

Effects of cardiac biological activities on low-intensity physical training in doxorubicin-induced cardiotoxicity rat models

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Objective: In the present study, we investigated the protective effects of low-intensity treadmill training in doxorubicin-induced cardiotoxicity rat models.

Design: Randomized controlled trial.

Methods: In this study, we randomly divided them into four groups. The normal group included non-cardiotoxicity normal control (n=10), the control group included non-treadmill training after doxorubicin-induced cardiotoxicity (n=10), the experimental group I included low-intensity treadmill training (3 m/min) after doxorubicin-induced cardiotoxicity (n=10), and the experimental group II included low-intensity treadmill training (8 m/min) after doxorubicin-induced cardiotoxicity (n=10). Rats in the treadmill training group underwent treadmill training, which began at 2 weeks after first intraperitoneal injection. We determined the body weight change for each rat on days 1 and 21. Biochemical markers (lactate dehydrogenase [LDH], creatine kinase [CK], glutathion, aspartate transaminase [AST], and alanine transaminase [ALT]) concentration in the serum change of rats from all four groups was examined at the end of the experiment.

Results: The results showed that the experimental group I and II showed a significant increase in body weight as compared with that of the control group ($p < 0.05$). We observed that the biochemical markers (LDH, CK, glutathion, AST, and ALT) were improved in the experimental group I than the experimental group II ($p < 0.05$). There was no difference between the experimental groups.

Conclusions: In conclusion, our data suggest that low-intensity treadmill training applied after doxorubicin treatment protects against cardiotoxicity following treatment, possibly by enhancing antioxidant defenses and inhibiting cardiac muscle cell apoptosis.

Key Words: Cardiotoxicity, Doxorubicin, Low intensity treadmill training

Introduction

The incidences of geriatric illnesses and heart-related diseases are increasing owing to today's stressful lifestyle, dietary habits, and chronic physical inactivity. Heart disease has a high mortality rate and serious physical consequences; thus, maintaining a healthy heart is as important as early diagnosis and treatment [1]. Heart disease is well known to be associated with an individual's physical activity level, and it is important that cardiac rehabilitation from heart disease is managed appropriately. Diet, smoking, and exercise are the

main adjustable cardiac risk factors, and exercise in particular is important in cardiac rehabilitation. Swift *et al.* [2] and Hansen *et al.* [3] reported that exercise is an important part of the treatment of cardiovascular diseases and suggested that exercise training is the central element of cardiac rehabilitation. A recent meta-analysis conducted in relation to exercise-centered management of cardiac rehabilitation training found reduced rates of myocardial infarction and improved cardiac function in patients who exercised regularly [4,5]. Soares *et al.* [6] reported that regular aerobic and cardiac rehabilitation exercises have been reported to im-

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prove cardiovascular function, in patients with cardiovascular diseases, a significantly decreased coronary artery index was found when regular exercise was completed at least three times a week, while an increased index was found in those who did not exercise [7]. However, despite the commitment to cardiac rehabilitation, lack of time to exercise is a limiting factor [8].

The effect of variable changes in different exercise protocols, even those of low intensity, is not yet clear. There is currently insufficient research on the effect of physical therapy in cardiac rehabilitation in patients with weakened cardiac function due to chemotherapy. Doxorubicin, a widely used anticancer drug, has the serious side effects of congestive heart failure and cardiomyopathy [9]. The intracellular and extracellular pathways are known to be involved in the side effects, and a complex mechanism of action is reported to lead to myocardial cell death, including oxidative stress related to reactive oxygen species (ROS) [10,11]. Thus, the purpose of this study was to evaluate the effect of low-intensity treadmill training on cardiac muscle toxicity in animal models with doxorubicin-induced conditions as a physical therapy intervention for reducing toxicity levels within the muscle tissues.

Methods

Subjects

Forty male 8-week-old 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°C (1.0°C) and a humidity level of 55% (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. All rats were divided randomly into 4 groups. The normal group included non-cardiotoxicity normal control (n=10), the control group included non-treadmill training after doxorubicin-induced cardiotoxicity (n=10), the experimental group I included low-intensity physical training (3 m/min) after doxorubicin-induced cardiotoxicity (n=10), and the experimental group II included low-intensity physical training (8 m/min) after doxorubicin-induced cardiotoxicity (n=10) (Table 1).

Treadmill exercise and biochemical analysis

Doxorubicin (10 mg/kg, Sigma-Aldrich Co., New York, NY, USA) was dissolved in normal saline. The doses of dox-

orubicin used in this study were based previous reports [12]. 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 [13]. The treadmill velocity was set at 3 m/min and 8 m/min treadmill exercise was performed during the 21 day period at a 0° degree incline. At that same time, the rats in the control group were allowed to move freely in their cages, but no additional treadmill running was employed. The rats in both the experimental group I and experimental group II underwent treadmill exercise during the 21-day. We determined the body weight change for each rat on days 1 and 21. Biochemical markers (lactate dehydrogenase [LDH], creatine kinase [CK], glutathion, aspartate transaminase [AST], and alanine transaminase [ALT]) concentration in the serum change of rats from all four groups was examined at the end of the experiment.

Data analysis

Data analysis was performed using IBM SPSS Statistics 22.0 (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 interval was considered significant.

Results

As shown in Table 2, rat body weight decreased significantly in doxorubicin-induced cardiotoxicity rat models compared to control group ($p<0.05$). The treadmill exercise groups showed a significant increase in rat body weight as compared with that of the control group ($p<0.05$). The effects of treadmill exercise on the oxidative stress related biological factors doxorubicin-induced cardiotoxicity rat mod-

Table 1. Classification of experimental groups (N=40)

Groups	Design of each group
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

Table 2. The change of body weight in each group (g) (N=40)

Group	Normal group (n=10)	Control group (n=10)	Experimental group I (n=10)	Experimental group II (n=10)
Starting body weight	164.60 (6.26)	146.11 (11.85) ^a	141.00 (6.05) ^a	144.75 (18.61) ^a
Final body weight	266.00 (4.58)	238.22 (15.04) ^a	275.00 (13.29) ^b	257.50 (36.59) ^b

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.

^a $p < 0.05$ compared with normal group on pre-low intensity physical training, ^b $p < 0.05$ compared with control group on post-low intensity physical training.

Table 3. Effect of treadmill exercise on biomarker in doxorubicin-induced cardiotoxicity rat (U/L) (N=40)

Group	Normal group (n=10)	Control group (n=10)	Experimental group I (n=10)	Experimental group II (n=10)
Lactate dehydrogenase	289.40 (94.28)	656.57 (184.24) ^a	322.75 (56.78) ^b	387.50 (10.50) ^b
Creatine kinase	774.80 (59.60)	1104.57 (310.38) ^a	667.80 (82.39) ^b	721.50 (99.14) ^b
Glutathione	164.60 (6.26)	146.11 (11.85) ^a	158.00 (11.25) ^b	151.75 (12.60) ^b
Aspartate transaminase	82.83 (13.30)	158.33 (49.00) ^a	98.00 (9.20) ^b	120.75 (12.41) ^b
Alanine transaminase	29.33 (3.27)	36.80 (9.09) ^a	22.75 (4.19) ^b	21.20 (6.97) ^b

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.

^a $p < 0.05$ compared with normal group, ^b $p < 0.05$ compared with control group.

els are shown in Table 3. The treadmill exercise groups had a significantly lower LDH, CK, glutathion, AST, and ALT than in the control group, assessed at 21 days after treadmill exercise began ($p < 0.05$). There was no difference between the experimental groups (Table 3).

Discussion

Causes of cardiomyopathy are various and complex; however, most are related to a lack of oxidative homeostasis due to the formation of oxygen free radicals and increases in lipid peroxidation that increase oxidative stress within an individual and reduce levels of intracellular antioxidant enzymes within cells [14]. While diverse approaches have been taken for treating cardiomyopathy, the therapeutic efficacy and mechanism of action of the ideal approach have yet to be elucidated, for which many studies are underway. Therefore, this study evaluated the effect of low-intensity training, one of many available physical therapy intervention approaches, on cardiac toxicity levels doxorubicin-induced ROS formation, the main cause of myocardial damage, destroys protective myocardial cell enzymes and leads to creation of a three-dimensional membrane-bound protein structure, denaturation, and increased

ion release by interacting with regulating proteins, resulting in myocardial toxicity [15]. Doxorubicin-induced oxidative stress activates heat shock protein-1 in the muscle cells and leads to increased heat shock protein-25 expression, which activates anti-apoptotic protein p53 [16]. These changes in myocardial cells can also be seen with changes in myocardial membrane intracellular electrolyte concentration and changes in the stable current and repolarization of myocardial cells as found in the altered diseased state, and could result from an unstable state consisting of heavy breathing after drug use. In the experimental group than in the control group performed the exercise is weight loss caused by doxorubicin dose was reduced. Dudka *et al.* [17] reported increases in oxidative stress and oxidative enzyme concentrations during doxorubicin administration, the latter of which was significantly suppressed by resveratrol administration.

Damage to proteins, nucleic acids, and membranes by ROS generated in vivo has been reported to be suppressed by antioxidant enzymes such as superoxide dismutase and catalase, and regular moderate exercise stimulates the tissues to relieve stress, decrease blood pressure, reduce cardiac burdens, decrease blood lipid levels, and prevent atherosclerosis in addition to many other positive effects.

Oxygen consumption during moderate strength training is increased by 10-20 fold compared to that in the resting state and induces intracellular oxidative stress and ROS generation from the actions of various metabolites, the immune and endocrine systems, muscle injury, and ischemia-reperfusion injury [18]. Such increases in free radicals lead to damage in cellular proteins, nucleic acids, and cell membranes. This damage can be limited by the actions of antioxidant enzymes, while proper training can increase the ability of intracellular antioxidants to minimize harmful free radicals. According to recent hypotheses, doxorubicin can directly cause cardiac toxicity and oxidative damage in a concentration-dependent manner and lead to the induction of myocardial toxicity and cardiac toxicity due to mitochondrial dysfunction through damage to mitochondrial DNA [19]. Inadequate mitochondrial function over time leads to synthesis acceleration within the adenosine triphosphate pathway, myocardial cell death after a few months, and eventually cardiomyopathy [10,20-22]. According to our study, the LDH, CK, and glutathione concentration in serum were reliably different in each experimental groups and depended on a exercise. Glutathione, an antioxidant enzyme that effectively suppresses free radicals, is known to be present in the mitochondrial matrix and eliminate the oxygen and reactive oxygen compound toxicities that play a role in homeostasis [23]. Physically inactive people generally display signs of chronic inflammatory responses [24]; in contrast, adequate activity leads to reduced intracellular inflammatory responses, while high-intensity exercise increases immune cell inflammatory responses [25,26]. This is thought to be due to the increased concentrations of antioxidant enzymes that result from treadmill training. Sureda *et al.* [27] reported that with regular training, the oxidative defense capabilities in the cytoplasm and mitochondria increase; even with exhaustion exercise, blood lipid peroxidation levels are decreased and lipid peroxidation and tissue damage are reduced [28]. The findings of this study using animal models suggest that low-intensity training as a therapeutic intervention can increase serum 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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