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The effect of swimming training on the expression of HIF-1α in the lung tissue of mice with lung cancer induced by benzo[a]pyrene (B[a]P)

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Abstract

Introduction: Hypoxia-inducible factor 1-alpha (HIF-1α) is commonly highly expressed in tumors under conditions of hypoxia or activation of oncogenic pathways. This study aimed to investigate the effect of swimming training on the expression of HIF-1α in the lung tissue of mice with lung cancer induced by B[a]P.

Materials and Methods: In this study, 18 male Balb/c mice (mean age: 8 months; weight: 18 to 22 grams) were divided into 3 groups (healthy control (HC), lung cancer control (B[a]P), and lung cancer + training (ST) group). Lung cancer was induced by injection of B[a]P (100 mg/kg). The ST group received swimming training for 12 weeks, 3 sessions per week. After the intervention period, lung tissue was dissected and HIF-1α gene expression was measured using real-time PCR. Statistical analysis was performed using one-way ANOVA with Bonferroni's post- hoc test.

Results: a significant difference was observed between the study groups (P=0.003). Based on the results of Bonferroni post hoc test, HIF-1α in the healthy control group was significantly lower than in the B[a]P and ST groups (P = 0.007; P = 0.01, respectively). Further investigation showed that HIF-1α was not significantly different between the B[a]P and ST groups (P = 0.99).

Conclusion: The findings of this study indicate that swimming training intervention does not reduce HIF-1α gene expression.

Keywords: Lung cancer, Angiogenesis, HIF-1α, swimming training

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Introduction

The most common malignant tumour with a high fatality rate is lung cancer. The main causes of lung cancer are tobacco inhalation, chronic inflammation, and oxidative stress. It is becoming more common globally, with an annual increase of 0.5 %. In developed countries, men are more susceptible to lung cancer, mostly because of smoking (1). Polycyclic aromatic hydrocarbons (PAHs), which are among the more than 60 carcinogens in tobacco smoke, are critical in the development of lung cancer. Among PAHs, by benzo[a]pyrene (B[a]P) is one of the most potent carcinogens that cause the development of lung cancer. BaP is metabolically activated into an epoxide derivative, which reacts with DNA and is combined to form a DNA adduct that induces carcinogenesis. It leads to oxidative damage to DNA by inducing reactive oxygen species (ROS) (1). Angiogenesis is the development of new blood vessels that originate from already existing vasculature and is an important process in both normal and pathological conditions. It has a significant impact in the progression and spread of cancers, particularly lung cancer (2). Recently, it has been identified that hypoxia-inducible factor-1 alpha (HIF-1α) is a major transcriptional regulatory factor that responds to hypoxic environments and modulates the expression of many genes in the organism and is closely associated with the biological behavior of malignant tumors. Importantly, HIF-1α is commonly highly expressed in tumors under conditions of hypoxia or activation of oncogenic pathways (3). In fact, HIF is pivotal in the transcriptional response to hypoxia. HIF is composed of a hypoxia-inducible α subunit and a constitutively expressed β subunit. In normoxic conditions, the HIF-α is degraded through the PHDs. Under hypoxic conditions, the activity of PHDs is inhibited, resulting in the accumulation of HIFα and initiation of downstream transcription. HIF-α has 3 isoforms: the ubiquitous HIF-1α, the tissue-specific HIF-2α and HIF-3α.10 HIF-1α activates several important signaling pathways related to metabolism and inflammation. However, the HIF-1α protein is unstable in normoxic conditions, with a short half-life of 5 min, which increases the difficulty of in situ and in vivo detection of tissue hypoxia and HIF-1α expression (4).

Various studies have been performed on the effect of exercise on tumor growth via alterations in the gene expression profile. Moreover, the preventive effects of exercise on cancers have been demonstrated. The angiogenesis of tumor tissue and blood supply is a paramount event for the persistence and growth of the tumor tissue (5). In hypoxia, the blockage of proline hydroxylation due to the inactivation of PHD and factor inhibiting HIF-1 (FIH-1) prevents the hydrolysis of HIF-1α, inducing activation of HIF-1 to affect the expression of VEGF, which drives tumor cell proliferation. Increased shear stress on endothelial cells during aerobic exercise is associated with tumor vascular remodeling, prompting greater blood flow to the tumor. Aerobic exercise alleviates hypoxia by improving tumor perfusion volume, serves to reduce the accumulation of mitochondrial ROS, which favors HIF-1 inactivation (6).

Therefore, given the proposed benefits of exercise and its management effects on cancer, the effects of swimming training on lung cancer remain unstudied. Also, HIF-1α, as a critical regulator of tumorigenesis, is dysregulated in cancer and alters the rate of tumorigenesis in both tumor and

healthy tissues. Therefore, it is essential to investigate how therapeutic interventions such as exercise affect HIF-1α. Therefore, considering the existing gaps, the present study aimed to investigate the effect of swimming training on HIF-1α expression in lung tissue of mice with B[a]P -induced lung cancer.

Methods

Study Design and Animals

This study was fully approved by the Animal Ethics Committee (IR.IAU.SHIRAZ.REC.1403.027). This study included a post-test design with 18 male Balb/c mice (mean age: 8 months; weight: 18-22 g). BALB/c mice were purchased from the Pasteur Institute of Iran and transferred to the laboratory, and a week of familiarization with the new environment was performed. After a week of familiarization, 18 mice were randomly divided into 3 groups (healthy control (HC), lung cancer control (B[a]P induction), and lung cancer + swimming training (ST) group).

Cancer Induction

In this study, lung cancer induction was performed using B[a]P (Sigma-Aldrich, Germany), dissolved in olive oil and then injected intraperitoneally (100 mg/kg) into fasted mice. To confirm the successful induction of lung cancer, initially in a pilot study, 8 mice (4 mice injected with B[a]P and 4 healthy mice as controls) were sacrificed after 3 weeks and then lung tissues were analyzed for tumorigenesis using pathological examinations. After confirming the induction of lung cancer, another 18 mice received B[a]P (7).

To examine the expression of HIF-1α gene using Real-time PCR, all primers were designed by Allele IDv7.8 software and TATA-binding protein (TBP) gene was used as an internal control. The primers were designed as exon-exon junctions. To ensure the absence of genomic DNA amplification, 25 ng of cDNA and 25 ng of RNA were used in separate tubes of PCR reaction and 1.5% agarose gel was used. Amplification of cDNA and observation of the desired band by specific primer and lack of RNA amplification after PCR reaction indicates the absence of genomic DNA amplification.

Table 1. Primers used in the study

Gene	Primer sequences	Size (bp)
HIF-1α	Forward: 5’- ACCTTCATCGGAAACTCCAAAG -3’ Reverse: 5’- CTGTTAGGCTGGGAAAAGTTAGG -3’	228

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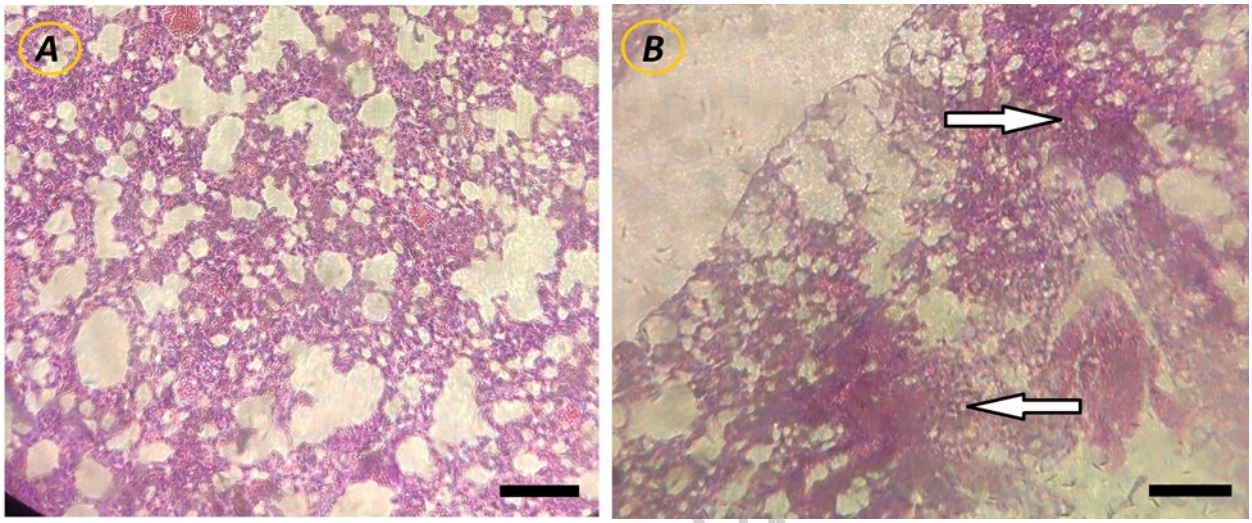

Figure 1. H&E staining of mouse lung paraffin sections. Representative pictures of H&E-stained lung sections. A. lung in the control group; B. lung in the BZP group. Arrowheads indicate tumors. Images acquired at ×100 magnification and scale bars represent 1 mm.

Swimming Training

In this study, swimming trainings were performed for 12 weeks, each week including 3 sessions, and each session lasted from 5 minutes to 38 minutes. The swimming trainings were not the same in the 12 weeks and varied each week. In this way, the intensity of the exercise in the first 4 weeks was the same as swimming trainings without weights (8). And weeks 5 to 8 with a tail weight of 2% of body weight and weeks 9 to 12 and the end of the study with a tail weight of 5% of body weight (9,10). The duration of the exercise was as follows: the first week was 5 minutes, the second week was 8 minutes, the third week was 11 minutes, the fourth week was 14 minutes, the fifth week was 17 minutes, the sixth week was 20 minutes, the seventh week was 23 minutes, the eighth week was 26 minutes, the ninth week was 29 minutes, the tenth week was 32 minutes, the eleventh week was 35 minutes, and the twelfth week was 38 minutes (11).

Tissue Collection and Analysis

48 hours post-last training session, mice were anesthetized (ketamine: 100 mg/kg; xylazine: 10 mg/kg), and lung tissues were excised and stored at −80°C. Total RNA was then isolated using the FavorPrep Total Tissue RNA Kit (FATRK 001, Taiwan), and HIF-1α gene expression was measured via Real-time PCR and calculated using the ΔΔCt method.

Statistical Analysis

Data were analyzed by one-way ANOVA followed by Bonferroni post hoc tests SPSS v25 ($P \leq 0.05$).

Results

Table 1. The result of comparison of HIF-1α levels between research groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.284	2	4.642	8.470	0.003
Within Groups	8.221	15	0.548		
Total	17.505	17			

According to the results of one-way analysis of variance (Table 1), a significant difference was observed between the study groups (P=0.003). In order to find the location of the difference, the Bonferroni post hoc test was used (Figure 1). Based on the results of Bonferroni post hoc test, HIF-1α in the healthy control group was significantly lower than in the B[a]P and ST groups (P = 0.007; P = 0.01, respectively). Further investigation showed that HIF-1α was not significantly different between the B[a]P and ST groups (P = 0.99).

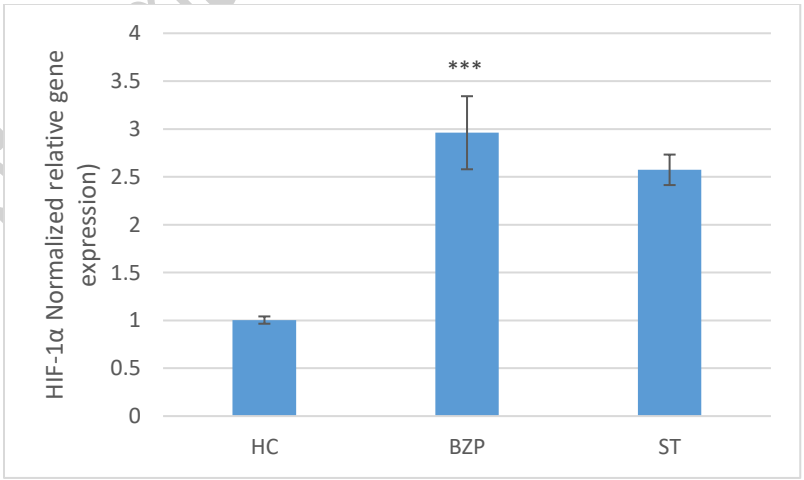

Figure 2. Comparison of HIF-1α gene expression between research groups

Discussion

The aim of the present study was to investigate the effect of swimming training on HIF-1α expression in lung tissue of mice with benzo(a)purine-induced lung cancer. The results showed that cancer induction with B[a]P caused an increase in HIF-1α gene expression in lung tissue. HIF can directly stimulate tumor cell proliferation, migration, and invasion (12). In fact, HIF-1α induces abnormal angiogenesis, further impairing blood flow and oxygenation, thereby reinforcing the cycle of hypoxia and increasingly aggressive cancer behavior (13). In particular, β[α]P, the most widely studied pollutant, has been implicated in both cancer initiation and progression. In a study by Mavrofrydi et al. (2016), B[a]P increased HIF-1α (14), which was consistent with the results of this study. Therefore, it can be inferred that cancer induction led to increased expression of the HIF-1α gene and that this gene plays a pivotal role in the tumorigenesis pathway. Angiogenesis has a significant impact on tumorigenesis and metastatic processes by allowing tumor cells to establish new blood circulation pathways. Angiogenic factors including VEGF, FGF-2, HIFs can directly stimulate tumor cell proliferation, migration, and invasion (12). Hypoxia activates hypoxia-related signaling pathways that are controlled by HIFs. In fact, HIFs are heterodimeric transcription factors consisting of three distinct members (HIF-1, HIF-2, HIF-3) which are composed of an α subunit and a β subunit. Among them, HIF-1 plays a key role in regulating the cellular response to hypoxia. HIF-1α and HIF-1β dimerize to form HIF-1. While HIF-1α is upregulated under hypoxic conditions, HIF-1β is constitutively expressed (15). It should be noted that HIF-1α is responsible for transcriptional activity because it contains trans-active domains, while HIF-1β is the only dimerization partner and is not required for induction. Detection of protein expression in various human tissues can show low levels of HIF-1α protein in normoxic cells (due to proteasomal degradation), even when HIF-1α is overexpressed, but there is a high induction in hypoxic cells (16). In sports and physical activity, the stimulation and activation of VEGF and HIF-1 signals can cause the formation of new blood vessels and angiogenesis, which in turn can lead to cell survival in various tissues and success in sports competitions, especially endurance sports (17). On the other hand, hypoxia, or lack of oxygen in tissues, is a characteristic of the tumor microenvironment that has a major impact on treatment resistance and cancer progression. In cancer, HIF-1α is often regulated not only due to hypoxic conditions, but also through genetic alterations that stabilize and activate the protein regardless of oxygen levels. Mutations in oncogenes and tumor suppressor genes can lead to increased HIF-1α activity, causing a more malignant phenotype and contributing to poor clinical outcomes (18).

In a study by Soltani et al. (2019), it was shown that 8 weeks of aerobic exercise caused a significant difference in tumor volume growth and a significant increase in HIF-1α gene expression, which was inconsistent with the results of this study, which showed that incremental swimming trainings caused a slight decrease in HIF-1α, which was not significant compared to the lung cancer control group, which is probably due to the type and intensity of the exercises (19). In a study by Li et al. (2022), it was shown that swimming significantly reduces tumor growth in mice with colorectal cancer by inhibiting tumor angiogenesis through suppression of the HIF-

1α/VEGFA pathway, in this study, the effect of swimming trainings was not shown to be significant compared to the lung cancer group (20), which is probably due to the type of tissue involved in the tumorigenesis process. Therefore, given these contradictory results and the lack of research that is consistent with that study, it needs to be addressed in future research to ensure the results of this study. Given that lung cancer is the product of deviations in normal cell function, including oxidative stress, genetics, and multiple signaling pathways (21). Reducing the angiogenic factor induced by HIF-1α activity could be a key therapeutic target in lung cancer. Although HIF-1α partially reduced the increase induced by B[a]P, this intervention did not effectively and significantly reduce this angiogenic factor.

Conclusion

The findings of this study indicate that swimming training intervention does not reduce HIF-1α gene expression and, consequently, swimming training in this study did not reduce the tumorigenic pathway in lung cancer tissue induced by B[a]P. Therefore, it is suggested that future research should examine the effects of various types of exercise training with different intensities and other supplements on lung tissue tumorigenic pathways in relation to cancer.

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