



## Original Article

Treadmill Exercise Alleviates Aging-induced Apoptosis in Rat Cardiac Myocytes<sup>☆</sup>Il-Gyu Ko<sup>1,2</sup>, Sung-Eun Kim<sup>2</sup>, Chang-Ju Kim<sup>2</sup>, Yong-Seok Jee<sup>1\*</sup><sup>1</sup> Department of Exercise Physiology Prescription, Graduate School of Health Promotion, Hanseo University, Chungcheongnam-do 356-706 <sup>2</sup> Department of Physiology, College of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

## ARTICLE INFO

## Article history:

Received 30 July 2012

Received in revised form

6 November 2012

Accepted 30 November 2012

Available online 15 February 2013

## Keywords:

aging,  
apoptosis,  
cardiomyocyte,  
heat shock protein 70 (HSP70),  
treadmill exercise

## SUMMARY

**Background:** The incidence and prevalence of heart failure increases with age. Cardiomyocyte apoptosis contributes to the pathogenesis of heart failure. In the end-stage of human heart failure, increased cardiomyocyte apoptosis is observed. Exercise training is one of the nonpharmacological treatments for chronic heart failure.**Methods:** In the present study, we investigated the effect of treadmill exercise on the aging-induced apoptosis within cardiac myocytes in relation to the expression of heat shock protein 70 (HSP70) using rats. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining and Western blotting for the expression of Bcl-2, Bax, HSP70, and phosphorylated p38 (p-p38) in the cardiac myocardium were conducted.**Results:** Aging induced apoptosis in the myocardium, which was confirmed by increased TUNEL-positive cells and the enhancement of Bax. Expression of HSP70 was suppressed and p-p38 expression was enhanced by aging. Treadmill exercise alleviated aging-induced apoptosis with enhancing HSP70 expression and suppressing p-p38 expression in the cardiac myocytes.**Conclusion:** Based on the present results, it can be inferred that treadmill exercise can provide a cardioprotective effect on aging-induced apoptosis through the enhancement of HSP70 expression in the heart. Thus, regular exercise may be a useful strategy for preventing heart problems in the elderly.

Copyright © 2013, Taiwan Society of Geriatric Emergency &amp; Critical Care Medicine. Published by Elsevier Taiwan LLC. All rights reserved.

## 1. Introduction

Aging is a complicated physiological process caused by the progressive decline of multiple biological functions and disrupted homeostasis. These alterations to homeostasis cause various diseases, including stroke, dementia, and heart disease. The incidence and prevalence of heart attacks such as myocardial infarction increases with age, and developed countries are increasingly faced with an aging population. Aging is associated with a dramatic increase in the incidence and prevalence of heart failure: heart failure is four times more common in those over 85 years compared to those aged 65–75 years<sup>1</sup>. It can thus be concluded that as our

population continues to age, the burden of heart failure will continue to rise.

In normal aging of both humans and animals, postmitotic heart tissue has been associated with a decrease in the total number of cells<sup>2,3</sup>. Therefore, a reduction in the total number of viable cells may lead to an accelerated decline in heart function. Cardiomyocyte apoptosis has been shown to contribute to the pathogenesis of heart failure. In the end stage of human heart failure, increased cardiomyocyte apoptosis is observed<sup>4</sup>.

Apoptosis is known as programmed cell death, and it is distinct from necrosis in terms of a type of cell death involved in cellular development. Apoptosis plays a crucial role in normal development and tissue homeostasis<sup>5</sup>. In addition to cell loss via necrosis, apoptosis may be a major factor contributing to the loss of post-mitotic cells with age<sup>6</sup>. The morphological characteristics of apoptotic cell death are cell shrinkage, chromatin condensation, membrane blebbing, and DNA fragmentation. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining is an assay that detects DNA fragmentation, which is characteristic of apoptotic cell death<sup>7,8</sup>. Cardiomyocyte dysfunction

<sup>☆</sup> All contributing authors declare no conflicts of interest.

\* Correspondence to: Dr Yong-Seok Jee, Department of Exercise Physiology Prescription, Graduate School of Health Promotion, Hanseo University, #360 Daegok-ri, Heami-myun, Seosan-si Chungcheongnam-do 356-706, Republic of Korea.

E-mail address: jeeys@hanseo.ac.kr (Y.-S. Jee).

caused by apoptosis has been associated with various molecules, including heat shock protein 70 (HSP70), mitogen-activated protein (MAP) kinase, and the Bcl-2 family.

Heat shock proteins are known as “stress-induced proteins,” and they are ubiquitous, highly conserved chaperones that are involved in the folding of newly synthesized or damaged proteins<sup>9</sup>. HSP70 plays a critical role in maintaining cellular homeostasis and protecting cells during episodes of stress, damage, and aging<sup>10</sup>. An increase in the level of HSP70 counterbalances the age-related antioxidant system in the rat heart<sup>11</sup>. The rate of apoptosis increases and HSP70 expression decreases with age in rats<sup>12</sup>. Many studies have indicated that the heart survival promoting effect of HSP70 can be ascribed to the suppressive effect of HSP70 on apoptosis<sup>13,14</sup>.

MAP kinases, members of discrete signaling cascades, serve as a focal point in response to various extracellular stimuli, and are divided into four subtypes: extracellular signal-regulated kinase (ERK), c-jun N-terminal or stress-activated protein kinase (JNK/SAPK), ERK/big MAP kinase 1 (BMK1), and p38 MAP kinase (p38)<sup>15</sup>. Of these, p38 regulates a variety of cellular processes including cell growth processes, cell differentiation, apoptosis, and cellular response to inflammation. HSP70 is also involved in the inhibition of p38 within the myocardium<sup>16</sup>. Activation of p38 in cardiomyocytes was reported to lead to a rapid onset of lethal cardiomyopathy, including cardiomyocyte hypertrophy, interstitial fibrosis, and contractile dysfunction<sup>17</sup>. Activation of p38 was ascertained through the phosphorylated p38 (p-p38) that is involved in the initiation of apoptosis<sup>18</sup>.

The Bcl-2 family proteins also play an important role in the regulation of apoptosis. The Bcl-2 family proteins are classified into antiapoptotic proteins, including Bcl-2 and Bcl-xL, and proapoptotic proteins, such as Bax and Bid. Bcl-2 inhibits apoptosis by preventing the release of cytochrome-c from the mitochondria. The antiapoptotic Bcl-2 and the proapoptotic Bax are important regulators of mitochondrial function in cardiomyocyte apoptosis<sup>19</sup>. Bcl-2 and Bcl-xL form heterodimers with the main proapoptotic member, Bax, and can be incapacitated in their protective function<sup>20</sup>. The balance between Bcl-2 and Bax is one of the crucial factors determining whether the cells undergo apoptosis, and the Bcl-2 to Bax ratio can be changed during the aging process<sup>21–23</sup>.

Regular exercise is known to decrease cardiovascular morbidity and mortality in both adults and elderly<sup>11,24,25</sup>. Regular exercise training improves exercise capacity, endothelial function, and collateralization in patients with coronary artery disease and chronic heart failure<sup>26</sup>. In addition, exercise ameliorates the aging-induced decrease in the capillary density of the heart<sup>27</sup>. The exercise training-induced increase in the capillary density may be a beneficial adaptation for the aged heart because the capillary network is involved in maintaining the supply of oxygen and energy to the heart<sup>28</sup>. Exercise is one of the nonpharmacological treatments for chronic heart failure, and it improves the exercise capacity and quality of life in heart failure patients<sup>29</sup>.

Kwak et al<sup>30</sup> demonstrated the protective effect of exercise training against elevated apoptosis through reducing the caspase-9 level and the ratio of Bax to Bcl-2. However, the effect of exercise on age-induced apoptosis in cardiac myocytes in relation with the expression of HSP 70 and p-p38 has rarely been observed. In the present study, TUNEL staining and Western blotting for the expression of Bcl-2, Bax, HSP70, and p-p38 in the cardiac myocardium were conducted.

## 2. Materials and methods

### 2.1. Experimental animals

Ten-week-old Sprague-Dawley rats ( $n = 20$ ; weighing  $250 \pm 10$  g) were used as the young-aged group and 24-month-old rats ( $n = 20$ ; weighing  $420 \pm 20$  g) were used as the old-aged group. The

experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The rats were housed under controlled temperature ( $22 \pm 2^\circ\text{C}$ ) and lighting (08:00 to 20:00 hours) conditions with food and water available *ad libitum*. The rats were randomly divided into four groups ( $n = 10$  in each group): the young-aged sedentary group, the young-aged exercise group, the old-aged sedentary group, and the old-aged exercise group.

### 2.2. Treadmill exercise protocols

The rats in the exercise groups were forced to run on a motorized treadmill for 30 minutes once a day for 6 weeks. The exercise load consisted of running at a speed of 2 m/min for the first 5 minutes, 5 m/min for the next 5 minutes, and 8 m/min for the last 20 minutes, with no incline. The rats in the control groups were left on the treadmill without running for the same time period as the exercise groups.

### 2.3. Tissue preparation

The animals were sacrificed immediately after the last treadmill exercise performed. The animals were anesthetized using Zoletil 50 (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), and the left ventricle area in the cardiac muscle was removed. Cardiac muscles were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, treated in xylene, and infiltrated and embedded in paraffin. Coronal sections of 5- $\mu\text{m}$  thickness were made by a paraffin microtome (Thermo, Co, Cheshire, UK), mounted on coated slides, and then dried at  $37^\circ\text{C}$  overnight on a hot plate.

### 2.4. TUNEL staining

In order to visualize DNA fragmentation, a marker of apoptosis, TUNEL staining was performed using an In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol<sup>8</sup>. The sections were postfixed in ethanol-acetic acid (2:1) and rinsed. The sections were then incubated with proteinase K (100  $\mu\text{g}/\text{mL}$ ), rinsed, and incubated in 3%  $\text{H}_2\text{O}_2$ , permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. Then, the sections were rinsed and visualized using Converter-POD with 0.03% 3,3'-diaminobenzidine (DAB). Mayer's hematoxylin (DAKO, Glostrup, Denmark) was used as a counterstain, and the sections were mounted onto gelatin-coated slides. Slides were air-dried overnight at room temperature, and coverslips were mounted using Permount.

### 2.5. Western blot analysis

Western blotting was performed as the method described previously<sup>22</sup>. The left ventricle tissues in the cardiac muscle were collected and immediately frozen at  $-70^\circ\text{C}$ . The tissues were homogenized with a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 1 mM EGTA, 1 mM PMSF, 1 mM  $\text{Na}_2\text{VO}_4$ , and 100 mM NaF, and then centrifuged at 14,000 rpm for 30 minutes. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad). Forty micrograms of protein were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred onto a nitrocellulose membrane. A rabbit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:5000; AbFrontier, Seoul, Korea), mouse Bax antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse Bcl-2 antibody (1:1000; Santa Cruz Biotech.), rabbit p38 antibody (1:1000; Cell Signaling Technology, Beverly,

Download English Version:

<https://daneshyari.com/en/article/3325094>

Download Persian Version:

<https://daneshyari.com/article/3325094>

[Daneshyari.com](https://daneshyari.com)