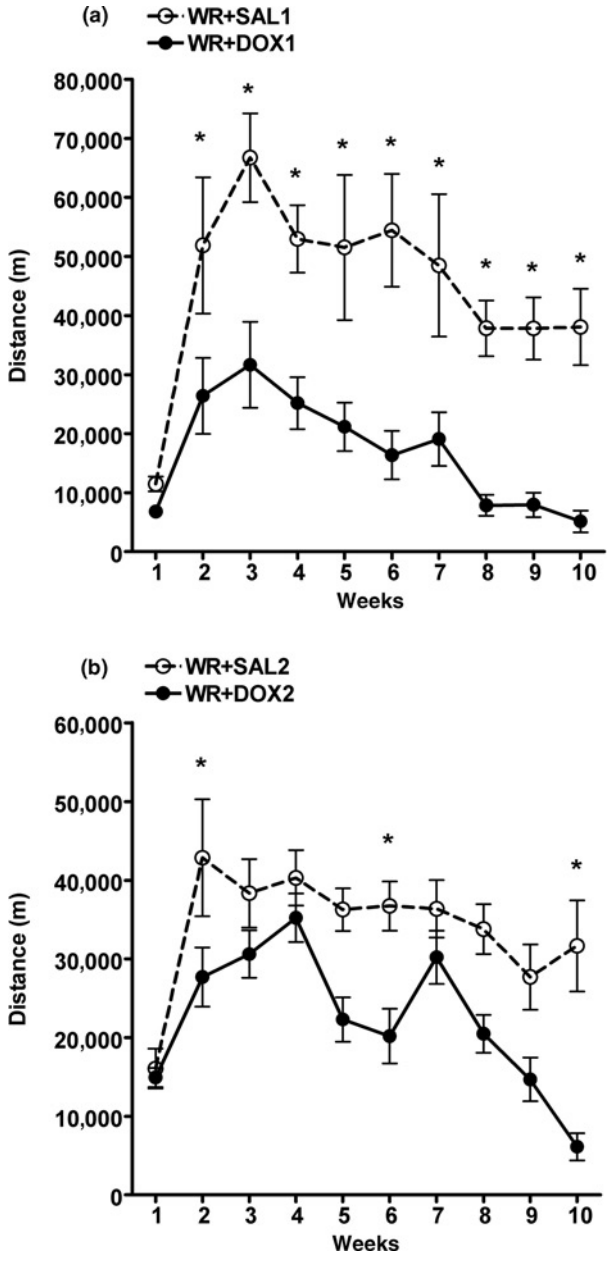

Figure 1 Weekly running distances for rats included in wheel run groups. (a) Data obtained from rats receiving daily injections of saline or DOX; (b) data obtained from rats receiving weekly injections of saline or DOX; WR, wheel running; SAL1, daily saline injections for 15 consecutive days; DOX1, daily 1 mg/kg doxorubicin injections for 15 consecutive days (15 mg/kg cumulative); SAL2, weekly saline injections for six consecutive weeks; DOX2, weekly 2.5 mg/kg doxorubicin injections for six consecutive weeks (15 mg/kg cumulative). (a) *Significant difference between WR + SAL1 and WR + DOX1 ($P < 0.05$); (b) *significant difference between WR + SAL2 and WR + DOX2 ($P < 0.05$)

Cardiac dimensions

Whole heart mass and echocardiography derived cardiac geometry parameters for the daily injection groups (DOX1 and SAL1) are presented in Table 1. Significant DOX main effects were present for whole heart mass, SWs, PWs, LVDs, LV mass and FS ($P < 0.05$) with no interactions or between-group differences observed. A significant activity main effect was observed in LVDd ($P < 0.05$), and SED + DOX1 LVDd was found to be significantly lower than WR + DOX1 LVDd ($P < 0.05$). Table 2 presents heart mass and cardiac geometry parameters obtained from the weekly injection groups (DOX2 and SAL2). Significant DOX main effects were detected for whole heart mass, SWs, SWd, PWs, LV Mass and FS ($P < 0.05$) with no activity main effects, interactions or between-group differences observed in any of the measured parameters.

Doppler blood flow velocities

Mitral and aortic blood flow velocities for the daily dosing groups (DOX1) are illustrated in Figure 2. Significant AV_{max} and AV_{mean} WR (activity) main effects, significant MV_{max}, MV_{mean}, AV_{max} and AV_{mean} DOX (drug) main effects, and significant WR × DOX interactions were observed for MV_{max}, AV_{max} and AV_{mean} ($P < 0.05$). Additionally, SED + DOX1 possessed significantly lower MV_{max}, AV_{max} and AV_{mean} than WR + DOX1 ($P < 0.05$). Figure 3 illustrates mitral and aortic blood flow velocities for the weekly dosing groups (DOX2). DOX main effects were observed in all measured blood flow velocities with significant WR × DOX interactions detected in MV_{max}, MV_{mean} and AV_{max} ($P < 0.05$). Additionally, maximal and mean MV and AV blood flows were significantly lower in SED + DOX2 than in WR + DOX2 ($P < 0.05$).

Ex vivo cardiac function

Data obtained for the isolated working heart model for daily and weekly injection groups are illustrated in Figures 4 and 5, respectively. With the daily injection groups (DOX1), significant WR main effects were observed for LVESP and $-dP/dt_{max}$, and significant DOX main effects were observed for LVEDP, LVDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ ($P < 0.05$, Figure 4). Significant LVESP, LVDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ WR × DOX interactions were also observed, and these parameters were found to be significantly lower in SED + DOX1 when compared with WR + DOX1 ($P < 0.05$). DOX main effects were observed in the weekly injection groups (DOX2) for all *ex vivo* cardiac function parameters measured with a significant activity main effect observed with LVDP ($P < 0.05$, Figure 5). Activity × drug interactions were apparent in LVESP, LVEDP and LVDP with these variables being significantly different in hearts from SED + DOX2 compared with WR + DOX2 ($P < 0.05$).

Myosin heavy chain

Figure 6 illustrates the left ventricular β-MHC isoform expression with representative gel images from rats receiving daily DOX dosing and weekly DOX dosing. Although a significant activity main effect was observed in the daily injection groups ($P < 0.05$), no DOX main effect or interaction was observed. However, LVs from SED + DOX1 expressed significantly higher β-MHC than WR + DOX1 ($P < 0.05$, Figure 6a). No WR or DOX main effects were observed in the weekly injection groups, but a significant

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Table 1 Heart mass and echocardiography derived cardiac geometry for daily injections groups

	SED + SAL1	WR + SAL1	SED + DOX1	WR + DOX1
Whole heart mass (g)*	1.23 ± 0.02	1.36 ± 0.03	1.05 ± 0.05	1.01 ± 0.04
SWs (mm)*	3.16 ± 0.11	3.18 ± 0.16	2.64 ± 0.11	2.50 ± 0.09
SWd (mm)	1.74 ± 0.13	1.74 ± 0.16	1.60 ± 0.09	1.54 ± 0.07
PWs (mm)*	3.45 ± 0.24	3.23 ± 0.17	2.69 ± 0.16	2.84 ± 0.17
PWd (mm)	1.86 ± 0.15	1.71 ± 0.13	1.60 ± 0.09	1.56 ± 0.11
RWT (mm)	0.65 ± 0.05	0.62 ± 0.09	0.59 ± 0.04	0.47 ± 0.03
LVDs (mm)*	2.21 ± 0.16	2.53 ± 0.31	3.05 ± 0.18	3.70 ± 0.40
LVDd (mm)†	5.57 ± 0.16	5.83 ± 0.29	5.55 ± 0.19‡	6.54 ± 0.42
LV Mass (mg)*	624 ± 39	670 ± 66	515 ± 45	477 ± 70
FS (%)*	60 ± 2	59 ± 5	45 ± 3	46 ± 4

Data are means ± SEM. SED, sedentary; WR, wheel running; SAL1, daily saline injections for 15 consecutive days; DOX1, daily 1 mg/kg doxorubicin injections for 15 consecutive days (15 mg/kg cumulative); SWs, septal wall thickness at end systole; SWd, septal wall thickness at end diastole; PWs, posterior wall thickness at end systole; PWd, posterior wall thickness at end diastole; RWT, relative wall thickness; LVDs, left ventricular dimension at end systole; LVDd, left ventricular dimension at end diastole; LV Mass, left ventricular mass; FS, fractional shortening

*Significant drug effect ($P < 0.05$)

†Significant activity effect ($P < 0.05$)

‡$P < 0.05$ versus WR + DOX1

Table 2 Heart mass and echocardiography derived cardiac geometry for weekly injections groups

	SED + SAL2	WR + SAL2	SED + DOX2	WR + DOX2
Whole heart mass (g)*	1.23 ± 0.04	1.29 ± 0.04	1.13 ± 0.01	1.16 ± 0.04
SWs (mm)*	3.22 ± 0.19	3.30 ± 0.13	2.49 ± 0.25	3.10 ± 0.18
SWd (mm)*	1.70 ± 0.09	1.84 ± 0.09	1.31 ± 0.14	1.68 ± 0.13
PWs (mm)*	3.21 ± 0.21	3.18 ± 0.06	2.48 ± 0.24	2.87 ± 0.24
PWd (mm)	1.66 ± 0.14	1.45 ± 0.13	1.36 ± 0.15	1.36 ± 0.11
RWT (mm)	0.60 ± 0.05	0.53 ± 0.04	0.49 ± 0.06	0.62 ± 0.11
LVDs (mm)	1.77 ± 0.30	2.16 ± 0.18	2.70 ± 0.40	2.29 ± 0.23
LVDd (mm)	5.68 ± 0.19	6.28 ± 0.15	5.65 ± 0.32	5.91 ± 0.31
LV Mass (mg)*	574 ± 20	651 ± 32	426 ± 53	553 ± 80
FS (%)*	69 ± 5	66 ± 3	52 ± 5	61 ± 4

Data are means ± SEM. SED, sedentary; WR, wheel running; SAL2, weekly saline injections for six consecutive weeks; DOX2, weekly 2.5 mg/kg doxorubicin injections for six consecutive weeks (15 mg/kg cumulative); SWs, septal wall thickness at end systole; SWd, septal wall thickness at end diastole; PWs, posterior wall thickness at end systole; PWd, posterior wall thickness at end diastole; RWT, relative wall thickness; LVDs, left ventricular dimension at end systole; LVDd, left ventricular dimension at end diastole; LV Mass, left ventricular mass; FS, fractional shortening

*Significant drug effect ($P < 0.05$)

activity × drug interaction was detected ($P < 0.05$, Figure 6b). Additionally, SED + DOX2 LVs expressed significantly higher β-MHC than WR + DOX2 ($P < 0.05$).

Discussion

Cancer patients receiving DOX as part of their chemotherapy regimens often experience fatigue and decreased quality of life,[18,19] and these debilitating side-effects are often attributed to cardiotoxicity. In fact, cardiotoxicity is the factor limiting DOX dosing in cancer patients, and as such, ameliorating DOX cardiotoxicity has received much attention. Exercise training's positive effects on the heart have been shown previously to apply to DOX cardiotoxicity,[3,16,17,23,28,30–34] but few of these reports have employed exercise training during and following DOX dosing (many employed exercise treatments prior to DOX administration). Furthermore, investigations examining the effects of exercise on DOX cardiotoxicity using incremental dosing (i.e. daily or weekly dosing) have been limited. The current report is novel in that exercise was employed during and following incremental DOX dosing which translates to cancer patients who do not have a history of exercise training prior to treatment.

Cardiotoxicity with incremental dosing DOX

Late-onset DOX cardiotoxicity is typically more severe than that of early-onset (or acute) DOX cardiotoxicity, but a large proportion of work investigating DOX cardiotoxicity has focused on the acute effects of DOX on the heart (for review see Ref.[35]). Although DOX's acute effect on the heart can be used to predict the severity of late-onset cardiotoxicity,[36] severe late-onset cardiotoxicity has been reported to manifest in the absence of severe acute cardiotoxicity.[37] The current study employed two different incremental DOX dosing schemes (daily injections and weekly injections) which our laboratory has shown previously to be associated with late-onset cardiotoxicity.[5] Besides these two incremental dosing schemes having improved survival rates when compared with bolus dosing,[5] these schemes are more clinically relevant than bolus dosing since cancer patients typically receive doses administered weekly or monthly until a cumulative dose is attained.[25,26]

Generalizations may be made in describing mechanisms responsible for acute and late-onset DOX cardiotoxicity (e.g. reactive oxygen species), but these two different forms of dysfunction are associated with unique characteristics. Simunek *et al.*[38] described acute and chronic DOX cardiotoxicity by further subdividing the pathology into

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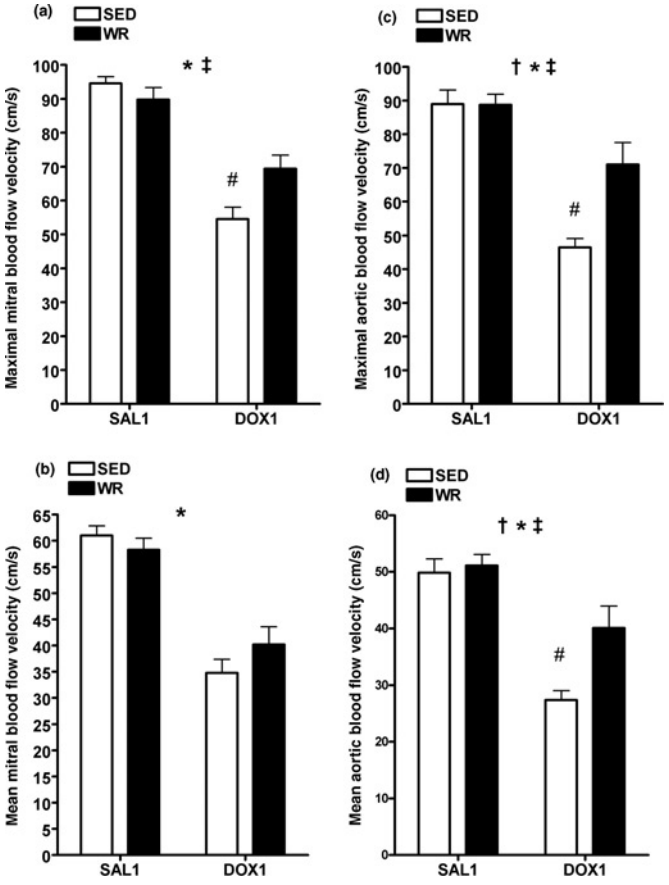

Figure 2 Echocardiography derived mitral and aortic valve blood flow from rats receiving daily injections of saline or DOX. (a) Maximal mitral blood flow velocities; (b) mean mitral blood flow velocities; (c) maximal aortic blood flow velocities; and (d) mean aortic blood flow velocities. SED, sedentary; WR, wheel running; SAL1, daily saline injections for 15 consecutive days; DOX1, daily 1 mg/kg doxorubicin injections for 15 consecutive days (15 mg/kg cumulative); †significant wheel running (activity) main effect ($P < 0.05$); *significant DOX (drug) main effect ($P < 0.05$); ‡significant activity × drug interaction ($P < 0.05$); #significantly different than WR + DOX1 ($P < 0.05$)

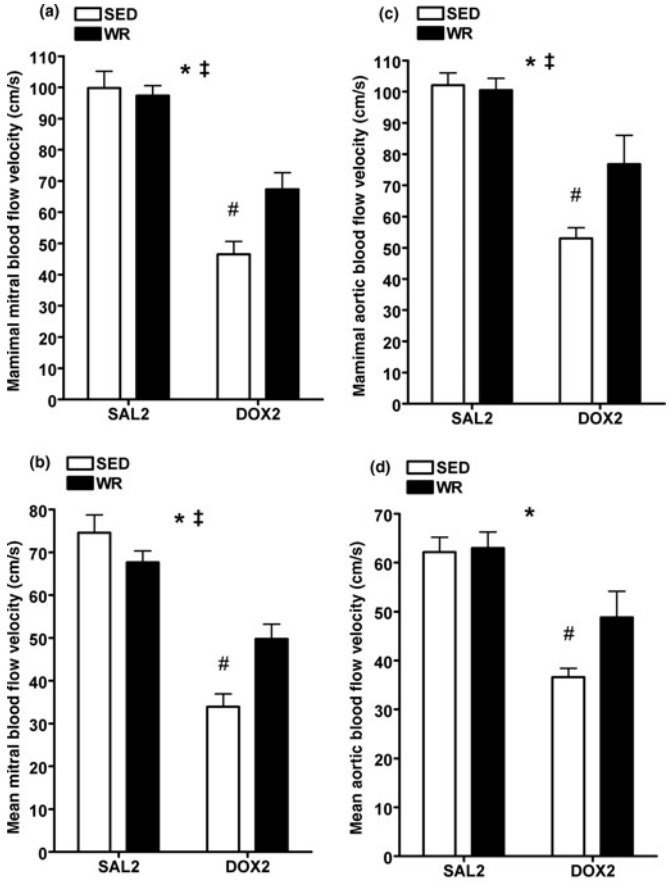

Figure 3 Echocardiography derived mitral and aortic valve blood flow from rats receiving weekly injections of saline or DOX. (a) Maximal mitral blood flow velocities; (b) mean mitral blood flow velocities; (c) maximal aortic blood flow velocities; and (d) mean aortic blood flow velocities. SAL2, weekly saline injections for six consecutive weeks; DOX2, weekly 2.5 mg/kg doxorubicin injections for six consecutive weeks (15 mg/kg cumulative); *significant DOX (drug) main effect ($P < 0.05$); ‡significant activity × drug interaction ($P < 0.05$); and #significantly different than WR + DOX1 ($P < 0.05$)

acute, subchronic, early chronic and delayed. Based on this classification system, acute DOX cardiotoxicity occurs during or immediately following treatment and generally manifests as vasodilation, hypotension and arrhythmias. Subchronic cardiotoxicity sets in 1–3 days following a high-dose treatment and is characteristic of a pericarditis–myocarditis syndrome. Much of the DOX cardiotoxicity research focuses on subchronic cardiotoxicity (1–3 days following a bolus dose), but this form of toxicity is very rare in cancer patients[38] since DOX is typically administered clinically in small incremental doses until reaching a cumulative dose. Early chronic cardiotoxicity, as exhibited in the current study, sets in later on during incremental DOX dosing or weeks to months following the completion of treatment, and it typically manifests as dilated cardiomyopathy and congestive heart failure (i.e. left ventricular contractile dysfunction). Lastly, delayed DOX cardiotoxicity is common in childhood cancer survivors where heart failure can develop years to decades following the completion of treatment.

Differences in the nature of the early chronic cardiotoxicity associated with daily and weekly DOX dosing in the current study, however, were apparent. Although significant SWs, PWs and FS DOX main effects were observed for both daily and weekly dosing, only the daily dosing resulted in significant LVDs effects signifying a dilated LV chamber at end systole. Nonetheless, these findings suggest that these incremental dosing schemes have a greater impact on systolic LV morphology when compared with diastolic LV morphology. Similarly, significant DOX main effects were observed for measured *in vivo* mitral and aortic blood flow velocities. Data obtained from *ex vivo* analysis in DOX1 and DOX2 did show a different trend, however. Although daily and weekly DOX dosing groups had similar LVDPs (77 ± 9 mmHg and 76 ± 12 in SED + DOX1 and SED + DOX2, respectively), stark differences in LVESP and LVEDP were observed. LVESP and LVEDP were observed to be higher in SED + DOX1 than in SED + DOX2 (LVESP: 91 ± 7 versus 79 ± 12 mmHg, respectively; LVEDP: 14 ± 6 versus 2 ± 4 mmHg, respectively). It appears that the daily DOX dosing (delivered with less time between doses) had a greater impact on

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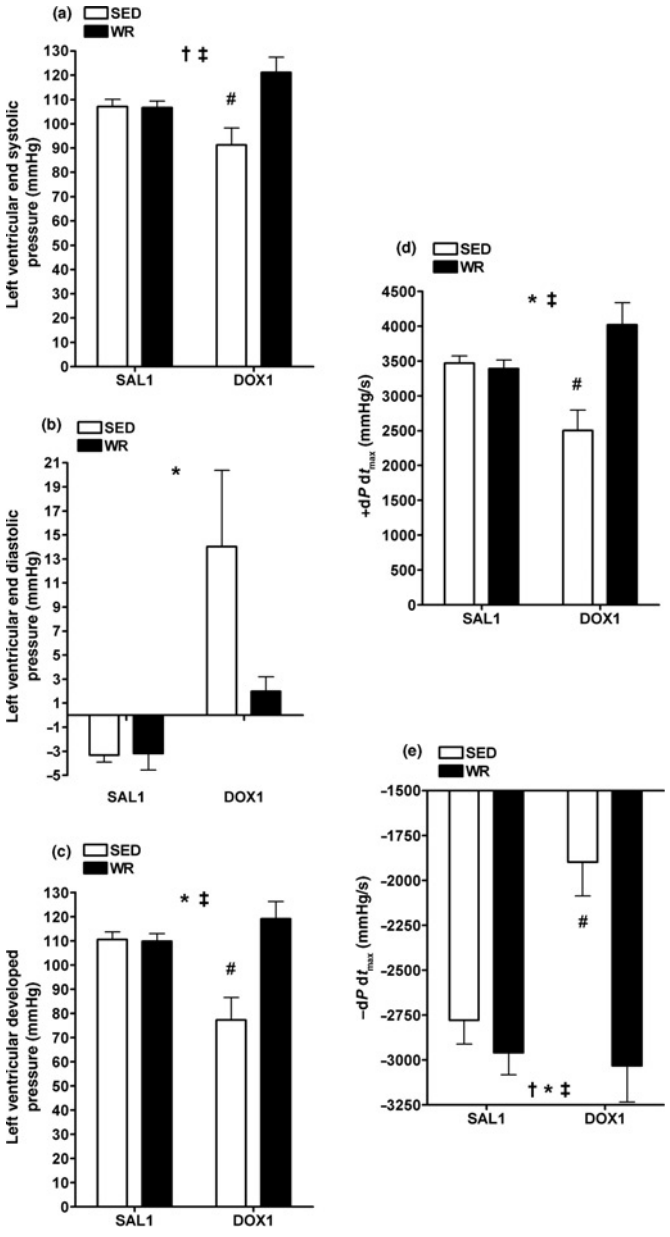

Figure 4 Left ventricular pressures obtained from an isolated working heart model from rats receiving daily injections of saline or DOX. (a) Left ventricular end systolic pressure; (b) left ventricular end diastolic pressure; (c) left ventricular developed pressure; (d) $+dPdt_{max}$; (e) $-dPdt_{max}$. SED, sedentary; WR, wheel running; SAL1, daily saline injections for 15 consecutive days; DOX1, daily 1 mg/kg doxorubicin injections for 15 consecutive days (15 mg/kg cumulative); †significant wheel running (activity) main effect ($P < 0.05$); *significant DOX (drug) main effect ($P < 0.05$); ‡significant activity × drug interaction ($P < 0.05$); and #significantly different than WR + DOX1 ($P < 0.05$)

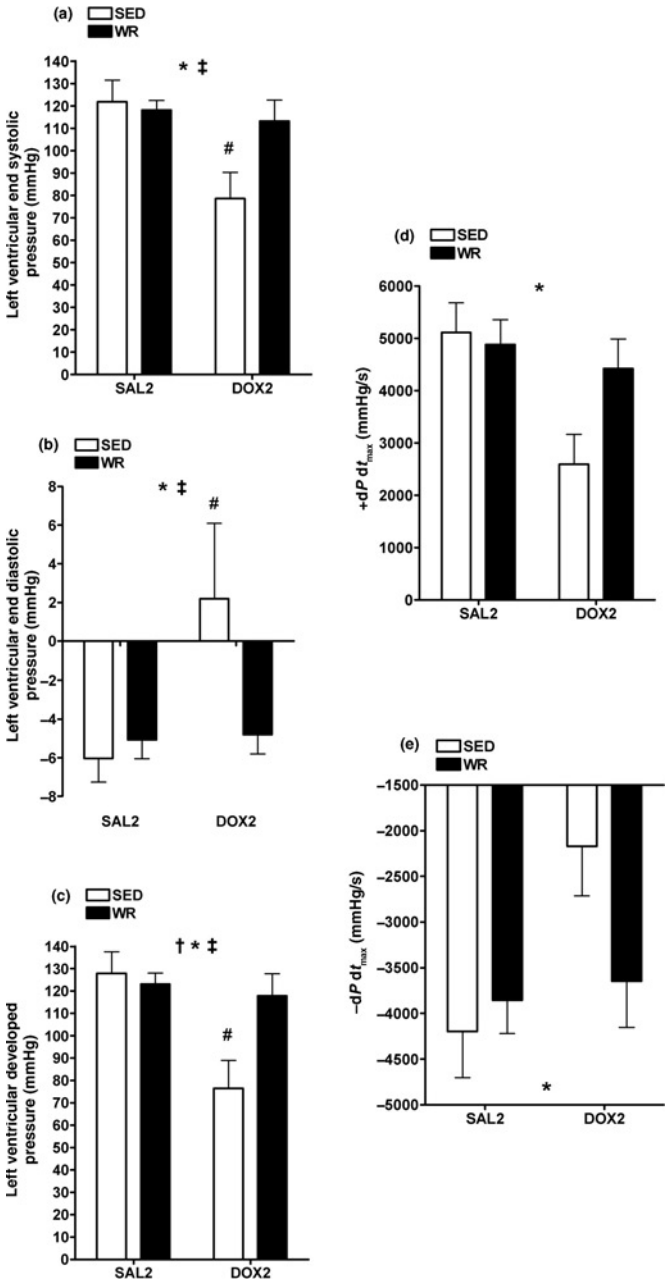

Figure 5 Left ventricular pressures obtained from an isolated working heart model from rats receiving weekly injections of saline or DOX. (a) Left ventricular end systolic pressure; (b) left ventricular end diastolic pressure; (c) left ventricular developed pressure; (d) $+dPdt_{max}$; (e) $-dPdt_{max}$. SED, sedentary; WR, wheel running; SAL2, weekly saline injections for six consecutive weeks; DOX2, weekly 2.5 mg/kg doxorubicin injections for six consecutive weeks (15 mg/kg cumulative). †Significant wheel running (activity) main effect ($P < 0.05$); *Significant DOX (drug) main effect ($P < 0.05$); ‡significant activity × drug interaction ($P < 0.05$); #significantly different than WR + DOX1 ($P < 0.05$)

ex vivo diastolic function than the weekly DOX dosing (delivered with more time between doses). With this, it should also be noted that different levels of sensitivity associated with the *in vivo* and *ex vivo* techniques employed in the current study could have contributed to the differential findings.

Voluntary wheel running-induced cardioprotection

It should first be noted that no significant wheel running effects were observed for whole heart mass and LV mass in either experimental subgroup. Although wheel running-induced cardiac hypertrophy in the rat is a common observation (for review see Ref.[39]), it has been reported previously that long-term voluntary wheel running did not result in significant cardiac hypertrophy.[28,40] Variability in running distances associated with the voluntary wheel running model in the current study must not be overlooked, and it is possible that animals

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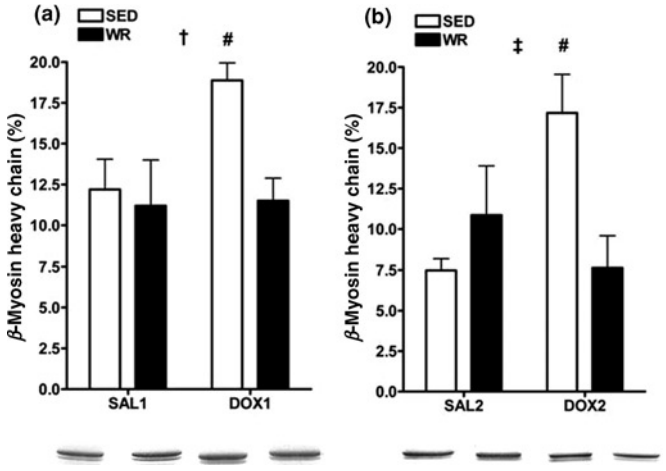

Figure 6 α- and β-myosin heavy chain expression in left ventricular homogenates with representative gel scans. The upper band on the gel scan is the α-isoform and the bottom band is the β-isoform. (a) Myosin heavy chain expression from rats receiving daily injections of saline or DOX; (b) myosin heavy chain expression from rats receiving weekly injections of saline or DOX. SED, sedentary; WR, wheel running; SAL1, daily saline injections for 15 consecutive days; DOX1, daily 1 mg/kg doxorubicin injections for 15 consecutive days (15 mg/kg cumulative); SAL2, weekly saline injections for six consecutive weeks; DOX2, weekly 2.5 mg/kg doxorubicin injections for six consecutive weeks (15 mg/kg cumulative). †Significant wheel running (activity) main effect ($P < 0.05$); ‡Significant activity × drug interaction ($P < 0.05$); (a) #Significantly different than WR + DOX1 ($P < 0.05$); (b) #significantly different than WR + DOX2 ($P < 0.05$)

running at higher volumes had greater cardiac hypertrophy signaling than animals running at lower volumes which resulted in no overall change in whole heart mass collectively.

The dose of exercise necessary for cardioprotection during DOX treatment is a topic of interest because of the likelihood that DOX treatment would render a cancer patient unable to exercise at high intensities or volumes. It is well known that exercise protects against cardiac insults, but low doses of exercise may not be adequate to provide cardioprotection against certain stresses such as ischemia reperfusion injury.[41] By spreading out DOX exposure over the course of days or weeks, the heart experiences smaller, incremental stressors when compared with bolus dosing schemes. In addition, the model employed in the current study provided what could be described as 'quasi-preconditioning' in that animals were exercising before the entire cumulative DOX dose was completed. Thus, while exercise volume was reduced in DOX-treated animals (weeks 2–10 for DOX1 and weeks 2, 6, and 10 for DOX2), this lower level of exercise combated many of the decrements in cardiac performance variables analyzed.

Although there was overall protection against DOX-induced cardiotoxicity with voluntary wheel running in both the daily and weekly doing schemes, it must be noted that differences in the nature of exercise-induced cardioprotection likely occurred. Dosing for the daily DOX group (DOX1) was completed in 15 days whereas dosing for the weekly DOX group (DOX2) was completed in six weeks. This rendered DOX1 having exposure to running wheels for ~8 weeks after the cessation of treatment until cardiac function analysis whereas DOX2 only had exposure to running wheels for four weeks after the cessation of treatment until cardiac function analysis. Therefore, besides the aforementioned differential effects of DOX dosing on running wheel distances, differences in post-treatment wheel running exposure should not be overlooked. With that, it is recommended that the effects of wheel running on the cardiotoxicity associated with the two different DOX dosing schemes be interpreted separately as opposed to collectively.

Nonetheless, the findings are especially important in translating to cancer rehabilitation practice because cancer patients experiencing fatigue are likely unable to exercise at the same level as their healthy counterparts, and as such, low to moderate intensity exercise is recommended for this population.[20–22] Although the current study did not monitor exercise intensity (only total distance data were collected), our laboratory reported previously that low-intensity exercise on a motorized treadmill during DOX treatments was protective against DOX cardiotoxicity analyzed at two weeks (at the time of DOX treatment completion).[23] The current study, however, expands on this previous work by extending the exercise intervention beyond the DOX dosing to get a sense of exercise-induced protective effects on early-chronic cardiotoxicity.

One mechanism responsible, at least in part, for the preserved left ventricular function observed in hearts from WR + DOX animals lies in the MHC isoform profile. DOX treatment is associated with a down regulation of the fast ATPase activity α-MHC isoform with a concomitant upregulation of the slow ATPase activity β-MHC isoform which has been observed both with acute[3] and chronic exposure.[24,32,42] Although the ventricular MHC profile in rats (expressing a high percentage of α-MHC with a low percentage of β-MHC) differs substantially from that of the cardiac MHC profile in humans (high percentage of β-MHC with a very low percentage of α-MHC), a small increase in β-MHC expression (with a concomitant decrease in α-MHC) results in substantial decrements in myocardial function.[43]

Nonetheless, the promotion of this DOX-induced MHC shift may partly be the result of DOX inducing metabolic disruptions (i.e. impaired oxidative phosphorylation), and the resulting limitation in ATP production promotes upregulation of the more metabolically efficient β-MHC isoform. This metabolic efficiency, however, comes at the cost of depressed cardiac function as the heart expressing more of the β-isoform contracts with lower force and slower velocities when compared with hearts expressing more of the α-isoform.[43,44] Left ventricles from wheel running animals receiving DOX had significantly lower levels of β-MHC when compared with left ventricles from sedentary animals receiving DOX regardless of the dosing scheme (DOX1 or DOX2). An extremely powerful stimulus for promoting α-MHC gene upregulation is exercise,[45] and it is likely that the wheel running stimulus during DOX treatment was sufficient to preserve α-MHC gene expression resulting in maintenance of MHC distribution.

It is likely that wheel running's protection against DOX cardiotoxicity extends to additional mechanisms including,

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but not limited to, Ca^{2+} handling, oxidative stress-induced damage and apoptosis. DOX cardiotoxicity is associated with reduced expression of calcium translocation proteins such as SERCA2a, Na/Ca^{2+} and ryanodine receptor[46] resulting in impaired Ca^{2+} homeostasis.[47,48] Because endurance exercise has a positive effect on calcium handling,[49] it is plausible that wheel running during and following incremental DOX treatment in the current study combated disrupted Ca^{2+} regulation. In addition, DOX treatment is associated with radical-induced cellular damage which may have been ameliorated by voluntary wheel running. Exercise is protective against oxidative stress induced injury elicited by ischemia reperfusion injury,[50] and exercise has been shown previously to protect against DOX-induced lipid peroxidation[33] and protein carbonyl formation.[16] Similarly, exercise plays a role in minimizing myocardial apoptotic signaling[51] which is one factor shown to be associated with DOX cardiotoxicity.[52]

Conclusion

The two incremental DOX dosing schemes administered in the current study resulted in cardiac dysfunction analyzed both *in vivo* and *ex vivo*. Allowing rats free access to running wheels during and following incremental DOX treatment was effective at attenuating much of the cardiac dysfunction despite overall suppressed running distances. Wheel running animals receiving DOX treatments had significantly lower β-MHC expression than sedentary animals receiving DOX treatments which helps to explain one mechanism behind wheel running-induced cardioprotection. Since DOX-induced fatigue is a major challenge for cancer patients, it is promising that protection against DOX-induced cardiac dysfunction does not necessarily require high volumes of endurance exercise.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data, and review of the manuscript; DSH, CYL, BTJ, TLP and RH conducted the experiments and DSH, CMS and RH wrote the manuscript.

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