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Original Article

Effect of eight weeks of swimming training and CBD oil consumption on PI3K and ERK gene expression in the heart tissue of rats with myocardial infarction

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Abstract

Background and aims: Cannabidiol (CBD) oil consumption can positively contribute to controlling the cardiovascular risk factors. Exercise can also be effective in rehabilitating myocardial infarction by strengthening muscle tissue. This study, therefore, aimed to evaluate the effect of eight weeks of swimming training together with CBD oil consumption on PI3K and ERK gene expression in the heart tissue of rats with myocardial infarction.

Methods: In this experimental trial, 25 ovariectomized rats with myocardial infarction were divided into five groups, including 1) control, 2) MI 3) MI +training, 4) MI + supplement, and 5) MI +training+ supplement groups. Myocardial ischemia was induced by subcutaneous injection of isoproterenol (50 mg/kg intravenously) in myocardial infarction rats. Groups 3 and 4 received 50 mg/kg CBD as gavage on a daily basis for eight weeks, and groups 2 and 4 performed swimming training five days a week. One-way analysis of variance (ANOVA) with Tukey's post hoc test was performed to analyze the findings ($P<0.05$).

Results: Induction of myocardial infarction contributed significantly to reducing PI3k and ERK gene expression in the heart tissue ($P=0.001$). However, swimming training with CBD oil consumption contributed significantly to increasing PI3K ($P=0.003$) and ERK ($P=0.001$) gene expression in the heart tissue of rats with myocardial infarction.

Conclusion: Seemingly, swimming training along with CBD oil consumption had more favorable effects on improvement of PI3K and ERK gene expression levels in myocardial infarction than either alone. Therefore, it was recommended that CBD oil together with swimming training should be employed when dealing with myocardial infarction.

Keywords: Swimming training, CBD oil, PI3K, ERK, Myocardial infarction

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Introduction

Myocardial infarction is one of the most fatal forms of ischemic heart disease in the world. The number of people with myocardial infarction worldwide reaches about three million, and it is argued that about 50% of deaths from cardiovascular disease are associated with myocardial infarction (1). Myocardial infarction is caused by insufficient oxygen-rich blood flow to the heart, which results in an imbalance in the ratio of oxygen-to-oxygen demand and, ultimately, in the damage to heart tissue (2). The benefits of exercise training on metabolic, cardiovascular, anti-inflammatory, and other factors have led many researchers to recognize exercise as an extremely effective, non-pharmacological method for preventing and treating cardiovascular disease (3). The effectiveness of exercise training as a reliable means of treating myocardial infarction disorders has been documented in clinical and experimental conditions. Swimming increases sarcomeres and encourages the growth of heart cells to compensate for the chronic increase in hemodynamic load (4). Cardiovascular diseases lead to incompatible regeneration in heart cells by increasing fibrosis and decreasing aerobic capacity. Some reports have suggested that the PI3K/AKT signaling pathway is an important regulator of cell growth that regulates cell growth and proliferation processes, gene expression, and protein synthesis (5,6). Many studies have indicated that the PI3K enzyme is activated by specific stimuli in the heart, and it plays an important role in regulating heart muscle growth by binding to tyrosine kinase receptors. Inhibition of PI3K enzyme in the embryonic period has been also shown to result in smaller hearts but with similar function to healthy rats (7,8). Other studies have demonstrated the hypertrophy matched in rats with the PI3K precursor gene and those without the insulin-like growth factor-1 (IGF-1) precursor gene compared to rats with the PI3K precursor gene with IGF-1. These studies have also documented the major role of PI3K in occurrence of hypertrophy following a continued activity (9,10). Studies have found that PI3K gene expression decreases following cardiovascular disease, especially myocardial infarction (5). Lin et al indicated that PI3K activation protects the heart against myocardial infarction-induced heart failure, an event that takes place through positive effect of PI3K/Akt pathway activation

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on the gene expression of *growth factor receptor*-bound protein *14* in the heart (11). The ERK signaling pathway, belonging to the MAPK family, also represents a cascade containing a sequence of successive kinases that ultimately leads to phosphorylation and activation of terminal kinases, such as p38, JNK, and ERK (12,13). Furthermore, the ERK signaling pathway is initiated by several receptor families containing tyrosine kinase receptors (insulin growth factor receptors and fibroblast receptors), G protein receptors (angiotensin II, adrenergic receptors and endothelin-1), and cardiotrophin-1 (130 gp receptors) in cardiac myocytes; and the ERK signaling pathway is also caused by various stresses and tensile stimuli (14,15). Each ERK, once activated, phosphorylates a wide range of intracellular targets, including transcription factors, resulting in reprogramming of heart gene expression (13). Moreover, once activated, ERK is transported to the nucleus and phosphorylates several layers, including transcription factors such as CREB and Elk1. Activation and suppression of nuclear targets lead to induction of growth as well as proliferation and prevention of cell death (4). Palabiyik et al showed that growth hormone combined with swimming exercise affected the PI3K/AKT/mTOR signaling pathway in the left ventricular tissue of rats (15). A study found that post-MI exercise training was effective in improving heart function and pathological regeneration by reducing oxidative stress and activating the PI3K/Akt pathway (16). Yan et al revealed that ventricular myocyte size and ERK1/2 phosphorylation increased after one week and four weeks of high-intensity running (17). Studies have also demonstrated that long-term exercise training has beneficial effects on human health; however, due to the rapid progression of metabolic disorders as well as the increase in risk factors among these people, it seems that proper diet is also of particular importance in addition to physical activity. Due to the nature of exercise training and its challenging effect on oxidative stress and inflammation, moreover, it seems that using natural anti-inflammatory and antioxidant factors along with performing exercise training can have a more favorable effect on the health of these people (18). In recent years, researchers have developed an interest in the therapeutic potential of cannabidiol (CBD) phytocannabinoid, naturally found in Cannabis sativa/indica, commonly known as marijuana. Several studies have shown that CBD is involved in the modulation of the immune system; it is an anti-inflammatory and anti-psychotic muscle relaxant, protective against myocardial ischemia and re-injury of blood flow, and involved in nerve protection (19). A study found that consuming CBD oil reduced myocardial damage (20). Given the importance of time management in myocardial infarction as well as the optimal role of each intervention (i.e., exercise and CBD) in myocardial infarction, there is limited information regarding the simultaneous effect of exercise training and CBD oil consumption on the subsequent ERK/PI3K axis in the heart tissue following exercise. Therefore, the present study aimed to investigate the effect of eight weeks of aerobic training with CBD oil consumption on PI3K and ERK gene expression in the heart tissue of isoproterenol-induced MI rats.

Materials and Methods

Maintenance of laboratory animals

In this experimental trial, 25 Wistar rats were obtained from the Pasteur Institute of Iran and, after transferring them to the laboratory, were kept there for one week for adaptation. In the course of the study, the animals were maintained in standard conditions including 12-hour dark-light cycle, ambient temperature of 20-22°C, relative humidity of 55%, and *ad libitum* access to water and food. In addition, all ethical principles of working with animals in this research were followed in accordance with the Declaration of Helsinki and under the supervision of the Ethical Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

Modeling method and induction of myocardial infarction

When the animals adapted well to the environment and the isoproterenol was subcutaneously injected at a dose of 85 mg/kg as a normal saline solution (1 mg/mL) for two consecutive days with 24 hours of interval in order to induce myocardial infarction in the experimental rats. One day after injection, four animals died but were replaced immediately; thus, the induction of myocardial infarction was directed based on the standard protocols. In this vein, a number of rats were randomly anesthetized two days after myocardial infarction, and samples of cardiac tissue from their left ventricle were extracted and examined using histochemical fans of hematoxylin and eosin staining; furthermore, the appearance of white areas indicating necrotic damage due to MI in the heart tissue was confirmed. Finally, 25 rats were included in the study. That is, 25 rats with myocardial infarction were randomly assigned to five groups, including: 1) control, 2) MI 3) MI +training, 4) MI + supplement, and 5) MI +training+ supplement groups.

Preparation of CBD oil

As for the CBD oil preparation, 2 mL of CBD oil was prepared in normal saline solution at a dose of 50 mg/kg (20).

Rats training protocol

Swimming training protocol was performed for eight weeks, three days a week and 30 minutes a day at a given time between 14:00 and 17:00 in a 150 × 90 × 70 cm plastic tank with a water temperature of 28±1°C. Other groups were kept *in vitro* during the implementation of the protocol. To implement the training protocol, the animals in the training and training and supplementation groups were introduced to animal having swum for two weeks. In the first week, referred to as the adaptation week, swimming training was performed so that the duration

of swimming was 10 minutes on the first day; however, 10 minutes was added to the time every session in the following days so that the rats' swimming time reached 30 minutes per day after a week, and it was maintained until the end of week eight (21).

Dissection and sampling

To perform the dissection and sampling, the rats were anesthetized with a combination of 50 mg/mL ketamine and 20 mg/mL xylazine solution 48 hours after the last training session and in a 12-hour fasting state. To diagnose anesthesia, laboratory experts used pain testing methods. After ensuring the complete anesthesia, the animal chest cavity was opened and other tissues were removed. Then, the arteries entering and leaving the heart were cut, and the heart tissue was carefully extracted and immediately immersed in a nitrogen tank. The heart tissue was then kept at -80°C until the variables were measured.

Molecular analysis of myocardial tissue by real-time PCR

Molecular analysis was performed at gene expression level. To this end, initially the RNA was extracted from tissues in all studied groups, based on the manufacturer's protocol (Qiagen, Germany). Thus, 200 µL Landa Chiazol was added to the samples and incubated at -80°C for 24 hours.

The plaque in cryotube was crushed in semi-freezing state and 100 µL chloroform was added to the samples for 1 minute to lyse the samples. The ensuing solution was centrifuged at 12 000 rpm for 10 minutes. The clear liquid at the top of the tube containing the RNA was lightly removed and placed in a DEPC microtube.

Next, 1 cc of isopropanol was poured onto clear RNA and stirred by hand for 1 minute. The samples were centrifuged at 12 000 rpm for 10 minutes. Then the supernatant was discarded and 1 cc of 70% alcohol was added to the sediment. After extracting RNA with high purity and concentration from all samples, cDNA synthesis was performed in accordance with the protocol of the manufacturer (Fermentas, USA) and, then, the synthesized cDNA was used for applying reverse transcription reaction. Measurement of PI3K and ERK1/2 expression levels of heart tissue was performed by adopting real-time PCR quantitative method (Table 1).

Statistical analysis

To report descriptive data, the mean and standard deviation were presented. After confirmation of the data normality by the Shapiro-Wilk test, one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to determine the significant difference between the means of the variables of the research groups. The required data were collected and analyzed by SPSS version 22 at $P \leq 0.05$. Excel software was used to draw the graphs.

Results

Levels of PI3K and ERK gene expression in the heart tissue of rats are shown in Figures 1 and 2. The results of one-way ANOVA showed that there was a significant difference among different research groups in terms of the levels of PI3K and ERK gene expression in the heart tissue of rats ($P \geq 0.05$) (Table 2).

The results of Tukey's post hoc test demonstrated that PI3K levels in the MI group were significantly lower than those in the control group ($P = 0.01$). In the MI + training ($P = 0.05$) and MI +CBD+ Exe groups ($P = 0.01$), PI3K levels were significantly higher than those in the MI group.

The results of Tukey's post hoc test showed that ERK levels in the MI group were significantly lower than those in the control group ($P = 0.001$). In the training ($P = 0.05$) and training + MI groups ($P = 0.001$), ERK levels were significantly higher than those in the MI group.

Discussion

The results of the present study revealed that eight weeks of swimming training along with CBD oil consumption significantly increased the levels of PI3K gene expression in the heart tissue of the rats with myocardial infarction. The IGF1-PI3K-Akt signaling pathway is considered to be the main signaling pathway responsible for mediating physiological cardiac hypertrophy resulting from prolonged exercise. Activation of this signaling cascade has also been shown to protect the heart in rat models of cardiovascular injury and cardiovascular disease, while decreased IGF1-PI3K-Akt signaling is detrimental to heart function and accelerates the disease progression (22). The PI3K/AKT signaling pathway is an important regulator of cell growth that regulates cell growth and proliferation processes, gene expression, and protein synthesis. It has been discovered that physiological

Table 1. Primer sequences

Gene name	Oligo sequence
PI3K	F ATATCCACCTGTCCTCTCCCT
	R CTAATCTTCTCCCTCTCCTTCCA
ERK	F GTGCTACAGAGGGGTGGAGGG
	R GCTTGAGAGGGAGAGGGTTAGG
GAPDH	F AAG TTC AAC GGC ACA GTC AAG G
	R CAT ACT CAG CAC CAG CAT CAC C

Table 2. One-way ANOVA for PI3k and ERK

Variable	Group					
	Control	MI	MI + training	MI + supplement	MI + training + supplement	*P* value
PI3K	0.0186±0.0076	0.0061±0.0018	0.0122±0.0036	0.0084±0.0041	0.0150±0.0042	0.003
ERK	0.0035± 0.0015	0.0011±0.0005	0.0021±0.0004	0.0017±0.0010	0.0036±0.0009	0.001

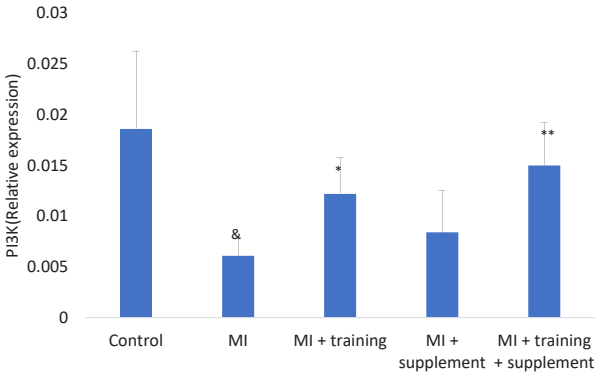

Figure 1. The mRNA expression of PI3k in the heart tissue of different study groups. The obtained values are displayed as means and standard deviation (mean ± SD). Statistically significant differences between the mean values ($P<0.05$). * $P\leq0.05$, ** $P\leq0.01$ Significant increase compared to group MI; $^{\&}P\leq0.01$ Significant decrease compared to control group.

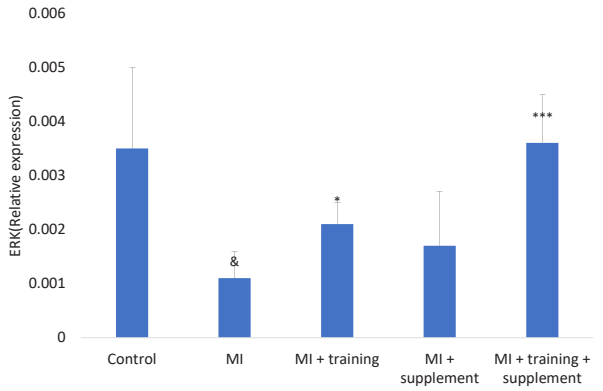

Figure 2. The mRNA expression of PI3k in the heart tissue of different study groups. The obtained values are displayed as means and standard deviation (mean ± SD). Statistically significant differences between the mean values ($P<0.05$). * $P\leq0.05$, ** $P\leq0.01$ Significant increase compared to group MI; $^{\&}P\leq0.01$ Significant decrease compared to control group.

hypertrophy of the heart occurs with the interaction of the simultaneous presence of IGF and PI3K and through direct regulation of key components of the Z plates in the structure of heart cells (23). Many studies have suggested that the PI3K enzyme is activated by specific stimuli in the heart, and it plays an important role in regulating heart muscle growth by binding to tyrosine kinase receptors. Inhibition of PI3K enzyme in the embryonic period has been also detected to result in smaller hearts but with similar function to healthy rats (14,24). Numerous studies have confirmed the role of PI3K in cardiac hypertrophy. McMullen et al, for instance, showed that rats with predominantly negative PI3K had smaller hearts and their cardiac hypertrophic responses to swimming activity were higher than those in healthy rats. Rats with a lower PI3K also developed a faster myocardial infarction (9). Hildick-Smith and Shapiro found that mutant rats had a much smaller heart with a 77% reduction in PI3K production, while their heart muscle function did not change; on the other hand, the heart weight to body weight ratio (HW/BW) in rats with higher mutant PI3K showed an increase of up to 20%. In addition, myocardial function was normal and no cardiac fibrosis was observed (25). All these results were consistent with our study findings regarding the development of cardiac hypertrophy with increased expression of PI3K gene. McMullen et al reported a positive role for PI3K in cardiac hypertrophy following endurance activity. Furthermore, McMullen et al showed that after 4 weeks of regular swimming training, the rate of myocardial hypertrophy and PI3K levels were higher in healthy trained rats compared to those in healthy non-trained rats (9). The researchers have reported that swimming training causes physiological hypertrophy and PI3K inhibition in mutant rats. The findings of Weeks et al also has confirmed the essential role of PI3K in providing cardiac protection due to exercise. These researchers have found that an increase in PI3K following continuous activity can lead to improved heart function (26). This result was in line with our study results concerning the effects of swimming and CBD supplementation.

The results of the present study also showed that eight weeks of swimming training along with CBD oil consumption significantly increased the levels of ERK1/2 gene expression in the heart tissue of the rats with myocardial infarction. Some previous studies have reported that the ERK1/2 signaling pathway is activated in cardiac myocytes in response to almost any type of stress stimulation being studied to date. For example, endocrine neurotransmitter glands, G protein receptor agonists, tyrosine kinase receptor agonists, cytokines, reactive oxygen species, and tensile all activate ERK1/2 and, in most cases, the hypertrophic response, indicating that ERK1/2 directly manages to plan its own growth (27,28). It is generally accepted that ERK1/2 activation is essential for cardiac hypertrophy (29). However, several partially contradictory studies have determined that ERK1/2 may not only lead to maladaptive cardiac hypertrophy but also to physiological hypertrophy (30,31) or that it may have no effect on cardiac hypertrophy (13). In addition, Lu et al showed that the ERK1/2 signaling pathway uniquely regulated the balance between eccentric and concentric growth of the heart (32). Yan et al revealed that high-intensity running resulted in the activation of ERK1/2 after one week. They also reported that the amount of phosphorylated ERK1/2 increased significantly by 134.3%, 137.1%, and 237.1% when the rats ran at low, medium, and high intensity running, respectively, for four weeks (17). In another study, and despite the fact that ERK1/2 phosphorylation was initially eliminated after stimulation of excess pressure, it did not reduce the hypertrophic response to exercise-induced overload stimulation (13). Exercise has been documented to activate several MAPK pathways in the heart, an effect that gradually diminishes with the development of exercise-induced cardiac hypertrophy. Taking into account the results from previous studies and the current evidence, therefore, it is argued that ERK1/2 is a positive regulator in the progression of cardiac hypertrophy (31). Our study results showed a significant increase in PI3K and ERK1/2 gene expression in the heart tissue of the group treated with CBD oil and training compared to those in the model

group. Chronic use of CBD is well tolerated in humans without side effects. Cannabidiol has several therapeutic effects including antioxidant, anti-inflammatory and anticoagulant effects (20). Cannabis has been discovered to contain more than 20 types of flavonoids. CBD has a cardioprotective effect against myocardial ischemia and re-damage to blood flow. Rajesh et al showed that CBD administration reduced myocardial damage by preventing a systemic inflammatory response. Walsh et al revealed that a single acute dose of CBD (50 mg/kg intravenously) reduced myocardial I/R damage. CBD may increase adenosine signaling and, therefore, may lead to activation of the adenosine A1 receptor (33).

One of the limitations of the present study was the small number of the study groups together with strict control of the received diet. Due to the role of PI3K and ERK isoforms in cardiac hypertrophy and their affectability in exercise as well as the failure to measure different isoforms, moreover, it was recommended that different measurement methods such as Western blotting and ELISA should be adopted when conducting future studies.

Conclusion

In was concluded that CBD supplementation along with exercise training – regular swimming, in particular – was effective in controlling the damage to the heart muscle by positively regulating PI3K and ERK in the heart tissue. Therefore, consuming CBD oil together with performing swimming training was highly recommended in case of myocardial infarction.

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Authors' Contribution

MS carried out tests and collected the data. KH and FT designed the study and analyzed the data. MS and KH wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interests

The authors declare that they have no conflict of interests to disclose.

Ethical Approval

The study protocol was approved by the Research Ethics Committee of Isfahan (Khorasgan)
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