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ORIGINAL ARTICLE

Endurance exercise training preserves cardiac function in rats receiving doxorubicin and the HER-2 inhibitor GW2974

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Abstract
Purpose To determine if endurance exercise training performed prior to administration of the anticancer drugs DOX and GW2974 would be cardioprotective.
Methods Rats remained sedentary or exercise trained for 10 weeks. Following the exercise or sedentary period, rats were randomly assigned to treatment groups. Rats in sedentary and exercise groups received saline or a combination of 10 mg/kg DOX and 30 mg/kg GW2974. Cardiac function was assessed 2, 5, or 10 days following treatments.
Results Sedentary animals receiving DOX/GW2974 experienced significant cardiac dysfunction. At 2-, 5-, and 10-days post, cardiac function in trained, drug-treated animals was significantly preserved. Additionally, animals exercised prior to DOX/GW2974 injections had significantly lower levels of myocardial lipid peroxidation and caspase-3 and -8 activities compared to their sedentary counterparts.
Conclusions Exercise training protected against the cardiac dysfunction associated with DOX/GW2974 administration and may be related to an inhibition in apoptotic signaling.

Keywords Anthracyclines · Cardiotoxicity · Caspase · Heart · Physical activity

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Introduction

Doxorubicin (DOX) is a highly effective anthracycline antineoplastic agent used to treat a variety of malignancies and tumors. Despite being one of the most effective chemotherapeutic agents, its use is limited by a serious and sometimes life-threatening cardiotoxicity [28]. DOX cardiotoxicity is the single most important factor in determining whether a patient will undergo, or continue with, this cancer treatment regimen. Utilization of this drug is often abandoned upon evidence of cardiac dysfunction which, considering its effectiveness, could decrease the likelihood of cure and increase the likelihood of mortality. Although improvements in cancer treatment strategies have been witnessed in recent decades, DOX cardiotoxicity remains a clinical dilemma.

The human epidermal growth factor receptor-2 (HER2) belongs to a family of genes involved in the regulation of normal breast growth and development. Overexpression of HER2 occurs in approximately 30% of breast cancers and is associated with metastasis, early relapse, and shorter survival [12, 45]. In response to this, attempts to develop drugs targeted at HER2 have resulted in the production of the anti-HER2 antibody trastuzumab (Herceptin®). Incorporation of trastuzumab into treatment regimens for patients with HER2-positive tumors has significantly improved disease-free survival and overall survival [20, 35, 42].

Most clinical studies support the use of anthracyclines and trastuzumab in the treatment of HER2-positive breast cancers [30, 39, 47], and, when used in combination, these drugs have proven to be effective in the treatment of breast cancer [6, 31]. However, use of these treatments is limited by unacceptably high rates of cardiotoxicity. When used independently, DOX [48] and trastuzumab [44] can induce cardiac dysfunction, and there appears to be an exacerbation

of this cardiotoxicity in patients who have undergone concurrent or sequential treatment with trastuzumab and anthracyclines [32, 33].

There is a growing body of work with animal models indicating that exercise training can protect the heart against DOX cardiotoxicity [4, 5, 8, 9, 18]. However, no studies have been conducted to determine if exercise preconditioning can protect against the cardiotoxicity of DOX when it is combined with an additional treatment known to induce cardiac dysfunction, namely HER2-inhibitors. Thus, the primary purpose of this study was to determine whether exercise training results in cardioprotection against the cardiac dysfunction that accompanies combined DOX/HER2-inhibitor treatment. We hypothesized that exercise training would protect against DOX/HER2-inhibitor-induced cardiotoxicity and that the cardioprotective effects would be associated with an attenuation of cardiac oxidative stress and apoptotic signaling.

Methods

Animal care

Female Sprague–Dawley rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). All animals were housed two per cage in a temperature-controlled facility, provided standard rat chow and water ad libitum, and were adapted to a 12:12 h light-dark cycle. Exercise was performed on a motorized treadmill (Exer 3/6 model; Columbus Instruments, OH). One week prior to beginning the exercise training protocol, all rats were habituated to treadmill exercise by walking at a speed of 13 m/min, 15 min per day, for 5 days. Rats in the exercise groups were trained for 10 weeks while rats in the sedentary groups were restricted to cage activity for the 10 weeks prior to drug treatment. All proposed protocols were approved by the University of Northern Colorado Institutional Animal Care and Use Committee (IACUC) and were in compliance with the Animal Welfare Act guidelines.

Exercise training protocol

Following the acclimation period, all animals in the exercise groups ran on a motorized rodent treadmill following a progressive training protocol adapted from Powers et al. [37]. Animals trained 5 days per week (Monday–Friday) for 10 weeks during their dark cycle. Rats initially ran at 13 m/min up a 5% grade for 20 min. Exercise intensity and duration was gradually increased during weeks 1–4 until reaching 30 m/min, 18% grade, for 60 min. This workload was maintained for the remainder of the study.

Drug treatment

GW2974 was selected as the HER2 inhibitor for these experiments due to the fact that Herceptin® does not effectively bind to rat HER2 (neu). GW2974 is not an antibody, but rather, it is a small molecule inhibitor that blocks the action of HER2 (neu). In vivo, administration of 30 mg/kg GW2974 inhibits average tumor growth by 95% after 21 days of treatment in tumors over-expressing HER2 [43]. We conducted a series of pilot experiments to assess the degree of cardiac dysfunction associated with combined treatment with DOX and GW2974. In these experiments, nine groups of rats ($N = 5$ per group) were treated with increasing concentrations of DOX (0, 5, or 10 mg/kg) and GW2974 (0, 15, or 30 mg/kg). At both the 15 and 30 mg/kg doses, GW2974 exacerbated the cardiac dysfunction associated with DOX treatment. DOX alone at 10 mg/kg resulted in a 22% decline ($p \leq 0.05$) in left ventricular developed pressure (LVDP) while a combination of DOX at 10 mg/kg and GW2974 at 30 mg/kg resulted in a 38% decline ($p \leq 0.05$) in LVDP. With this dosage combination, animals appeared to tolerate treatment well and all animals survived. Thus, for all drug treatments, DOX was administered at 10 mg/kg and GW2974 was administered at 30 mg/kg.

Twenty-four hours following the completion of the exercise training or sedentary period, animals were randomized into one of four experimental groups: sedentary + saline (SED + SAL), exercised + SAL (EX + SAL), SED + DOX/GW2974 (SED + D/GW), and EX + DOX/GW2974 (EX + D/GW). The SED + D/GW and EX + D/GW groups were administered a 10 mg/kg i.p. bolus injection of DOX-HCl (Bedford Labs, Bedford, OH) immediately followed by a 30 mg/kg i.p. injection of GW2974 (Sigma-Aldrich, St Louis, MO). Rats in the SED + SAL and EX + SAL groups received two separate 1 mL injections of 0.9% sterile saline in lieu of DOX and GW2974. Animals were sacrificed 2, 5, or 10 days following injections.

Cardiac function

At the scheduled time of sacrifice, each rat was anesthetized with heparinized sodium pentobarbital (50 mg/kg, i.p.). Ex vivo cardiac function was analyzed using a constant flow isolated perfused working heart preparation in which the perfusate was not recycled. Following confirmation of anesthesia, the heart was rapidly excised and immersed in ice-cold Krebs–Henseleit buffer, consisting of 120 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM MgCl, 25 mM $NaHCO_3$, 17 mM glucose, and 0.5 mM EDTA. Within 1 min, the aorta was cannulated and the heart was subjected to retrograde perfusion with the Krebs–Henseleit buffer. The buffer was saturated with 95% O_2 and 5% CO_2 and

maintained at 37°C in a water-jacketed reservoir throughout the duration of the experiment. To assess cardiac function using the working heart preparation, the pulmonary vein was cannulated and flow was re-directed to enter through the left atrium and exit through the aorta. Preload was set at 10 cmH_2O and afterload was set at 100 cmH_2O. Following cannulation of the pulmonary vein, each heart was allowed to equilibrate for 15 min before data collection.

To assess left ventricular function, a microtip catheter pressure transducer (Millar Instruments Inc., Houston, TX) was inserted directly into the left ventricular cavity through the apex of the heart. Each heart was paced at 300 bpm using an electrode clipped to each cannula. After a 2–3 min stabilization period, four-second samples of LVDP, diastolic pressure, and the rate of pressure development and relaxation (dP/dt_{max} and dP/dt_{min}, respectively) were recorded using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO). At the end of the data collection period, the heart was blotted dry, and weighed. The left ventricle was then isolated, frozen in liquid nitrogen, and stored at –80°C for biochemical analyses.

Myocardial lipid peroxidation

Approximately 100 mg of frozen left ventricular tissue was pulverized in liquid nitrogen using 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 w/v. The tissue was homogenized using a glass tissue homogenizer. Sample homogenates were then centrifuged at 3,000*g* for 10 min at 4°C and the supernatant was assessed for malondialdehyde and 4-hydroxy-alkenals (MDA + 4-HAE) analysis. Total protein concentration was determined using the method of Bradford (27) with bovine serum albumin (BSA; Pierce, Rockford, IL) as the reference standard.

A commercially available assay kit (OXIS International, Inc., Portland, OR) was used to measure myocardial lipid peroxidation as an indicator of DOX-induced oxidative injury in the heart. The MDA + 4-HAE assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA at 45°C. To perform the assay, 200 μL of left ventricular homogenate was added to 10 μL of the antioxidant probucol and 640 μL N-methyl-2-phenylindole in acetonitrile and briefly vortexed. Next, 150 μL of concentrated HCl was added, vortexed, and incubated at 45°C for 60 min. Samples were then centrifuged for 10 min at 10,000*g* 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 a MDA standard curve to yield the final concentration of MDA + 4-HAE (μM). All samples were assayed in duplicate and any samples varying more than 5% were reassayed.

Caspase-3 and caspase-8 activity

Apoptosis is associated with increases in the activity of both caspase-3 and caspase-8. Two activity assay kits were used to assess caspase-3 and caspase-8 activities in cardiac tissue in vitro (Chemicon International, Temecula, CA). For each assay, tissue samples from the left ventricular free wall were suspended in a lysis buffer, homogenized using a glass tissue homogenizer for approximately 5 min, and centrifuged at 14,000*g* for 10 min. The supernatant was then diluted with the assay buffer specific to each kit and incubated with a colorimetric substrate per instructions in a 96-well microtiter plate and read using a microtiter plate reader (Molecular Devices, Sunnyvale, CA). Both caspase-3 and caspase-8 activities were determined by measuring the cleavage of the p-nitroaniline (p-NA) dye from the protein-substrate solution at an absorbance of 405 nm using the plate reader. A higher absorbance reading with the plate reader indicated increased caspase activity.

Statistical analyses

A three-factor (group × drug × time) analysis of variance (ANOVA) was used to determine differences due to the main effects (group, drug, and time) and interaction of these three factors. Two levels of group, two levels of drug, and three levels of time were analyzed. In the presence of significant interactions, Tukey post-hoc tests were completed to identify where differences occurred. A significance level of $p < 0.05$ was used for all statistical analyses.

Results

General observations

Table 1 presents animal characteristics observed before the exercise/sedentary period, at the time of drug treatment, and at the time of sacrifice. At the time of D/GW or saline injections, there were no significant differences in body mass between any of the sedentary or exercise groups. The combination of DOX and GW2974 treatment resulted in significant weight loss at 2, 5, and 10 days post injections for both the sedentary and exercise groups (Table 1).

Table 1 Animal characteristics

	SED ± SAL	EX ± SAL	SED ± D/GW	EX ± D/GW
2-Day				
Starting BM (g)	189 ± 3	179 ± 3*	187 ± 2	188 ± 2
Injection BM (g)	254 ± 6	258 ± 5	252 ± 3	253 ± 4
Sac BM (g)	256 ± 5	264 ± 6	241 ± 2*	240 ± 4*
Change in BM (%)	+0.66 ± 0.2	+2.4 ± 0.16*	–4.4 ± 0.5*	–5.2 ± 0.1*
Heart Mass (mg)	1,079 ± 27	1,128 ± 20	1,007 ± 29	1,080 ± 26
Heart Mass (mg/g BM)	4.24 ± 0.18	4.27 ± 0.09	4.19 ± 0.15	4.49 ± 0.08
5-Day				
Starting BM (g)	188 ± 4	191 ± 4	184 ± 3	183 ± 4
Injection BM (g)	261 ± 3	256 ± 3	251 ± 6	249 ± 6
Sac BM (g)	258 ± 5	271 ± 3	235 ± 7*	229 ± 6*
Change in BM (%)	+1.0 ± 0.4	+5.3 ± 0.1*	–6.7 ± 0.14*	–8.7 ± 0.1*
Heart Mass (mg)	1,024 ± 31	1,201 ± 22*	957 ± 19	1,056 ± 39
Heart Mass (mg/g BM)	3.96 ± 0.12	4.43 ± 0.09	3.71 ± 0.17	4.63 ± 0.18*
10-Day				
Starting BM (g)	184 ± 3	186 ± 3	191 ± 2	186 ± 3
Injection BM (g)	245 ± 5	255 ± 3	253 ± 4	254 ± 4
Sac BM (g)	256 ± 7	270 ± 4	225 ± 10*	236 ± 4*
Change in BM (%)	+4.7 ± 0.28	+5.3 ± 0.25	–12.6 ± 0.6*	–7.7 ± 0.1*
Heart Mass (mg)	1,038 ± 34	1,137 ± 42	860 ± 90	971 ± 25
Heart Mass (mg/g BM)	4.05 ± 0.12	4.22 ± 0.17	3.75 ± 0.43	4.06 ± 0.11

SED + SAL = sedentary saline, EX + SAL = exercise saline, SED + D/GW = sedentary DOX/GW2974, EX + D/GW = exercise DOX/GW2974, BM = body mass, Sac = sacrifice. Values are means ± SE

* $p < 0.05$ versus SED + SAL

Cardiac function

At the scheduled time of sacrifice, each rat was anesthetized with heparinized sodium pentobarbital and ex vivo cardiac function was analyzed using a constant flow isolated perfused working heart preparation. All cardiac function data are presented in Figs. 1 and 2.

As expected, GW2974 and DOX treatment resulted in significant cardiac dysfunction that worsened over time. This was evidenced by significant decreases in LVDP in the sedentary drug-treated groups (Fig. 1). In comparison to the SED + SAL groups, the SED + D/GW groups experienced a 27% decrease in LVDP 2 days after injections, a 49% decrease 5 days post injections, and a 55% decrease 10 days post injections ($p < 0.05$). Ten weeks of exercise training protected against this dysfunction, as LVDP values from the EX + D/GW groups were not significantly different from SED + SAL at 2 and 5 days after injections ($p > 0.05$). At 10 days post, LVDP had declined by 35% from SED + SAL values in the EX + D/GW group ($p < 0.05$).

A similar trend was observed with dP/dt_{max} and dP/dt_{min} values, as well (Fig. 2). In comparison to the SED + SAL groups, the SED + D/GW groups experienced a 27, 23, and

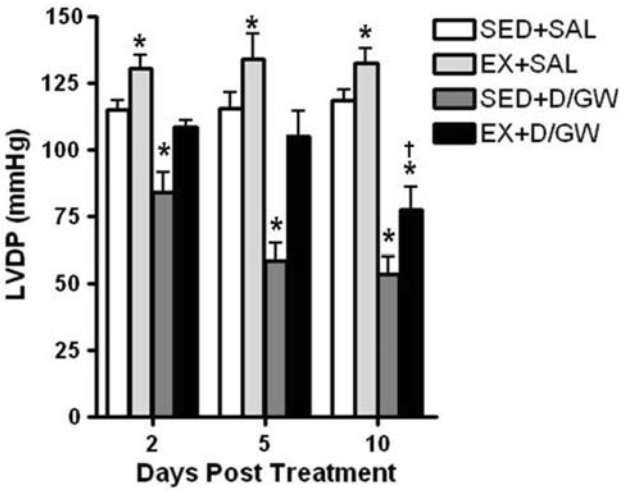

Fig. 1 Left ventricular developed pressure in sedentary and treadmill trained rats receiving SAL or a combination of DOX and GW2974. Values are mean ± SE. *$p < 0.05$ versus SED + SAL at same time point. †$p \leq 0.05$ versus SED ± D/GW at same time point, versus EX ± D/GW at 2 days, and versus EX ± D/GW at 5 days. *SED* sedentary, *EX* exercise trained, *SAL* saline, *D/GW* doxorubicin in combination with GW2974

34% decrease in dP/dt_{max} at 2 ($p < 0.05$), 5, and 10 days ($p < 0.05$) after injections, respectively. Likewise, dP/dt_{min} was 26, 28, and 21% lower in the SED + D/GW group

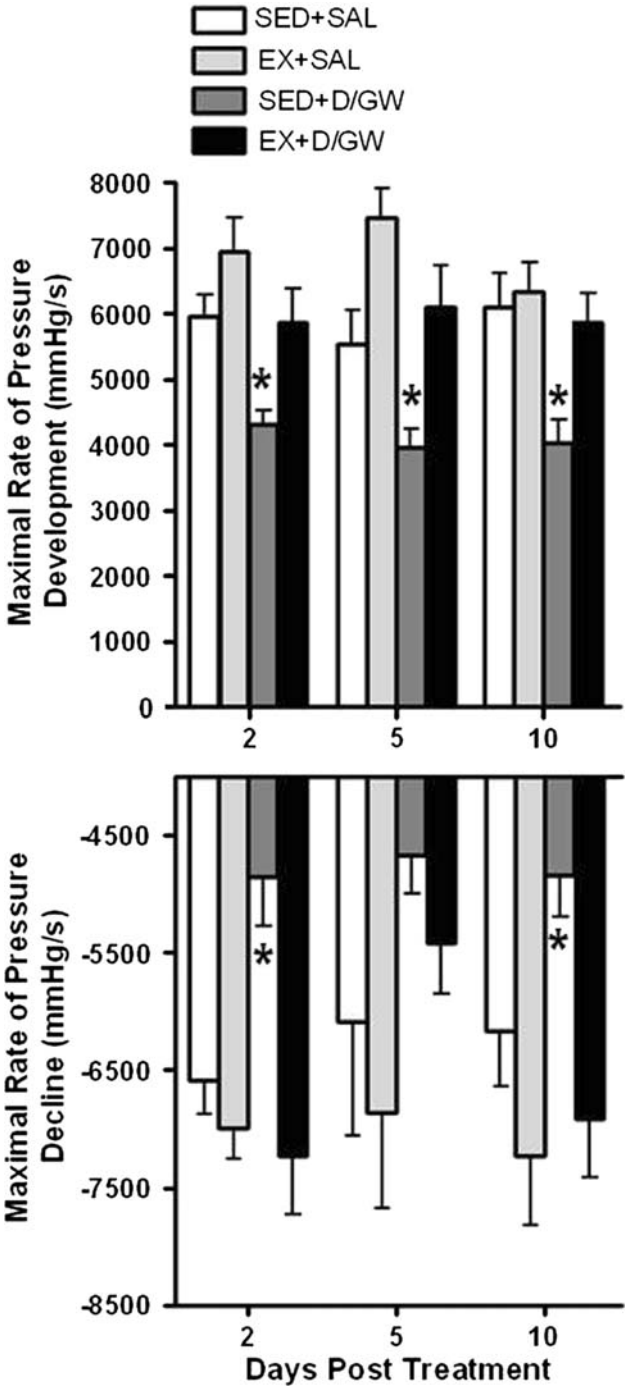

Fig. 2 Rate of left ventricular pressure development (dP/dt_{max}, *top panel*) and decline (dP/dt_{min}, *bottom panel*) in sedentary and treadmill trained rats receiving SAL or a combination of DOX and GW2974. Values are mean ± SE. *$p < 0.05$ versus SED + SAL at same time point. *SED* sedentary, *EX* exercise trained, *SAL* saline, *D/GW* doxorubicin in combination with GW2974

compared to SED + SAL values at 2, 5, and 10 days post injections, respectively ($p < 0.05$). dP/dt_{max} and dP/dt_{min} values from the EX + D/GW did not differ significantly from the SED + SAL groups at any time point ($p > 0.05$).

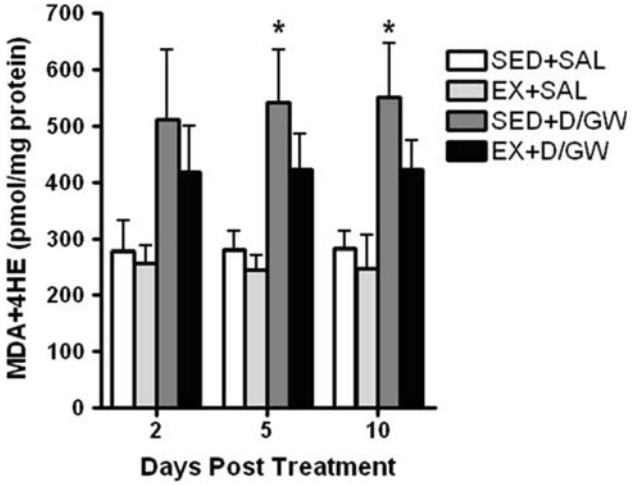

Fig. 3 Myocardial lipid peroxidation in left ventricular homogenates. Values are mean ± SE. *MDA* malondialdehyde, *4-HAE* 4-hydroxy-alkenals. *$p < 0.05$ versus SED + SAL at same time point. *SED* sedentary, *EX* exercise trained, *SAL* saline, *D/GW* doxorubicin in combination with GW2974

Biochemical analyses

Left ventricular homogenates prepared from rats following their scheduled time of sacrifice were analyzed for indices of oxidative stress and apoptosis. All data from biochemical analyses are presented in Figs. 3 and 4.

MDA + 4-HAE, products of lipid peroxidation, were examined to provide an index of oxidative stress in the myocardium (Fig. 3). At 2 days post treatment, there were no significant differences observed among any group. However, at 5 and 10 days after injections, SED + D/GW was 48 and 49% higher than SED + SAL, respectively ($p < 0.05$). Myocardial MDA + 4-HAE levels in the EX + D/GW did not significantly differ from SED + SAL at any time point.

Caspase-3 and -8 activities (Fig. 4) were used to measure apoptosis in the myocardium. Caspase-3 activity in the SED + D/GW group was significantly higher than all other groups at each time point. At 2 days after injections, caspase-3 activity in the SED + D/GW group was 56% higher than the SED + SAL group ($p < 0.05$). At 5 days post injections, caspase-3 activity was 59% higher ($p < 0.05$), and at 10 days post, caspase-3 activity was 55% higher than the SED + SAL group ($p < 0.05$). On other hand, caspase-3 activity in the EX + D/GW groups was not significantly different than the saline treated groups at any time point ($p > 0.05$).

Caspase-8 activity in the SED + D/GW groups were significantly higher than the saline treated groups at each time point. At 2 days after injections, the caspase-8 activity of SED + D/GW was 69% higher than the SED + SAL group ($p < 0.05$). At 5 days post injections, caspase-8 activity was

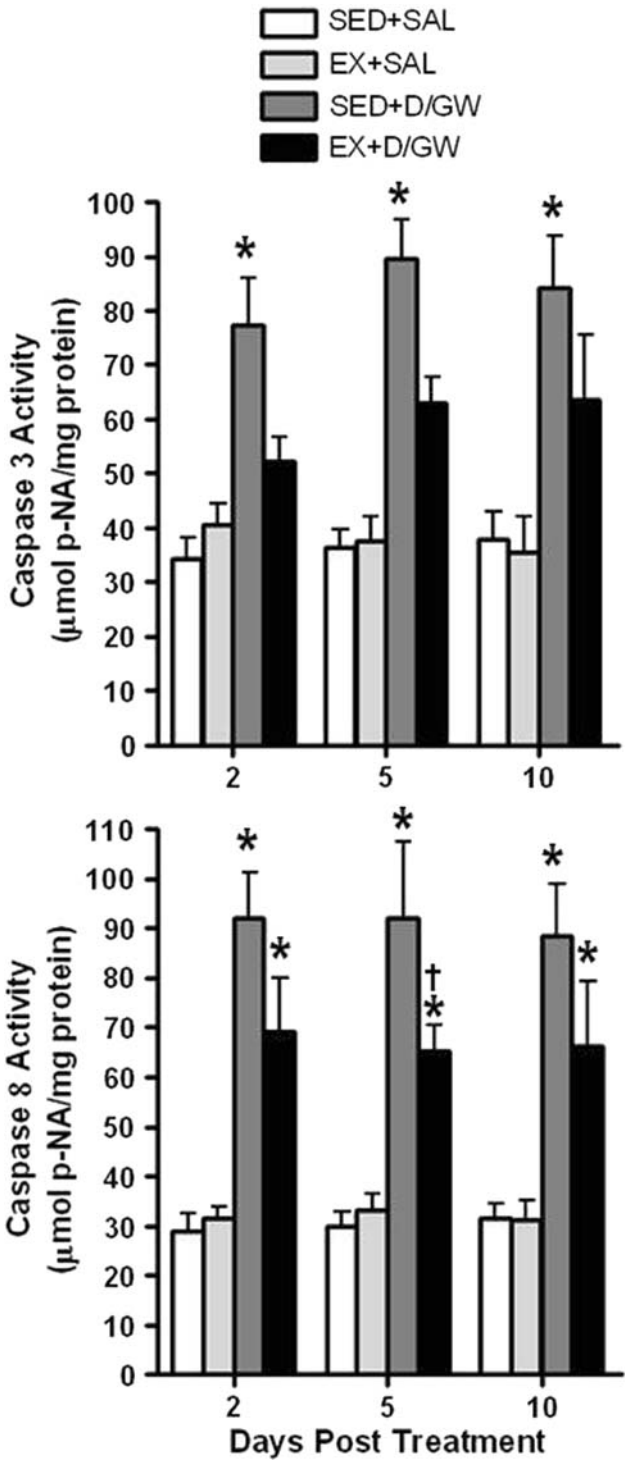

Fig. 4 Caspase-3 and -8 activities in left ventricular homogenates. Values are means ± SE. *$p < 0.05$ versus SED + SAL at same time point. †$p < 0.05$ versus SED + D/GW at same time point. *SED* sedentary, *EX* exercise trained, *SAL* saline, *D/GW* doxorubicin in combination with GW2974

68% higher ($p < 0.05$), and at 10 days post, caspase-8 activity was 64% higher in the SED + D/GW groups ($p < 0.05$), compared to the SED + SAL groups ($p < 0.05$). In addition, caspase-8 activity for EX + D/GW was significantly higher than the SED + SAL groups at each time point ($p < 0.05$). However, the rise in caspase-8 activity observed in the SED + D/GW groups was blunted in the EX + D/GW groups, which were 25, 29, and 25% lower than the SED + D/GW groups at 2, 5, and 10 days, respectively.

Discussion

This study examined the effects of exercise training on DOX/GW2974-induced cardiac dysfunction. Results indicated that combined treatment with DOX and the HER2-inhibitor GW2974 caused cardiac dysfunction, evidenced by significant decreases in LVDP, dP/dt_{max}, and dP/dt_{min} at 2, 5, and 10 days after treatment. In addition, the combination of DOX/GW2974 resulted in significant increases in myocardial lipid peroxidation, as well as increases in caspase-3 and caspase-8 activities at all time points. Ten weeks of treadmill exercise training prior to DOX/GW2974 treatment mitigated the decline in cardiac function. This exercise-induced cardioprotection was associated with less myocardial lipid peroxidation and lower caspase-3 and caspase-8 activities. This is the first study to demonstrate that exercise training, when conducted prior to treatment, is cardioprotective against DOX/GW2974-induced cardiac dysfunction.

There is an abundance of evidence indicating that breast cancer treatments with trastuzumab increase the incidence of cardiac dysfunction and heart failure [13, 36, 44, 49, 51]. The mechanisms responsible for trastuzumab-induced cardiac dysfunction have yet to be elucidated, but it has been suggested that HER2 inhibition may interfere with cardiac survival and repair mechanisms that are mediated by HER2 heterodimers [32, 47]. This is supported by studies in mice demonstrating that HER2 is critical for normal cardiac development [25] and, if selectively deleted, will result in severe dilated cardiomyopathy [10, 29]. In addition to its effects on normal cardiac development, inhibition of HER2 has been shown to directly impair cardiomyocyte contraction and induce apoptosis [40]. Additional evidence for the role of this pathway in normal cardiac function can be found in heterozygous neuregulin (NRG)-1 knockout mice. Neuregulins are a family of growth factors, produced by the endocardium and endothelium of the cardiac vasculature, that bind HER heterodimers in order to promote growth and survival of cardiomyocytes [25]. $NRG^{+/-}$ mice exhibit a significantly lower survival rate and are rendered more susceptible to DOX induced cardiac dysfunction when compared to wild-type mice [27]. Kuramochi et al. [24] demonstrated that oxidative stress in cell culture and in the intact mouse heart activated NRG/HER signaling

pathways, suggesting that this pathway may be important in cardiac adaptations to oxidative stress.

This adaptation is particularly important in our model since DOX is known to induce cardiac oxidative stress. While several mechanisms are likely responsible for DOX-induced cardiac dysfunction (e.g., impaired calcium handling [3], alterations in cardiac contractile proteins [11], impaired mitochondrial respiration [5]), a large body of work has been completed indicating that DOX cardiotoxicity is initiated by oxidative stress [1, 2, 14, 15, 21–23]. The results of DOX-induced oxidative stress are widespread and may be manifested as altered cellular function (via oxidation of cellular components such as membranes and proteins) or the induction of apoptosis. It is generally believed that DOX-induced cardiotoxicity is mediated in large part by the production of reactive oxygen species (ROS). Specifically, DOX has been reported to produce hydroxyl radical, superoxide, as well as hydrogen peroxide [26], resulting in an increase in numerous indicators of oxidative stress.

The underlying hypothesis of DOX/HER2-inhibitor cardiotoxicity is that DOX treatment elicits cardiac oxidative stress and leads to an induction of apoptosis, both of which impair contractile function. Cardiac dysfunction is then exacerbated by HER2-inhibitor treatment which may (1) directly reduce cardiomyocyte contractility, (2) induce cardiac apoptosis independent of any interaction with DOX, and (3) inhibit one of the primary compensatory mechanisms that may protect against DOX-induced oxidative stress; specifically, NRG/HER signaling pathways. Data presented here support the hypothesis that combined DOX/HER2 treatment increases cardiac oxidative stress and stimulates apoptotic pathways.

Our laboratory has completed a series of studies investigating the utility of exercise preconditioning in alleviating the cardiotoxicity of DOX [8, 9, 18], yet this is the first study to show that exercise training can protect against DOX-induced cardiac dysfunction even when it is combined with a HER2-inhibitor. Attenuations in cardiac MDA and caspase activities support our hypothesis that exercise training protects against cardiac oxidative stress and the accompanying upregulation of apoptotic pathways. Exercise training has been shown to protect the heart against oxidative stress by increasing glutathione and upregulating cardiac copper/zinc-superoxide dismutase, manganese–superoxide dismutase, catalase, and glutathione peroxidase activities, and protein expression [16, 17, 37, 38]. These exercise-induced increases in the antioxidant status of the heart have been shown by others to be beneficial under conditions of oxidative stress. Taylor et al. [50] reported that 9 weeks of endurance exercise training preserved cardiac output and coronary blood flow after exposure to hydrogen peroxide. Likewise, Brown et al. [7] demonstrated that exercise trained rats increased the expression of antioxidant proteins and this was associated with protection against myocardial ischemia-reperfusion injury, a condition mediated in large part by the release of ROS.

Considering that oxidative stress is a primary means by which apoptosis is activated, it follows that a reduction in DOX/GW2974-mediated oxidative stress may limit the activation of apoptotic pathways. Apoptosis depends on the activation of caspases, and there are two primary pathways of caspase stimulation: (1) by forming a complex between the cell surface receptor Fas, and Fadd (Fas-associated death domain) protein, leading to the activation of caspases, and (2) by the release of mitochondrial cytochrome c and its subsequent complexing with apoptosis activating factor (Apaf-1) and pro-caspase-9, thereby leading to caspase activation. The Bcl-2 family of proteins regulates the integrity of the outer membrane barrier and blocks cytochrome c release into the cytosol (anti-apoptotic), while Bax induces the release of cytochrome c by facilitating the formation of mitochondrial membrane pores. Exposure of myocytes to oxidative stress (e.g., H_2O_2 or O_2^-) can result in the translocation of Bax and the activation of p53, both of which stimulate cardiomyocyte apoptosis. While cardiomyocyte apoptosis may be a normal process of aging and possibly involved with organ maintenance [34], exercise training per se does not induce cardiomyocyte apoptosis [19]. In fact, exercise training has been shown to protect against cardiomyocyte apoptosis following ischemia-reperfusion and exposure to DOX [5, 41]. While the exact mechanism remains unclear, it is possible that exercise training modifies upstream regulation of the caspases. This is supported by observations that 8 weeks of exercise training increases Bcl-2 protein and transcript levels, decreases Bax transcript levels, and decreases Apaf-1 protein levels in rat cardiac muscle [46]. It is therefore possible that exercise-induced cardioprotection against DOX/GW2974 cardiotoxicity may be related to the regulation of pro- and anti-apoptotic signaling.

In conclusion, these data demonstrate that exercise training protected against DOX-induced cardiac dysfunction even when accompanied by a secondary treatment (i.e., the HER2 inhibitor GW2974) known to exacerbate DOX cardiotoxicity. Specifically, exercise training preserved cardiac function following DOX/HER-inhibitor treatment, and this preservation in function was associated with an attenuation of oxidative stress and apoptotic signaling.

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Conflict of interest statement None.

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