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Research Article

THE EFFECT OF AEROBIC AND ANAEROBIC EXERCISE ON BAX AND BCL-2 MYOCARDIUM APOPTOTIC MARKERS AFTER REPERFUSION ISCHEMIA

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ABSTRACT

The present research aims to examine the effect of aerobic and anaerobic exercise on Bax and Bcl-2 markers after the induced reperfusion ischemia by isoprenaline. In this study, there were used 26 male Wistar rats weighing approximately 200-250 g with two to three months old. The rats in pilot group were divided into two subgroups of reperfusion ischemia (n= 7) and healthy (n= 7) for study of heart-failure byisoprenaline. The rats in research group were divided into three subgroups ofaerobic (n= 4), anaerobic (n= 4) and control (n= 4) for study of gene expression. In rats of reperfusion ischemia group were confirmed heart-failure after injection isoprenaline by Trichromation staining technique. However, the rats in research group were familiar running on treadmill after one week, then they were exposed for one month training with frequency of three times weekly. After one month, all rats were rest for two consecutive days. Then within 24 hours, there were injected isoprenaline with dose of 150 and 125 mg per kg of their body weight. Data was analyzed using independent T, one way ANOVA and$2^{-\ ct}$ formula. The research results showed that aerobic and anaerobic exercise can reduce and increase Bax and Bcl-2 gene expression respectively, but these changes were not significant. However, it seems that one month training can't decrease amount of myocardial apoptosis. Therefore, study of effect increase of period physical activity is essential for decrease amount of heart-failure.

INTRODUCTION

Before 1900, infectious diseases and malnutrition were the most common causes of death in the world. Now, cardiovascular diseases (CVDs) are the most common causes of death in many parts of the world. If such a situation continues in the same way, death rate can be increased in the future by considering age. Of course, it is predicted that by 2020, number of deaths caused by cardio-hereditary diseases (CHDs) is the most important cause of death. According to research reports, accumulation of lipoproteins, endothelial damage and inflammation are some of multiple processes that are involved to initiate and progress atherosclerosis (Longo *et al*, 2012). However, hypoxia is one of the causes of damaging heart muscle cells and the programmed heart cell death called apoptosis; so that cells and mitochondria of an area will be inflated, lose their membrane integrity, as a result, in-cell material such as pre-apoptotic proteins are released that cause inflammatory reactions and intensify initial injuries. In contrast, anti-apoptotic proteins can reduce cell death caused by incentives through anti-apoptotic activity and influencing caspases. At the same time, Bax pre-apoptotic protein and Bcl-2 anti-apoptotic protein are the most important involved proteins in apoptosis, which they used as a measure to reduce or increase cell death. So any factor that can increase Bax/Bcl-2 ratio, it can be effective in reducing apoptosis (Shariatzadeh *et al*, 2008). However, several factors can reduce the severity of coronary artery lesions and myocardial apoptosis that physical activity is the most important one because the risk of developing coronary artery disease in ready or active adults is 30-40% lower than inactive individuals (Longo *et al*, 2012). Other studies showed that aerobic exercise increases anti-apoptotic factors such as Bcl-2 and decreases apoptotic factors such as Bax in myocardium (Santana *et al*, 2014). It seems, reducing apoptosis markers such as Bax and Bad and increasing anti-apoptotic markers such as Bcl-2 in myocardial cells are the possible causes of exercise benefits to protect the heart. In this regard, a study showed that exercise activities reduced increasing ratio Bax/Bcl-2 ratio, apoptosis and cardiac remodeling in old rats (Bum Kwak *et al*, 2006). Another study indicated that the Bax/Bcl-2 ratioin endurance training group was more than the control and acute exercise groups (Delchev *et al*, 2005). Finally, another research pointed out that exercise

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activity can increase protein expression of heart protection such as Bcl-2and HSP_{72} in rats with blood pressure (Lajoie *et al*, 2004). Therefore, we can say that regular aerobic exercise program can improve aerobic capacity of coronary patients. Versus, Jafary *et al* showed aerobic training for 12 week has no significant effect on Bcl-2 gene expression. Given that some studies have investigated apoptotic and anti-apoptotic proteins (no gene expression), while others have reported conflicting results according to various period of time training, the present research aims to examine the impact of aerobic and anaerobic exercise training on Bax and Bcl-2 gene expression markers to reduce amount of apoptosis in rats with reperfusion ischemia. Another goal of the study is to compare two different intensities of aerobic and anaerobic exericise to reduce heart-failure.

Procedure

In this study, there were used 26 male Wistar rats weighing approximately 200-250 g with two to three months old. The rats in pilot group were divided into two subgroups of reperfusion ischemia (n= 7) and healthy (n= 7).After inducing isoprenaline with dose of 150 and 125 mg per kg of their body weight, there was investigated amount of fibrosis in their left ventricular using Trichromation staining technique. Then it was found that the dose of isoprenaline causes heart-failure. However, rats in aerobic(n= 4) and anaerobic (n= 4)groups were familiar running on treadmill after one week, then they were exposed for one month training course with frequency of three times weekly (Høydal *et al*, 2007) (Table 1). Then within 24 hours, control, aerobic and anaerobic groups were injected isoprenaline with dose of 150 and 125 mg per kg of their body weight.

Table 1 Aerobic and Anaerobicexercise protocol

Exercise process Exercise components	Heat	Main body of aerobic exercise	Main body of anaerobic exercise	Cool
Exercise time (min)	6	30	15-23	6
Exercise severity (VO_{2max})	50-60%	70-75%	85-100%	50-60%
Speed (m/min)	15-20	23-25	30-38	15-20
Distance (m)	90-120	690-750	690-750	90-120
Treadmill gradient (degree)	0	5-20		0

For surgery, rats were anesthetized with chloroform. Then left ventricle of their heart was removed and rinsed with saline serum. Then they were placed in microtubes and liquid nitrogen capsule for freezing.

RNA Extraction

Samples of left ventricular were taken out from the nitrogen cylinders for homogenization. Then RNA were extracted using manual protocols of trisol. The extracted solution containing RNA was held in the freezer with -80 °C until cDNA synthesis. thenc DNA was synthesized using the Vivantis kit. In the following, there were obtained 20 ml cDNA solution that was diluted with ratio of 1:5. It was transferred to the freezer with -80 °C or -20 °C.

In order to find the frequency of primers, there were found articles in this field and there were evaluated the frequency of Bax and Bcl-2 genes as well as beta-actin (Gorji *et al*, 2013; Shi *et al*, 2016; Yang *et al*, 2006). Then they were examined through ncbi website (Table 2).

Table 2 Frequency of the used primers in real-time

Optimum temperature	Primer frequency		The required gene
60	CCAGGACGCATCCACCAAGAAGC	F	Bax
	TGCCACACGGAAGAAGACCTCTCG	R	
60	CAGAGATGTCCAGTCAGCTG	F	Bcl-2
	CAGTCATCCACAGAGCGATG	R	
60	CTAAGGCCAACCGTGAAAAGATG	F	Beta-actin
	TGGTACGACCAGAGGCATACAG	R	

Real-Time Preparation

In order to prepare materials of real-time, there were poured 4λ cDNA from each research group into wells of real-time device. Then there were added 6λ of master mix solution (0.5λ of the forward primer and rivers, 0.5λ water and 5λ master mix) to it. After that, the samples were vortex and then they were run for 5 minutes at 95 °C (initial formation), 10 seconds at 95 °C (secondary formation), 15 seconds at 60 °C (primers' connection) and 20 seconds at 72 °C (replication). After the second stage, the reaction was repeated for 40 cycles. The ct-related reactions were extracted and recorded by software.

Statistical Method

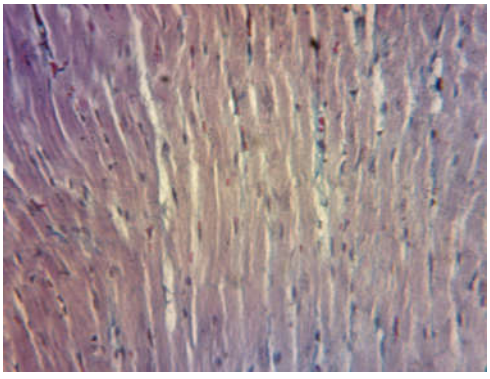
The independent t-test was firstly used to examine mean difference of fibrosis in healthy and reperfusion ischemia groups. Next, there was used $2^{-\Delta\Delta ct}$ formula to quantify expression levels of the considered genes. Then the fold change formula was used for multiple gene expression. Also, the one way ANOVA was used to examine differences in gene expression between the groups. Finally, there was considered 0.05 as the significance level for all tests.

RESULTS

The results showed a significant difference of fibrosis level in reperfusion ischemic with healthy group, so that compared to the healthy group, the obtained amount of fibrosis in reperfusion ischemic group is greater than the average level. The results show that isoprenaline injection at dose of 150 and 125 mg/kg BW on two consecutive days has the ability to cause fibrosis in the rats' heart (Table 3) (Fig. 1).

Table 3 Fibrosis amount in the pilot group

Statistical indicator Group	Number	Fibrosis mean	SD	Loon	Sig.
Reperfusion ischemia	7	107.85	11.95	0.098	0.00
Healthy	7	24.57	17.17		



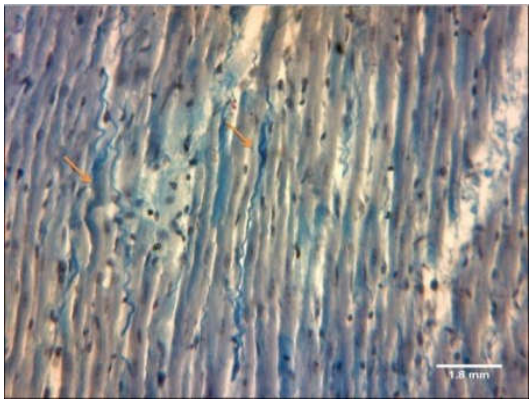


Fig 1 Images of healthy and induced groups by isoprenaline

RESULTS

The research results showed that Bax and Bcl-2 gene expression in the aerobic group were reduced (about 0.8 times) and increased (about 1.63 times) and also in the anaerobic group were reduced (about 0.9 times) and increased (about 3.39 times) respectively (Fig. 1).

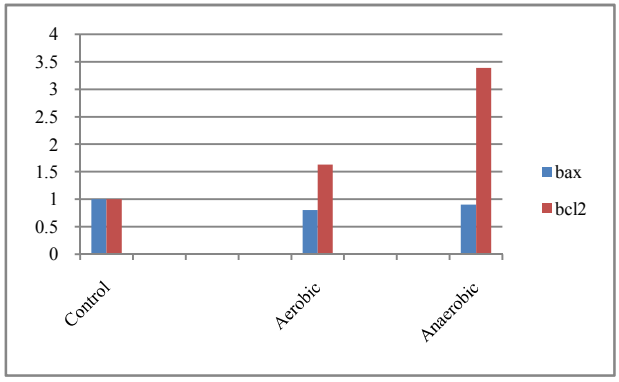


Fig 2 The Bax and Bcl-2 gene expression in the aerobic and anaerobic group

To evaluate the significance levels, there were used the values of $2^{-\Delta\Delta ct}$. Meanwhile, the one way Anovatest was used to compare groups (Table 4).

Table 4 Comparing Bax and Bcl-2 genes in the control, aerobic and anaerobic groups

Research groups	$2^{-\Delta\Delta CTBax}$	$2^{-\Delta\Delta CTBcl2}$	Bax mean	Bcl-2 mean	Bax SD	Bcl-2 SD	Bax Sig	Bcl-2 Sig
control	1.12 1.02 .91 0.96	2.99 .53 0.21 0.27	1	1	0.09	1.33		
Aerobic	1.11 .80 .37 0.95	5.04 0.58 0.25 0.66	0.80	1.63	0.31	2.27	0.495	0.386
Anaerobic	1.1 0.95 0.62 0.94	7.9 0.55 3.41 1.71	0.9	3.39	0.2	3.22		

According to the results of Table 4, $H_0$is confirmed that means of Bax and Bcl-2 gene expression have no significant changes in control, aerobic and anaerobic groups. Because statistic values of Bax and Bcl-2 have been reported 0.495 and 0.386 respectively, which they are greater than significant value (0.05). In other words, aerobic and anaerobic exercise has no significant effect on Bax and Bcl-2 gene expression. However, as seen in fold change chart, Bax and Bcl-2 gene expression with aerobic and anaerobic exercise showed reduction and increasing, compared to the control group.

DISCUSSION AND CONCLUSION

Findings of the present research showed that one month aerobic and anaerobic exercise will respectively decrease and increase Bax and Bcl-2 gene expression after ischemia reperfusion through isoprenaline, but they were not significant. Unlike the research results, Kang *et al* (2004) showed that exercise training increases Bax/Bcl-2 ratio. Also, Lajoie *et al* (2004) showed that exercise training for 5 days per week at a speed of 18 m/m for 120 minutes daily can increase hsp72 and Bcl-2 anti-apoptotic proteins. Delshev *et al* (2005) showed that Bcl-2 level had the most increase in endurance training for 8 weeks, compared to other research groups. To support history of research, Marfe *et al* (2012) showed that exercise training will reduce Bax and increase Bcl-2 by increasing SIRT1 and SIRT7. Bum Kwak *et al* (2013) demonstrated that exercise training can reduce Caspase-9 as well as Bax/Bcl-2 ratio and eventually, it can increase and decrease Bax and Bcl-2 protein. In this regard, Santana *et al* (2014) showed that exercise training for 13 weeks increased mRNA and protein of anti-apoptotic factors such as Bcl-2, Survivin, ILK and AKT. On the other hand, Jaafari *et al* (2015) showed that value of Baxgene and Bax/Bcl-2 ratio of exercise group was less than the control group. However, the study did not find significant differences in Bcl-2 genes between the two groups. Of course, there was reported higher level of Bcl-2 for the exercise group, compared to the control group. In another research, Chengji *et al* (2016) showed Bax and Bcl-2 gene expression was not significantly reduced in the aerobic exercise group with myocardial infarction, compared with MI. Given these results, it seems that different aspects of the present and previous studies such as exercise duration can affect significance of Bax and Bcl-2 gene expression because the more exercise duration, the better compatibility of practice and exercise useful aspects such as reducing fat lipoproteins and increasing antioxidants to oxidants factors and consequently, reducing inflammatory factors. Most of studies have considered duration of 8 and 13 weeks to practice, while the present research examined duration of 4 weeks. On the other hand, some studies have proposed five exercise sessions weekly, but the present research has examined three exercise sessions weekly. In this regard, Lajoie *et al* were trained rats for 120 minuts per each session; but present research were trained rats for 42 and 23 minuts.

In contrast, the results of this study showed one month anaerobic training were reduced and increased Bax and Bcl-2 gene expression by isopernaline. It seems that training intensity can also affect the results because the more intensity of exercise, the more profit for body. Now it seems that if we increase number and time of exercise sessions weekly, we can get favorable results of decreasing and increasing Bax and Bcl-2 genes. As a result, we can observe reducing volume of cardiac apoptosis because factors such as reducing lipoproteins (LDL, VLDL and leptin), increasing antioxidants (glutathione and superoxide dismutase), decreasing inflammatory factors such as TNF-α, IL-6 and increasing free radicals as well as increasing growth factors such as IGF-1 and AKT and NFAT that are created by total compatibility of exercise can reduce

volume of cardiac apoptosis, since they are associated with reducing cellular homeostasis disruptive factors such as caspases, calpain, ROS, RNS and increasing cell survival factors such as STATs, PI3K, PGC1-α, MEK and ERK. In this regard, scott *et al* (2004) mentioned exercise training is protection versus reperfusion ischemia injury. Because endurance training protect all levels of myocard reperfusion ischemia Injury. This research exercise-induced myocard protective mechanisms expressed increase in the coronary artery circulation, expression of ER stress proteins, increase the activity of cyclooxygenase-2, induction of heat shock proteins of myocard, increased myocardial cytosolic antioxidant capacity, increased signaling nitric oxide, changes in mitochondrial phenotypic, increase and changes in the ATP-sensitive potassium channels expression and increase the mitochondrial inner membrane. Finally, the researchers showed increased antioxidant levels and increased myocardial expression of ATP-sensitive potassium channels assist cardiac protection against reperfusion ischemic injury. Because these factors stimulate reduced damage to mitochondria and mitochondrial membrane integrity; however it increases the BCL2 gene expression in mitochondria. Finally, this study suggest aerobic and anaerobic exercise for 4 weeks cant reduce volume of heart-failure in patients prone to ischemic coronary artery; But given that the improvement in anaerobic exercise has been better, it seems to increase the duration and intensity of exercise can reduce the size of a possible stroke. Therefore, the present research suggests that patients with coronary artery ischemic should implement long-term aerobic and anaerobic exercise to reduce risk of heart attack and myocardial infarction.

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