Effects of aerobic exercise on antioxidants in rat models with cardiomyopathy

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Objective: In this study, we aimed to test the hypothesis that aerobic exercise might exert its cardio-protective effect by preventing oxidative stress and improving cardiac function in rat models with doxorubicin-induced cardiomyopathy.

Design: Randomized controlled trial.

Methods: We randomly divided experimental rats into four groups: the normal group was used as a non-cardiomyopathy normal control (n=10); the control group included non-aerobic exercise after doxorubicin-induced cardiomyopathy (n=10); the experimental group I included aerobic exercise (3 m/min) after doxorubicin-induced cardiomyopathy (n=10); and experimental group II included aerobic exercise (8 m/min) after doxorubicin-induced cardiomyopathy. Rats in the treadmill training groups underwent treadmill training, which began at 2 weeks after the first intraperitoneal injection. At the end of the exercise period, we determined the heart weight change for each rat. Changes in the levels of oxidative stress enzymes (superoxide dismutase [SOD], thiobarbituric acid-reactive substances [TBARS], and catalase) in the cardiac tissue of rats from all four groups were examined at the end of the experiment.

Results: Significant cardiac myocyte injury and increase in myocardial TBARS concomitant with a reduction in myocardial SOD and catalase were observed following cardiomyopathy ($p<0.05$). Significant cardiac tissue and increase in myocardial TBARS along with reduction in myocardial SOD and catalase were observed following cardiomyopathy ($p<0.05$). Oxidative parameters were significantly improved in the aerobic exercise groups compared with the control group.

Conclusions: These findings indicate that aerobic exercise effectively prevents oxidative stress in rat models with cardiomyopathy.

Key Words: Cardiomyopathies, Exercise, Oxidative stress

Introduction

Cardiomyopathy related to disease of the heart muscle has several causes, including obesity, excessive alcohol intake, inflammation, stress, nutritional deficiencies, genetic disorder, and post-childbirth [1]. Many pathological processes affect muscles and cause a loss of functional cardiac muscle cells. In cardiomyopathy, the heart muscle becomes enlarged, thick, or rigid [2,3]. As cardiomyopathy worsens, the heart becomes weaker. Oxidative stress plays a major role in the biochemical and pathophysiological changes associated

with cardiomyopathy. Cardiomyopathy has been shown to be associated with increased oxidative stress, as evidenced by an increase in myocardial thiobarbituric acid-reactive substances (TBARS) and depletion of myocardial endogenous antioxidants such as C-reactive protein, superoxide dismutase (SOD), catalase, glutathione, and glutathione peroxidase [4-6]. Similar observations have been made previously in different studies using similar models [7,8]. Cardiac rehabilitation focuses on relieving symptoms and improving function. This may include exercise training and diet supplementation as methods for regulating oxidative

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stress [9]. Gielen *et al.* [10] reported that exercise training improves cardiovascular function in individuals with cardiovascular disease. Exercise has beneficial effects on their activities of daily living, health-related quality of life, and ultimately their hospital admission rate and mortality for cardiovascular disease [11-14]. Doxorubicin, an anthracycline antibiotic, is widely used as an effective antineoplastic agent in the treatment of a variety of malignancies, including leukemia, lymphoma, and solid tumors [15]. Unfortunately, the clinical use of this drug is limited by cumulative dose-related cardiotoxicity, which may lead to a severe and irreversible form of cardiomyopathy [16]. Doxorubicin used to treat cancer has the serious side effect of dilated cardiomyopathy [17,18]. In view of this observation, the present study was designed to investigate whether aerobic exercise could offer protection against oxidative stress arising in a rat model out of cardiomyopathy.

Methods

Cardiomyopathy rat model

Forty 6-week-old male Sprague-Dawley rats, weighing 120.0 ± 5.0 g were used following a 1-week acclimatization period. The rats were housed at a temperature of $25.0^\circ\text{C} \pm 1.0^\circ\text{C}$ and a humidity level of $55 \pm 2\%$ with a 12-h light-dark cycle; they had free access to food and water. All animal experimental protocols were performed in accordance with the guidelines of the Dongshin University Animal Care and Use Committee. We randomly divided experimental rats into four groups: the normal group was used as a non-cardiomyopathy normal control ($n=10$); the control group included non-aerobic exercise after doxorubicin-induced cardiomyopathy ($n=10$); the experimental group I included aerobic exercise (3 m/min) after doxorubicin-induced cardiomyopathy ($n=10$); and experimental group II included aerobic exercise (8 m/min) after doxorubicin-induced cardiomyopathy.

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Aerobic exercise and biochemical analysis

Doxorubicin (10 mg/kg, Sigma-Aldrich Co., St. Louis, NY, USA) was dissolved in normal saline. The doses of doxorubicin used in this study were based previous reports [19]. Rats in the treadmill training group underwent treadmill training, which began at 2 weeks after first intraperitoneal injection. Treadmill exercise was performed according to a previously described method [20]. The treadmill velocity was set at 3 m/min (experimental group I) and 8 m/min (experimental group II) at a 0° degree incline, and treadmill exercise was performed by rats over a 21-day period. Rats in the control group were allowed to move freely in their cages, but no additional treadmill running was employed. We determined the heart weight change for each rat after the 21 days of exercise. TBARS measured as a marker of lipid peroxidation and endogenous antioxidants, e.g., SOD and catalase [21-23].

Data analysis

Data analysis was performed using IBM SPSS Statistics for Windows ver. 21.0 (IBM Co., Armonk, NY, USA). All the data are expressed as mean (standard deviation) of 3 replications. Differences between two groups were tested by one-way ANOVA, followed by the Student-Newman-Keuls multiple comparisons test when difference were detected. p -value less than 0.05 at 95% confidence level was considered significant.

Results

As shown in Table 1, heart weight decreased significantly rat models of doxorubicin-induced cardiomyopathy compared to the control group ($p < 0.05$). The effects of aerobic exercise on the oxidative stress-related physiological factors

Table 1. Rat body weight and heart weight

Variable (g)	Normal group	Control group	Experimental group I	Experimental group II
Body weight	266.00 (4.58)	238.22 (15.04) ^a	275.00 (13.29) ^b	257.50 (36.59) ^b
Heart weight	1.17 (0.12)	0.95 (0.08) ^a	1.15 (0.05)	1.00 (0.04)
Weight ratio	0.44 (0.01)	0.40 (0.02)	0.42 (0.02)	0.39 (0.01)

Values are presented as mean (SD).

Normal group: normal rat, control group: non-treatment after doxorubicin-induced cardiotoxicity, experimental group I: low-intensity treadmill training (3 m/min) after doxorubicin-induced cardiotoxicity, experimental group II: low-intensity treadmill training (8 m/min) after doxorubicin-induced cardiotoxicity.

Tested by one-way ANOVA; ^a $p < 0.05$ compared with normal group, ^b $p < 0.05$ compared with control group.

Figure 1. The effect of aerobic exercise on superoxide dismutase in cardiomyopathy rat model. Values are presented as mean (SD). Normal group: normal rat, control group: non-treatment after doxorubicin-induced cardiomyopathy, experimental group I: aerobic exercise (3 m/min) after doxorubicin-induced cardiomyopathy, experimental group II: aerobic exercise (8 m/min) after doxorubicin-induced cardiomyopathy. Tested by one-way ANOVA; ^a $p < 0.05$ compared with normal group, ^b $p < 0.05$ compared with control group.

Figure 2. The change of aerobic exercise on thiobarbituric acid reactive substances in cardiomyopathy rat model. Values are presented as mean (SD). Normal group: normal rat, control group: non-treatment after doxorubicin-induced cardiomyopathy, experimental group I: aerobic exercise (3 m/min) after doxorubicin-induced cardiomyopathy, experimental group II: aerobic exercise (8 m/min) after doxorubicin-induced cardiomyopathy. Tested by one-way ANOVA; ^a $p < 0.05$ compared with normal group, ^b $p < 0.05$ compared with control group.

in rats with cardiomyopathy are shown in Figures 1-3. In the aerobic exercise groups, oxidative stress factors related to heart muscle (e.g., SOD and catalase) were significantly increased after 21 days of aerobic exercise (Figures 1, 2), and there was a TBARS decrease ($p < 0.05$) (Figure 3). There

Figure 3. The change of aerobic exercise on catalase in cardiomyopathy rat model. Values are presented as mean (SD). Normal group: normal rat, control group: non-treatment after doxorubicin-induced cardiomyopathy, experimental group I: aerobic exercise (3 m/min) after doxorubicin-induced cardiomyopathy, experimental group II: aerobic exercise (8 m/min) after doxorubicin-induced cardiomyopathy. Tested by one-way ANOVA; ^a $p < 0.05$ compared with normal group, ^b $p < 0.05$ compared with control group.

was, however, no difference between the experimental groups.

Discussion

Cardiomyopathy can lead to an inability of the heart to effectively circulate blood through the body a state known as heart failure [24]. Physical activity is one of the core components in cardiac rehabilitation and secondary complication prevention [25]. While diverse approaches have been adopted for treating cardiomyopathy, the therapeutic efficacy and mechanism of action of the ideal approach have yet to be elucidated, although many studies are currently investigating this. Thus, the purpose of this study was to evaluate the effect of aerobic exercise training on cardiomyopathy in rats with doxorubicin-induced conditions as an exercise intervention for reducing oxidants within the muscle tissues.

Doxorubicin acts by forming an iron-anthracycline complex that generates free radicals, which in turn, causes severe damage to the muscle cell membrane, and interferes with cytoskeletal structure [26]. The decrease in body weight and heart weight in this study is in accordance with other studies [27] and it may be attributed to reduced dietary antioxidant intake and inhibition of protein synthesis due to doxorubicin treatment compared to the normal group. Oxygen free radical formation mediated by doxorubicin enhances the susceptibility of cardiac tissue to lipid peroxidation leading to a

progressive dose-related irreversible loss of myofibrils, dilation of the sarcoplasmic reticulum, cytoplasmic vacuolization, swelling of mitochondria, increased number of lysosomes, and myocyte necrosis [28]. Matysiak *et al.* [29] reported increases in superoxide dismutase-1 and glutathione peroxidase concentrations during regular physical exercise, the latter of which was significantly suppressed by interval cycloergometer training.

Therapeutic strategies, designed to augment cellular endogenous defense systems such as antioxidants have been identified as a promising approach to combat doxorubicin toxicity [30]. According to our study, the oxidant enzyme concentrations in cardiac muscle were consistently reliably different in each experimental group and depended on aerobic exercise. In the rats with induced cardiomyopathy, TBARS levels were highest when compared with the normal group. Myocardial SOD and catalase activities were significantly decreased compared with the control group, and were correlated with cell death and severity of left ventricular dysfunction [31]. Oxidative stress plays a central role in the etiopathogenesis of cardiac dysfunction, and protection against oxidative stress through a cellular mechanism such as like myocardial adaptation holds promise as an effective therapeutic approach. Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase, glutathione peroxidase, and glutathione [32]. We have also reported that low-intensity exercise causes a significant decrease in basal glutathione and lactate dehydrogenase activities in rat serum, which is associated with a concomitant decrease in myocarditis [33]. Oxidative stress in cardiac tissue is caused by histological changes in cardiomyocyte size. A marked increase in cardiomyocyte apoptosis and concomitant induction of myocardial fibrosis was observed. Increased extracellular matrix content contributes to diastolic stiffness, and ultimately promotes ventricular dysfunction [34]. Reduction in oxidative stress through aerobic exercise will lead to an improvement in functional changes. Thus, aerobic exercise would be a major contributor to improved cardiac function in doxorubicin-induced cardiomyopathy.

The results of this study cannot be generalized for human population, because the design of this study was a rat models with cardiomyopathy. Further study will conduct for human population with or without cardiopulmonary dysfunction to evaluate the effect of aerobic exercise on cardiac functions in persons with or without cardiopulmonary dysfunction. In

light of these results, aerobic exercise may play a role in decreasing TBARS and in increasing SOD and catalase. The findings of this study using animal models suggest that aerobic exercise as a therapeutic intervention can increase cardiac muscle antioxidant enzyme levels and reduce the levels of oxidative enzymes, which can suppress tissue inflammation and damage and promote heart function recovery.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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