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The interactive effect of swimming training and vitamin D supplements on Bcl-2 gene expression in heart tissue of cadmium-poisoned rats

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Abstract

Introduction: Apoptosis mechanism is one of the most important ways to remove unwanted cells, which is done in the body of porcelain organisms and even single cells. The aim of this study was to investigate the interactive effect of swimming training and vitamin D supplements on Bcl-2 gene expression in heart tissue of cadmium-poisoned rats

Material & Methods: Forty two male Sprague Dawley rats randomly divided into seven groups including, (1) control, (2) sham, (3) swimming, (4) vitamin D, (5) interval swimming + weight, (6) swimming + vitamin D, and (7) interval swimming + Vitamin D + weight. The Rats in groups 3,5,6,7 swam for 4 weeks and 5 sessions per week. 5 µg vitamin D3 was dissolved in 150 µl sesame oil and injected intraperitoneally to groups 4, 6, 7 every two days.

After the 4 weeks of intervention, rats in all groups were peritoneally exposed to 2mg/kg of cadmium. At the end of protocol, the rats were sacrificed and their heart tissues were removed to measure gene expression of Bcl-2. Data were analyzed by one-way ANOVA and Tukey's post hoc tests.

Results: The results showed that there was no significant difference in Bcl-2 gene expression between swimming groups, vitamin D and interval training swimming + weight with control group. Interactive effect of swimming and vitamin D on the increase of Bcl-2 gene expression in the cadmium-exposed rats is significant.

Conclusions: Based on the results, it was inferred that the combination of swimming training (especially swimming with weight) with vitamin D supplementation could increase the Bcl-2 gene expression as an anti-apoptotic protein in the heart.

Keywords: Apoptosis, Swimming, Bcl-2, Cadmium, Heart tissue

1. Introduction

Cadmium is one common harmful heavy metal that is widely distributed in our environment. It is still a major concern for public health because accumulating epidemiological studies have shown the associations between environmental level of cadmium exposure and adverse health risks (1,2). Food and smoking are the two main way for cadmium exposure in general population. Cadmium exposure can cause several adverse effects or diseases, including renal dysfunction, liver damage, cardiovascular disease, and osteoporosis (3).

Cadmium causes oxidative stress in the cell so that its toxic effects can cause the oxidation of membranes, organelles, or phospholipids, which subsequently leads to cellular dysfunction or apoptosis (4). Apoptosis plays a critical role in monitoring different types of non-pathological events and is a naturally planned biological process for the removal of damaged cells (5, 6). Cells that suffer from apoptosis are characterized

by numerous morphological changes, such as cellular shrinkage and contraction, germination of the plasma membrane, organized nucleic rupture, and cellular fragmentation. One of the pathways involved in the induction of apoptosis is controlled by Bcl-2 family members and, by activating Bax, leads to the permeability of the mitochondrial membrane (7). Expression of Bcl-2 or CrmA each suppressed cadmium-induced cell death although Bcl-2 was somewhat more effective than CrmA. In vitro assay of caspase activity carried out using poly(ADP-ribose) polymerase (PARP) as a substrate as well as intracellular caspase assays using a fluorigenic caspase-3 substrate confirmed that caspase-3 is activated in Rat-1 cells undergoing cadmium-induced apoptosis. Both Asp-Glu-Val-Asp-aldehyde (DEVD-cho) and Tyr-Val-Ala-Asp-chloromethylketone (YVAD-cmk), selective inhibitors of caspase-3 and caspase-1, respectively, suppressed significantly cadmium-induced cell death (8).

On the other hand, many studies have examined the antioxidant potential of some vitamins such as vitamin E, vitamin C, and vitamin D and their role in human health. Vitamin D can play multiple roles in the body, such as regulating cell growth, immune function, inflammatory reaction and antitumorogenic activity. The antioxidant effect of vitamin D is between the newest suggested non-calcemic roles of this compound (9,10). Although recently Amanpour et al. (2019) reported that cadmium exerts its apoptotic effects through the mitochondrial pathway by increasing the ratio of Bax/Bcl-2 and activating caspases in the rats' testes, and vitamin E plays a protective role against cadmium-induced apoptosis (11), the effect of vitamin D on cadmium-induced apoptosis is not well known.

Regarding the effect of exercise on apoptotic indices, it has been shown that in ovariectomized rats, long-term endurance training could significantly decrease Bax, Bad, t-Bid, activated caspase-9, activated caspase-3, and cytosolic cytochrome-C (12). 12 weeks of endurance training with 15% gradient significantly increased the Bcl-2 gene expression in trained rats (5). Three months of exercise on a rats' special treadmill significantly reduced Bad, Bax, and Bax to Bcl-2 protein ratio, as well as activated caspase-9 and activated caspase-3 (13). Recently, Ghajari et al. (2019) indicated that eight weeks of endurance training

can improve the Bcl-2 and Bax gene expressions in the heart tissue of rats exposed to cadmium (14). The interactive effect of exercise training and vitamin D supplements on Bcl-2 gene expression in heart tissue of cadmium-poisoned rats is unclear. Thus, the aim of present study was to examine the interactive effect of swimming training and vitamin D supplements on Bcl-2 gene expression in heart tissue of cadmium-poisoned rats.

2. Material & Methods

An experimental design was employed for this study and all experimental procedures were performed according to the guidelines of the Helsinki declaration and approved by the Regional Research Ethics Committee of Islamic Azad University, Marvdasht, Iran. Forty two male Sprague Dawley rats were obtained from the Pasteur Institute of Shiraz, Iran. The rats were housed in pathogen-free conditions at 22 ± 2°C, with a relative humidity of 50 ± 10% for 14 days. They were exposed to a reverse light condition of 12 hours of light and 12 hours of darkness each day and were fed rat chow and water *ad libitum* throughout the study period. Animals were randomly divided into seven groups including, (1) control, (2) sham, (3) swimming, (4) vitamin D, (5) interval swimming + weight, (6) swimming + vitamin D, and (7) interval swimming + Vitamin D + weight. The Rats in groups 3,5,6,7 swam for 4 weeks and 5 sessions per week. 5 µg vitamin D was dissolved in 150 µl sesame oil and injected intraperitoneally to groups 4,6,7 every two days. After the 4 weeks of intervention, rats in all groups were peritoneally exposed to 2mg/kg of cadmium. In the end of the study period, 48 hours after the final exercise session, all rats were anesthetized by xylazine and ketamine, and then their heart tissues were taken to measure their histochemical variables. All efforts were made to minimize animal numbers and suffering.

For molecular analysis at the gene expression level, the RNA of the heart tissues was first extracted according to the manufacturer's protocol (Cinna-Gen, Iran). Then, by drawing a standard curve based on light absorbance at the wavelength of 260 nm, the concentration and degree of

purity of the RNA sample were quantitatively determined using the following equation (14,15):

$$C\left(\frac{\mu g}{\mu L}\right) = A260 \times \epsilon \times \frac{d}{1000}$$

C: concentration 260;
A: absorbance at 260 nm wavelength;
ε: Molar offset for RNA 40, and for DNA 50;
d: dilution factor.

After extracting RNA with high purity and high concentration from all of the samples, cDNA synthesis steps were taken according to the manufacturer's protocol, and the synthesized cDNA was used for reverse transcription reaction. Initially, the designed primer for gene was examined and then, gene expression was examined by quantitative q-RT PCR method (14). The sequence of the primer used in the study was:

(f): ACTTTTAGGCGTGGCTGATG
(r): TTTTGCTGCTCACTGTATTTTATTTT

Results were expressed as the mean ± SD and distributions of all variables were assessed for normality. To analyze the results of the tests, one-way ANOVA and Tukey post hoc test were run using SPSS software for windows (version 22, SPSS, Inc., Chicago, IL). The level of significance in all statistical analyses was set at $P \leq 0.05$.

Results

Bcl-2 gene expression level is shown in Figure 1.

The results of one-way ANOVA in Table 1 showed significant differences in the Bcl-2 ($F = 18.29$, $P = 0.001$) gene expression levels between the seven groups of rats.

Table 1. Comparison of Bcl-2 gene expression in the rats of seven groups of research

Factor	Sum of Square	df	Mean of Square	F	P
Bcl-2				18.29	0.001
Between group	18.71	6	3.13		
Within group	5.98	35	0.17		
Total	24.75	41			

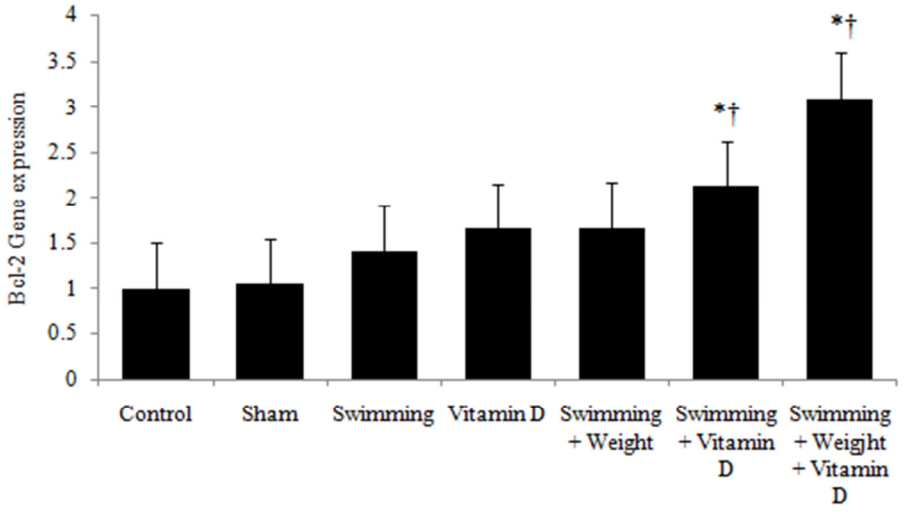

Figure 1. Comparison of the tau gene expression within the groups
* Significant differences with the control group (P <0.05)
† Significant differences with the sham group (P <0.05)

The results of Tukey's post hoc test showed that there was no significant difference in Bcl-2 gene expression between swimming groups, vitamin D and interval training swimming + weight with control group. Interactive effect of swimming and vitamin D on the increase of Bcl-2 gene expression in the cadmium-exposed rats is significant.

Discussion

Research results indicated that cadmium reduced the mRNA level of Bcl-2 in several tissues including: heart (14), osteoblasts (16) and hippocampus and cortex cells (17). Cadmium is a non-essential toxic metal, which can be absorbed through smoking, diet and occupational exposure in certain industries (18). The elimination of cadmium from the human body is a slow process – the biological half-life has been estimated to be 10 – 40 years (19,20). Multiple adverse health effects of cadmium exposure have been reported, such as increased risk of renal dysfunction (19,21), osteoporosis (21,22) and cancer (23). Clinical studies also found that cadmium was associated with the prevalence and future growth of atherosclerotic plaques (24) and with cardiovascular disease (25,26). The

mechanisms underlying the association between cadmium and heart failure are unclear. Cadmium has been associated with many cardiovascular risk factors, such as hypertension (27), smoking and kidney disease (28), which might partly explain the association between cadmium and heart failure. Borné et al. (2015) found that cadmium was significantly associated with baseline blood pressure, current smoking, history of a coronary event and plasma creatinine in Swedish population (18). One of the pathways involved in the induction of apoptosis is controlled by Bcl-2 family members and, by activating Bax, leads to the permeability of the mitochondrial membrane (7). Expression of Bcl-2 or CrmA each suppressed cadmium-induced cell death (8).

Regarding the effect of exercise and antioxidant vitamins on apoptotic indices, it has been shown that endurance training and vitamin E significantly increased the Bcl-2 gene expression (5,11,14). Our results indicated that combination of swimming training (especially swimming with weight) with vitamin D supplementation could increase the Bcl-2 gene expression as an anti-apoptotic protein in the heart.

Jafari et al. (2015) showed that 12 weeks of endurance training with a 15% gradient increased Bcl-2 gene expression and reduced Bad, Bak, and Bax in rats (5). Ghajari et al. (2019) also indicated that cadmium consumption significantly decreased the Bcl-2 gene expression and increased the Bax gene expression in rats. On the other hand, endurance training in cadmium-infected rats increased the Bcl-2 gene expression and decreased the Bax gene expression in rats when compared to the cadmium consumption group (14). The Bcl-2 family members, Bcl-2 associated X (Bax) protein and Bcl-2, have been identified as putative key proteins involved in the formation of mitochondrial apoptotic channels and also in the regulation of mitochondria permeability and mitochondrial-associated apoptotic signaling (29). The ratio of pro- to anti-apoptotic Bcl-2 family proteins (e.g., Bax/Bcl-2) regulates myonuclei integrity and cell survival by controlling mitochondrial membrane permeability and activation of caspases (30). The main pathway for increasing mitochondrial-dependent apoptosis is based on the activity of caspases mediated by the continuous release of mitochondrial cytochrome C. Following various pressures leading to the

release of Ca^{2+} ions from the endoplasmic network, mitochondria play an important role in the collection of these cytosolic calcium ions. In fact, the rapid release of Ca^{2+} from the endoplasmic lumen implies the permeability of the mitochondrial membrane and stimulates the apoptotic responses. Mitochondria are the main actor in the apoptosis process, and this is done through the integration of death signals by proteins belonging to the family of cell B lymphoma (31). Controlling the transfer of proteins through the endoplasmic- Golgi network secretion pathway or the accumulation of unfolded proteins in response to stressors leads to their competitive binding to the ER lumen chaperones, Bip/GRP 78 (binding protein)/(73 kDa glucose-regulated protein). This issue causes chaperone displacement and separation from serine-threonine Ire-1αkinase existed on the ER membrane, which allows the kinase to dimerize and phosphorylate its cytosolic trace (autophosphorylation). Then, the cytocellular part of this kinase, by presenilin-1 (PS-1), undergoes a proteolytic breakdown and is transmitted to the nucleus, where it is specified to increase the transcription of ER chaperones (such as Bip and calreticulin) and the transcription factor CHOP/GADD 153 (C/EBP homologous protein)/(growth arrest and DNA Damageinduciblegene 153). The transcription factor CHOP/GADD 153 reduces the Bcl-2 gene expression and leads to the initiation of mitochondrial apoptosis (7). Regarding the effects of exercise on cadmium-induced apoptotic indices, some studies have suggested that submaximal endurance training by modulating the negative effects of cadmium in the body can protect the heart tissue (32). The extent for exercise training effects on Bcl-2 family of factors that suppress apoptosis was previously reported by Siu et al. (2004). The authors showed a 68% increase in the Bcl-2 content for animals that were trained by running five days weekly for eight weeks (17).

At the end, our result demonstrated that vitamin D remarkably attenuated the cadmium-induced effect. By our knowledge there is no study about the effect of vitamin D on the cadmium effects. Recently, Amanpour et al. (2019) indicated that the other antioxidant vitamin (vitamin E) significantly decrease the mRNA expression of Bax and caspase-9 genes and increase the mRNA Mfn1, Mfn2, and Bcl-2 in the rats' testes receiving cadmium. They noted that cadmium exerts its

apoptotic effects through the mitochondrial pathway by increasing the ratio of Bax/Bcl-2 and activating caspases in the rats' testes, and vitamin E plays a protective role against cadmium-induced apoptosis (11).

Conclusion

Our findings suggested that the combination of exercise training with vitamin D supplementation could increase the Bcl-2 gene expression as an anti-apoptotic protein in the heart.

Conflict of interests: The authors declare that they have no conflict of interest.

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