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Studying the effect of a swimming training period on NF-κB gene expression in mice with Benzo[a]pyrene -induced lung cancer

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ABSTRACT

Introduction: NF-κB activation affects hallmarks of cancer and inflammatory diseases through the transcription of genes involved in cell proliferation, survival, angiogenesis, inflammation and tumor promotion and metastasis. This study aimed to investigate the effect of a swimming training session on NF-kB gene expression in mice with benzo[a]pyrene-induced lung cancer.

Material & Methods: In this study, 18 male Balb/c mice (mean age: 8 months; weight: 18–22 g) were divided into 3 groups (healthy control (HC), lung cancer (B[a]P), and lung cancer + training (ST) groups). Lung cancer was induced by injection of benzo[a]pyrene (B[a]P) (100 mg/kg). The intervention groups received swimming training for 12 weeks, 3 sessions per week. After the 12-week intervention period, lung tissue was dissected and NF-kB gene expression was measured using PCR. Statistical analyses were performed using one-way analysis of variance followed by Tukey's post hoc test.

Results: According to the results of one-way analysis of variance, there was a significant difference between the study groups in NF-κB gene expression (P=0.03). To find the location of the difference, Tukey post hoc test was used. According to the results of Tukey post hoc test, NF-κB gene expression in the HC group was not significantly different from the B[a]P group (P=0.190). Also, there was no significant difference between the HC group and the ST group (P=0.526). On the other hand, NF-κB gene expression in the ST group was significantly lower than that in the B[a]P group (P=0.02).

Conclusion: The findings of this study show that ST intervention reduces NF-κB gene expression and thereby improves inflammation in cancerous lung tissue.

Keywords: Lung cancer, Benzo[a]pyrene, Inflammation, NF-κB, Swimming training.

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1. Introduction

Benzo(a)pyrene (B[a]P), belonging to the polycyclic aromatic hydrocarbon family, is commonly found in cigarette smoke, food, and atmospheric pollutants. Benzo(a)pyrene-trans-7,8-diol-9,10-epoxide (BPDE), a metabolite of B[a]P, is a well-known mutagen and carcinogen. B[a]P exposure significantly correlates with lung cancer initiation (1). Lung cancer constitutes a formidable menace to global health and well-being, as its incidence and mortality rates escalate alarmingly. According to 2020 worldwide cancer data, lung cancer ranks among the most prevalent malignancies and exhibits the highest mortality rate among all major cancers (2). Environmental agents associated with elevated lung cancer risk, such as ambient particulate matter, may damage the lung by inducing chronic inflammation (3). The pro-inflammatory transcription factor, nuclear factor kappa B (NF-κB) contributes to malignancy by impacting cell senescence, apoptosis, metabolism, stress responses, and tumorigenesis. In adenocarcinoma and squamous cell carcinomas of the lung, NF-κB activation induced by smoking regulates the expression of downstream pathways such as COX-2, cyclin D1, and matrix metalloproteinase-9, thereby promoting cancer cell proliferation and survival (4). In fact, NF-κB activation affects hallmarks of cancer and inflammatory diseases through the transcription of genes involved in cell proliferation, survival, angiogenesis, inflammation and tumor promotion and metastasis (5).

In the realm of lung cancer therapy, exercise has unequivocally proven its efficacy in bolstering the quality of life for patients subjected to arduous treatment modes. A salubrious lifestyle characterized by regular exercise and physical activity is associated with a lower incidence of cancer (including lung cancer) and cancer mortality. Exercise also improves the overall physical condition of cancer patients undergoing chemotherapy or surgery, reducing treatment-related adverse effects and complications (6). Murphy et al. (2011) observed a reduction in tumor volume following aerobic exercise in cancer-bearing mice and attributed this to a decrease in inflammatory factors. A positive feedback loop plays a key role in the transformation of normal cells into cancer cells and cancer progression. NF-KB is a critical component of this loop. In fact, cellular change is initiated by an inflammatory signal that activates NF-KB. In fact, NF-KB is a key mediator of carcinogenesis caused by inflammation(7).

However, exercise's effects on this key inflammatory factor in cancerous lung tissue have not yet been studied. Therefore, considering the therapeutic effects of exercise, it is necessary to investigate the effect of this therapeutic intervention on NF-κB. Therefore, the present study aimed to investigate the effect of a swimming training session on NF-KB gene expression in mice with Benzo[a]pyrene-induced lung cancer.

2. Methodology

2.1. Materials and methods

In this experimental study, a post-test design.

2.2. Participants

In all experiments, the Helsinki' declaration guidelines for animal care was followed. This study was approved by the Azad Shiraz University. In this experimental study, a post-test design was used, which included 18 male Balb/c mice with an average age of 8 months; weight: 18-22 g. They were divided into 3 groups (healthy control (HC), lung cancer control (Benzo(a)pyrene (B[a]P) induction), and lung cancer + training (ST) group).

2.3. Measurements

Cancer induction: In this study, lung cancer induction was performed using benzo[a]pyrene (B[a]P) (Sigma-Aldrich, Germany). This substance was dissolved in olive oil and injected intraperitoneally (100 mg/kg) into mice. To confirm the induction of lung cancer by B[a]P, first in a pilot study, 6 mice (3 mice injected with B[a]P and 3 healthy mice as controls) were killed after 3 weeks and then the lung tissues were analyzed for tumorigenesis using pathological examinations and the results confirmed lung cancer induction and another 18 mice received B[a]P (8)..

2.4. Intervention

2.4.1 Exercise Protocol (Swimming Training, ST)

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

2.4.2 Tissue collection and analysis

48 hours after the last training session, male Balb/c mice were anesthetized using ketamine: 100 mg/kg; xylazine: 10 mg/kg, and lung tissue was isolated and stored at -80°C. Total RNA extraction was performed using FavorPrep™ Tissue Total RNA Kit (FATRK 001, Taiwan). NF-κB mRNA was then measured by real-time PCR and calculated by the [A1] ΔΔCt method.

Table 1. *Primers used in the study*

Gene	Primer sequences	Size (bp)
NF-κB	*Forward: 5'- GCTGCCAAAGAAGGACACGACA -3'*	131
	Reverse: 5'- GGCAGGCTATTGCTCATCACAG -3'	

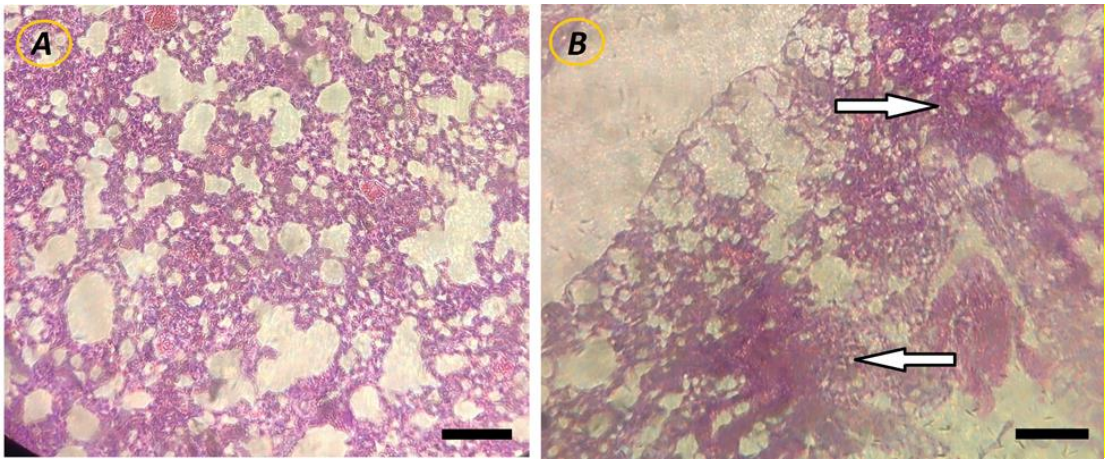

Fig 1. *Representative pictures of H&E-stained lung sections. A. lung in the control group; B. lung in the B[a]P group. Arrowheads indicate tumors. Images acquired at ×100 magnification and scale bars represent 1 mm*

2.5. Statistical Methods

Data were analyzed by a one-way ANOVA followed by Tukey's post-hoc test (SPSS v25). A significance level of $p < 0.05$ was considered for all statistical analyses, indicating the threshold for determining statistically significant results.

3. Results

Table 2. *The result of comparison of NF-KB between research groups*

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	8.956	2	4.478	4.435	0.03
Within Groups	15.145	17	1.010		
Total	24.101	17			

According to the results of one-way analysis of variance (Table 1), there was a significant difference between the study groups in NF-κB gene expression (P = 0.03). To find the location of the difference, the Tukey post hoc test was used (Figure 1). According to the results of the Tukey post hoc test, NF-κB gene expression in the HC group was not significantly different from the B[a]P group (P = 0.190). Also, there was no significant difference between the HC group and the ST group (P = 0.526). On the other hand, NF-κB gene expression in the ST group was significantly lower than in the B[a]P group (P = 0.02).

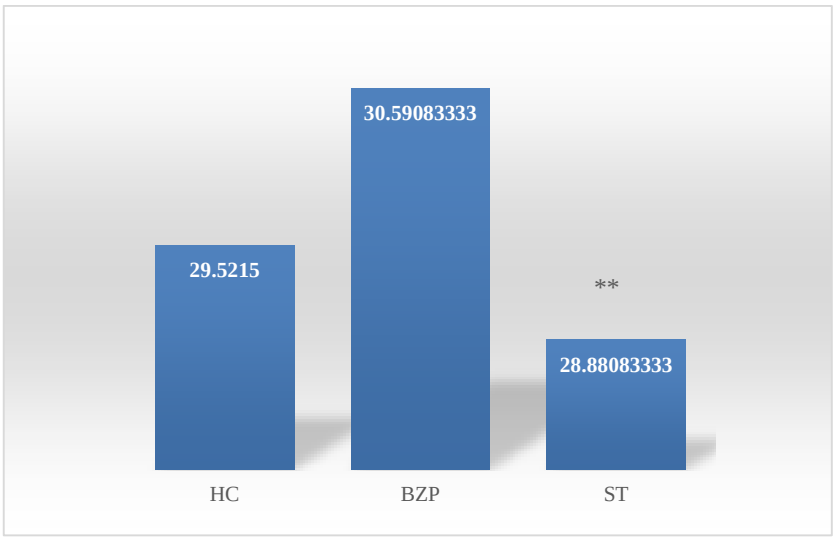

Figure 2. *Figure 1. Comparison of NF-κB gene expression between research groups (* Difference between BZP and ST groups (P = 0.02))*

4. Discussion

The present study investigated the effect of a swimming training period on NF-KB gene expression in mice with benzo[a]pyrene-induced lung cancer. Inflammation is a key factor in cancer development, initiating tumorigenesis but also promoting progression (12). In fact, the nuclear factor-κB (NF-κB) family of transcription factors control the expression of genes involved in many critical physiological responses such as inflammatory responses (5). Research has shown that moderate exercise reduces NF-κB gene expression and suppresses inflammatory factors (13). Results of this study showed that NF-κB in the HC group, despite being lower than in the B[a]P group, did not differ significantly. There was also no significant difference between the HC group and the ST group. On the other hand, there was a significant difference between the B[a]P and ST groups. This significant difference indicated an improvement in NF-B reduction in the ST group. Therefore, according to this study, 12 weeks of swimming training reduced this inflammatory factor in the lung tissue of B[a]P-induced cancer mice. NF-κB plays an essential role in the management of inflammation, proliferation, and survival of cell lines.NF-κB is a superfamily of TFs discovered in 1986 which includes NF-κB 1 (p50) NF-κB (p52), ReIA (p65), ReIB, and C-ReI.NF-κB is present in the cytoplasm as an inactive form by complexing with inhibitory subunits IKB-α, -β and -ϒ.The dissociation of inhibitory subunits (IκB s) results in the activation and rapid translocation of NF-κB heterodimer into the nucleus to bind with DNA. The subunit p65 then displays its transcriptional activity and induces the expression of NF-κB target genes that inhibit apoptosis, and forms a network that regulates the cell cycle, and promotes cell invasiveness, inflammation, tumorigenesis, metastasis, and eventually resistance to radio- and chemotherapy (14). Fashi et al. (2015) evaluated aerobic exercise's effect on lung inflammation. The results showed that aerobic exercise inhibits the expression of NF-κB and TNF-α genes (15) which was consistent with this study. Luo et al (2023), in a study investigating the mechanisms through which exercise exerts its anti-cancer effects against lung cancer, showed that in terms of prevention, exercise can reduce the risk of lung cancer. Exercise suppresses inflammatory responses, enhances immune function, regulates cellular autophagy, and decreasing oxidative stress. The results of this study were also consistent with the results of this study that ST reduced NF-κB in the lung tissue of mice with lung cancer (16). Therefore, given that the available evidence suggests persistent inflammation and cancer in various organs. Also, impairment of inflammatory responses in tumors occurs due to inadequate proinflammatory mediators. Therefore, inhibition of the excessive or abnormal production of proinflammatory mediators is one of the predicted effects of chemopreventive phytochemicals. A specific subset of proinflammatory mediators facilitates the neoplastic transformation of cells in an abnormal microenvironment created by chronic inflammation (17). In this study, ST reduced B[a]P-induced NF-κB.

5. Conclusion

This study shows that ST intervention reduces NF-κB gene expression and improves inflammation in cancerous lung tissue. ST counteracts cancer effects by reducing NF-κB expression, highlighting their potential as anti-cancer therapies.

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Conflict of interests: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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