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Original Article

The Effect of Short-Term Treadmill Exercise on the Expression Level of TFAM in the Heart of Nicotine-Sensitized Rats

Amir Abbas Lashgari[1], Mohammad Ali Azarbayjani[1,*], Maghsoud Peeri[1], Mohammad Nasehi[2]

[1]Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
[2]Cognitive and Neuroscience Research Center (CNRC), Amir-Almomenin Hospital, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

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Correspondence
Mohammad-Ali Azarbayjani
Email:m_azarbayjani@iauctb.ac.ir

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Abstract

Introduction: TFAM (mitochondrial transcription factor A) is involved in mitochondrial biogenesis and induces anti-oxidant and anti-apoptotic effects. Nicotine can also alter the function of cardiovascular system and induce heart failure and other heart diseases. Interestingly, it has been reported that exercise can interfere with the effects of nicotine, and change the expression pattern of different genes. The goal of the present study was to investigate the effect of short-term treadmill exercise on the expression level of TFAM in the heart of nicotine-sensitized rats.

Materials and Methods: Nicotine was administered intraperitoneally at the dose of 0.21 mg/kg. Treadmill exercise was performed during 14 days, according to the study's protocol.

Results: The results revealed that nicotine reduced the expression of TFAM. The treadmill (Fourteen-day training) increased the expression of TFAM in the heart of the control rats. Furthermore, 14-day training with treadmill restored the effect of nicotine on the expression of TFAM in nicotine-sensitized rats.

Conclusion: Nicotine induced pro-apoptotic and anti-oxidative stress effects via down-regulating the expression of TFAM. Fourteen -day training with treadmill induced a protective effect against nicotine-induced cardiac apoptosis and oxidative stress, via restoring the effect of nicotine on TFAM. The results are indicative of the fact that short-term treadmill exercise may decrease the risk of heart failure and other cardiac diseases.

Keywords: TFAM (mitochondrial transcription factor A), Nicotine, Treadmill, Heart, Rats

1. Introduction

TFAM (mitochondrial transcription factor A) is a member of HMGB (high mobility group box) subfamily, involved in the maintenance of mtDNA (mitochondrial DNA) and protection of mitochondrial biogenesis from the negative effects of oxidative stress [1-3]. In fact, TFAM is critically involved in mitochondrial biogenesis [4, 5]. Previous research has reported that in heart failure, the level of TFAM is significantly reduced and cardiomyocyte instability ensues [6]. Furthermore, TFAM can prevent NFAT (nuclear factor of activated T cells) activity, which decreases ROS production [6]. Thus, it seems that decrease in TFAM level can enhance the expression of protease and hypertrophic factors, leading to cardiomyocyte functional deficit [6]. Tobacco smoking is one of the leading causes of preventable morbidity and mortality worldwide. Nicotine, one of the

major active components in cigarettes may contribute to maternal smoking-induced developmental programming of cardiovascular dysfunction in offspring [7]. It has been revealed that perinatal nicotine exposure alters the function of cardiovascular system and induces a heart ischemia-sensitive phenotype in adult offspring [8]. Nicotine can also affect genes expression [9, 10]. For example, previous research has reported that nicotine enhances the expression of genes involved in mitochondrial biogenesis in the hippocampus [11]. However, there is no published paper about the exact effect of nicotine on the expression of TFAM.

On the other hand, exercise can alter the effect of nicotine. It has been reported that treadmill exercise improves nicotine withdrawal-induced anxiety, depression, and memory impairment [12]. Another study has shown that moderate-intensity exercise ameliorates nicotine-induced cognitive behaviors, and a possible mechanism is the role of prefrontal α7-nAChR-mediated signal transduction [13]. Additionally, exercise can affect gene expression. For example, treadmill exercise inhibits age-induced myonuclear apoptosis via activating SIRT1 (Silent information regulator 1)/PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) mRNA expression [14]. Note that the effect of exercise on the expression of TFAM is not properly studied.

According to the mentioned findings, the goal of the present study is to investigate the effect of short-term treadmill exercise on the expression level of TFAM in the heart of nicotine-sensitized rats.

2. Materials and Methods

2.1. Animals

In this study, male Wistar rats weighing 180-200g were used. All rats were transferred to the laboratory and placed in Plexiglas cages. All rats had ad libitum access to water and food (except during the tests) and their cages were cleaned every other day. The temperature in the lab was 22±3°C and the light/dark cycle was 12/12h. All experiments were done only in the light phase.

2.2. Nicotine Administration

Nicotine was administered intraperitoneally at the dose of 0.21 mg/kg with insulin syringe (1 ml/kg). Before administration, nicotine was diluted in normal saline 0.9%.

2.3. Treadmill Schedule

After the last injection of nicotine, treadmill exercise started for 14 days. At the first day of exercise, rats were placed on treadmill (10 m/s) and ran for 10 minutes. Then, 10 minutes was added to running for each day, until the total time of exercise reached 60 minutes (day 2: 20min, day 3: 30min… day 6: 60min). This was the first phase of exercise, also called habituation period. For the next days (day 7 to 14), rats ran on the treadmill for 60 minutes [15].

2.4. Experimental groups

This study consisted of 6 groups and 48 rats (each group included 8 rats):

Saline+Sedentary Group: Rats of this group received intraperitoneal administration of saline (1ml/kg) for 4 consecutive days. Then, they were placed on treadmill for 14 consecutive days when the treadmill apparatus was off.

Saline-Exercise Group: Rats of this group received intraperitoneal administration of saline (1ml/kg) for 4 consecutive days. Then, they were placed on treadmill for 14 consecutive days when the treadmill apparatus was on.

Nicotine Group: Rats of this group received intraperitoneal administration of nicotine (0.21 mg/kg) for 4 consecutive days.

Nicotine-Exercise Group: Rats of this group received intraperitoneal administration of nicotine (0.21 mg/kg) for 4 consecutive days. Then, they were placed on treadmill

for 14 consecutive days, when the treadmill apparatus was on

2.5. Real-time PCR

To assess mRNA expression, complementary DNA (cDNA) was prepared from the whole cellular RNA. The total RNA was extracted by using BioFACT™ Total RNA Prep Kit. The RNA was quantified through Picodrop Microliter Spectrometer. cDNA was prepared by using BioFACT™ OneStep RT-PCR Kit according to the manufacturer's method in the final volume of 40µl. Finally, the cDNA was stored at -20°C. To normalize target gene expression, beta-actin was used as the housekeeping gene. The primer which was used for the real-time PCR was TFAM. Real-time PCR reactions were performed through using Takara SYBR Premix Ex Taq II (Tli RNaseH Plus) (2X conc.) in the final volume of 20µl on StepOnePlus Real-Time PCR System (Applied Biosystems). The 2µl of the synthesized cDNA was used in all reactions. The annealing temperature optimized for primers pairs was 64°C. Standard curve method was used for quantification of target gene. Beta-actin was declared as the reference gene and used to normalize the results. All the samples were loaded in duplicate and the mean data were used for further analysis. The specificity of PCR products was verified through observing a single peak in melting curve analysis. For complementary length verification, PCR products were visualized on 2.5% agarose gel.

2.6. Statistical Analysis

For analyzing data, SPSS software was used. The results are indicated as Mean±S.E.M considering normal distribution of data and homogeneity of variance. One-way ANOVA followed by post hoc analysis was used to assess the differences between groups. $P<0.05$ was considered as statistically significance.

3. Results

The One-Way ANOVA followed by post hoc analysis showed that [$F(5, 18)= 3.35$; $P<0.05$] 14-day training with treadmill increased the expression of TFAM in the heart of the control rats. Nicotine reduced the expression level of TFAM in the heart. Furthermore, 14-day training with treadmill restored the effect of nicotine on the expression of TFAM in nicotine-sensitized rats (Fig.1).

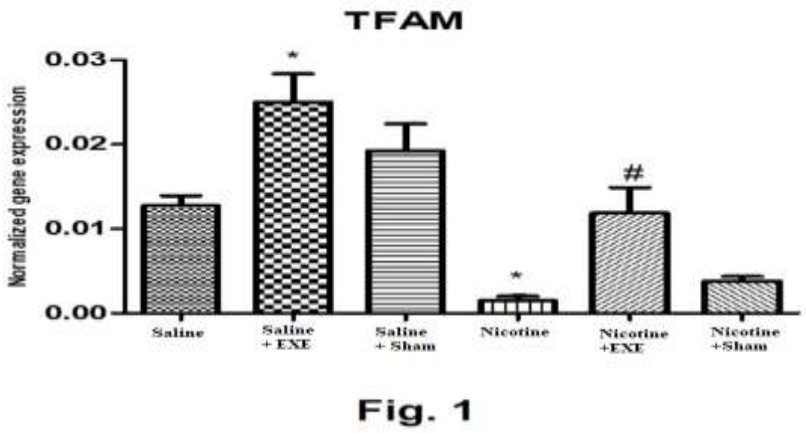

Figure 1. The expression level of TFAM in the heart of the rats in all experimental groups. ($P<0.05$* compare with saline group and $P<0.05$# compare with nicotine group).

4. Discussion

The goal of the present study was to investigate the effect of short-term treadmill exercise on the expression level of TFAM in the heart of nicotine-sensitized rats. The results showed that nicotine decreased the expression of TFAM, while treadmill exercise restored the effect of nicotine on TFAM. Mitochondrial dysfunction has been observed in a wide-range of heart diseases, such as myocardial ischemia, diabetic cardiomyopathy and heart failure [16]. It has been reported that mitochondrial dysfunction induces impaired myocardial energetics and enhanced apoptosis during myocardial injury [17]. As mentioned, TFAM (mitochondrial transcription factor A) is involved in the maintenance of mtDNA (mitochondrial DNA) and protection of mitochondrial biogenesis from oxidative stress [1-3]. TFAM regulates mitochondrial biogenesis [4]. Mitochondrial biogenesis also needs TFAM to protect mtDNA from oxidative damages [1]. Following heart failure, the level of TFAM is significantly decreased, leading to cardiomyocyte instability [6]. Previous research has suggested that HSP70 (the 70 kilodalton heat shock protein) transgenic mice are cardioprotective in a doxorubicin heart failure model [18]. Naka and et.al has shown that the up-regulation of HSP-70 enhances mitochondrial biogenesis via an increase in TFAM transport, leading to calcium regulation, decrease in protease activity and apoptotic signaling, and inducing a cardioprotective effect [18]. Furthermore, previous reports have shown that transgenic TFAM mice with volume-overload (VO) heart failure have cardioprotective effects, when compared to wild type mice [19] possibly due to the suppression effect of TFAM on ROS accumulation [20]. TFAM transgenic mice also have a higher survival rate after myocardial infarction [19]. Evidence shows that myocardial infarction in transgenic TFAM mice is accompanied by decrease in myocyte hypertrophy, oxidative stress, apoptosis and interstitial fibrosis, compare with wild type mice [19]. Additionally, in failing hearts, TFAM level initially goes up as a compensatory mechanism, but it is significantly reduced following calcium mishandling and ROS accumulation, which are observed in the later stages of heart failure [19]. In transgenic mice, the overexpression of TFAM has been reported to enhance the mitochondrial number and cope with cardiomyopathy [21]. Furthermore, TFAM overexpression attenuates myocyte hypertrophy and cardiac chamber dilation [22]. Interestingly, previous research has reported that the absence of TFAM in mitochondria of failing hearts has a critical role in mitochondrial dysfunction [19].

On the other hand, perinatal nicotine exposure alters various neurotransmitter systems in the central nervous system including the serotonergic and the cholinergic systems, which are significantly involved in cardiorespiratory function [23, 24]. Fetal nicotine exposure also impairs cardiac and respiratory responses to hypoxia [25]. Apoptosis is a mechanism which is considered as a main actor in the development of cardiac failure [26, 27]. Interestingly, nicotine can trigger cardiomyocyte apoptosis [28, 29]. Furthermore, nicotine can inactivate AMPK and augment oxidative stress [30]. As mentioned, AMPK activates PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1α) (and subsequently TFAM) and improves mitochondrial biogenesis [31]. In addition, oxidative stress can induce cardiac apoptosis. Previous reports have also declared that nicotine promotes cardiomyocyte apoptosis via induction of oxidative stress and disruption of apoptosis-related gene expression [29]. In the present study, nicotine induced a pro-apoptotic effect and augmented oxidative stress via down-regulating the expression of TFAM. Nicotine also decreased the expression of TFAM probably via inactivating AMPK and impairment of mitochondrial

biogenesis. On the other hand, the protective effect of exercise against apoptosis has been shown [32]. It has been revealed that low intensity exercise reduces apoptosis and oxidative stress in streptozotocin-induced diabetic rat heart [32]. Furthermore, treadmill exercise and L-arginine supplementation induce a protective effect against age-induced myocyte loss and fibrosis formation in the ventricle via suppressing oxidative stress, inflammation and apoptosis [33]. Treadmill exercise also inhibits age-induced myonuclear apoptosis via increasing SIRT1/PGC-1α mRNA expression [14]. Previous research has reported that 8 weeks of treadmill exercise increases angiogenic and mitochondrial signaling in mice, and increases the expression of PGC-1 and TFAM [34]. In line with these findings, in the present study, short-term treadmill exercise increased the expression of TFAM in both control and nicotine-sensitized rats. We suggest that short-term treadmill exercise can induce a protective effect against nicotine-induced cardiac apoptosis and oxidative stress. In addition, it can decrease the risk of heart failure and other cardiac diseases.

5. Conclusion

The results of the present study showed that nicotine decreased the expression of TFAM, while treadmill exercise restored the effect of nicotine on TFAM. The findings of this study suggest that nicotine induced a pro-apoptotic effect and augmented oxidative stress via downregulating the expression of TFAM. Further, nicotine decreased the expression of TFAM via inactivating AMP-activated protein kinase (AMPK) and impairment of mitochondrial biogenesis. Short-term treadmill exercise induced a protective effect against nicotine-induced cardiac apoptosis and oxidative stress via restoring the effect of nicotine on the expression of TFAM. Moreover, it is concluded that short-term treadmill exercise may decrease the risk of heart failure and other cardiac diseases.

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Conflict of interest

The authors declare no conflict of interest.

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