ISSN: 2538-5887; Pathobiology Research. 2025; 28 (4): 57-62
DOI: 10.48311/mjms.2025.117231.82608

The effect of six weeks aerobic training on sPLA2 and COX-2 genes expression In Balb/c mice with breast cancer

Review History:
Received: 2025-10-24
Revised: 2025-11-01
Accepted: 2025-11-15

Article Type:
Original Research

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ABSTRACT

Introduction: Clinical evidence points to the development of breast cancer in women as being rooted in genetic and hormonal factors. The present study aimed to determine the effect of aerobic exercise on the expression of some transcription factors (sPLA2, COX-2) that are effective in breast cancer tumor growth in Balb/c mice.

Materials and Methods: For this purpose, breast cancer was induced by 4T1 tumor cells in 14 Balb/c mice, then were randomly divided into exercise (aerobic training, n = 7) or control (n = 7) groups. Aerobic training was performed 5 sessions weekly in the form of running on a rodent treadmill for 6 weeks. sPLA2, COX-2 genes expression in Breast tumor tissue were measured at 48 hours after lasting exercise in 2 groups. Independent t-test was used for comparing variables between groups.

Results: Aerobic training induced significant decrease in sPLA2 (p = 0.001) and COX-2 (p = 0.001) expression in Breast tumor tissue compared to the control group.

Conclusion: These findings supports the effectiveness of aerobic training as a non-pharmacological treatment to inhibit the growth or severity of breast cancer. Further studies are required to understand the main mechanisms responsible for the exercise training on the factors affecting the growth and progression of breast cancer.

Keywords: Aerobic training, Gene expression, Breast cancer, Balb/c mice

Introduction

Breast cancer is one of the most common cancers among Iranian women, usually occurring between the ages of 35 and 55. In this cancer, uncontrolled growth of abnormal cells is observed in various tissues of the chest and non-glandular tissues (1). According to the World Health Organization, up to 2.3 million women will be diagnosed with this cancer by 2050. In Iran, cancer is the third leading cause of death, and breast cancer accounts for 32%. Also, unlike Western countries, Iranian women are at higher risk of developing this disease at younger ages, which shows the importance of diagnosing, screening, and controlling this disease in our country (2).

Several genes are involved in the development of breast cancer. One of these genes is PLA2, which increases cell proliferation in a wide range of normal and transformed tissues. PLA2 leads to the release of arachidonic acid. Arachidonic acid is a polyunsaturated fatty acid widely present in mammalian cell membranes that is released from the cell membrane after PLA2 activation (3). In addition to its catalytic role in the release of arachidonic acid, PLA2 also has noncatalytic functions such as activating membrane receptors

expressed on tumor cells and stimulating intracellular responses (4). The expression of sPLA2 is significantly higher in breast cancer patients than in healthy individuals, indicating that PLA2-related genes are activated in the initiation and progression of breast cancer. PLA2 is also likely to be involved in the pathogenesis of cancer (5). Therefore, understanding the role of secretory PLA2 in the molecular biology of cancer may help develop therapeutic strategies to control various tumors. Arachidonic acid is catalyzed by COX to produce prostaglandins and precursors of thromboxanes. Prostaglandin E2 (PGE2) has an important role in tumor progression (6).

PGE2 production leads to the intensification of various metabolic processes and inhibition of apoptosis. Therefore, a sharp increase in PGE2 expression plays a key role in inflammatory processes and imbalance in cell division, apoptosis and angiogenesis, and leads to cancer progression (7). Longitudinal studies indicate a close relationship between COX-2 levels and various types of cancer, and the use of its inhibitors leads to the inhibition of cancer progression. Based on this evidence, the metastatic stage of cancer, which reduces the patient's chance of survival, is associated with increased Cox-2 levels (8). It plays an important role in angiogenesis in tumor cells (1), and deletion of the sPLA2 and COX-2 enzyme genes in mice has led to a decrease in tumor growth (9).

Physical activity is one of the few behaviors that can be intervened in reducing the risk of breast cancer (10). Epidemiological evidence shows that increased physical activity is associated with a reduced risk of breast cancer. In such a way that the incidence of breast cancer in women who engage in regular physical activity is estimated to be about 25% lower than in sedentary women (11). Regular physical activity leads to a decrease in inflammatory markers and inhibits signaling pathways effective in the growth of cancer cells (12). In breast cancer, overexpression of COX-2 has made this factor a major factor in the initiation and progression of cancer through prostaglandin E2. This factor can activate the PI3K/AKT signaling pathway, which regulates cell survival, metabolism, and growth, so that the activation of this pathway contributes to the survival and proliferation of cancer cells (13).

Exercise has been shown to affect tumor growth by affecting COX-2 expression. Exercise leads to a reduction in proinflammatory cytokines and inflammatory molecules. Inhibition of these inflammatory signals through exercise reduces COX-2 expression and ultimately reduces the production of prostaglandin E2, which is essential for tumor growth (14). However, conflicting findings have been reported in this regard. For example, Aoi et al, (2010) showed that COX-2 expression was not altered by exercise in mice with colon cancer (15). However, Lee et al, (2015) indicated that exercise inhibits the COX-2-dependent inflammatory pathway (16). Winzer et al, (2011) cited their findings and stated that although exercise is more associated with improved survival among patients with colon cancer, the direct effects of exercise on COX-2 expression or PGE2 activity in cancer have not been determined (17). Despite the aforementioned evidence, there is no study to date aimed at determining the effect of exercise training on sPLA2 and COX-2 gene expression in mice with breast cancer. Therefore, in the present study, the effect of 6 weeks of treadmill training on sPLA2 and COX-2 gene expression in (where) Balb mice with breast cancer is measured.

Materials and Methods

The statistical population of this experimental-applied study consisted of all the Balb/c mice in the animal house of the Pasteur Institute of Iran. Among them, 14 mice aged 8 weeks were randomly selected to participate in the study. Then, after inducing breast cancer, they were randomly assigned to exercise (6 weeks of aerobic exercise) and control (no exercise) groups. This study was approved by Committee of Ethics in Research of Islamic Azad University of Islamshahr Branch, Tehran, Iran (Ethic Code: IR.IAU.PIAU.RC.1403.006). The studied rats were kept under controlled light conditions (12 hours of light and 12 hours of darkness, lighting starts at 6 in the evening and turns off at 6 in the morning) with temperature (22 ± 3 C) and humidity in the range of 30 to 60. For this purpose, plexiglass cages with a mesh door and dimensions of 25 x 27 x 43 cm were prepared so that the mice could have free access to water and standard food. Three rats were kept in Plexiglas cages with mesh doors measuring 25 x 27 x 43

cm in such a way that they had free access to water and standard food. Throughout the study period, the rats were moved by one person. All rats were familiarized with the living conditions in the animal house and how to run on the treadmill for 2 weeks.

Breast cancer induction

Breast cancer was induced by 4T1 tumor cells. 4T1 mammary carcinoma can grow as a primary tumor in Balb-C mice in vivo. To better simulate the disease, subcutaneous injection was performed in the abdominal mammary gland (18). To ensure cancer induction, tumor growth was monitored visually and palpated using a caliper. Swelling and redness appeared in the tumor area. In addition, histopathological analysis of the excised tumor also helped to determine and report the tumor grade.

Training protocol and tissue extraction

Exercise training was performed on a treadmill at a speed of 20 m/min and zero incline for 40 minutes per session, 5 days per week, for 6 weeks (19). 48 hours after the last training session (10 to 12 hours of fasting), the mice in each group were anesthetized by intraperitoneal injection of a mixture of 10% ketamine at a dose of 50 mg/kg and 2% xylazine at a dose of 10 mg/kg. Subsequently, breast tumor tissue samples were collected, chloroform was added to the samples, incubated, and centrifuged for 15 minutes at 12,000 rpm. The upper clear phase was separated, then isopropanol was added, the mixture was inverted, and incubated at -20°C. After that, it was centrifuged again at 12,000 rpm. The upper solution was discarded, and the sediment was washed with 75% ethanol before centrifugation at 7,500 rpm for 5 minutes. Finally, 20 to 30 µl of DEPC water was added to dissolve the RNA. After RNA extraction, cDNA synthesis was performed using a synthesis kit (Pars Toos Company) and according to the manufacturer's protocol. The expression levels of sPLA2 and COX-2 genes were measured by quantitative Real Time PCR. GAPDH was used as a control gene. The primer sequence pattern is shown in the table 1.

Statistical analysis

All the data are expressed as mean ± SD. Data were analyzed by computer using the Statistical Package for Social Sciences (SPSS) for Windows, version 22.0. Independent t-tests were performed to determine whether there were significant within-group changes in the outcomes. Differences were considered to be statistically significant when $p- < 0.05$.

Results

Pre and post training of body weight for 2 groups are shown in table 2. Data represented by Mean and standard deviation. Based on independent t test, no significant difference was found between 2 groups at pre-training. However, after the intervention, a significant difference in body weight was observed between the two groups. On the other hand, the findings of the paired t-test revealed that at the end of the intervention, despite no change in weight in the exercise group, it decreased significantly in the control group.

The effect of aerobic training on COX-2 and sPLA2 gene expression in Breast tumor was main aim of this study. Based on independent t-test data, COX-2 and sPLA2 expression was significantly lower in exercise group than control rats. In other words, 6 weeks aerobic training resulted in significant decrease in COX-2 and sPLA2 expression in Breast tumor compared with control subjects (table 3, Fig 1 and 2).

Table 1: The primer sequence pattern of the studied gene

Genes	Primer sequence
GAPDH	F: CAGAACATCATCCCAGCCTCC R: TTGGCAGGTTTCTCAAGACGG
sPLA2	F: GACCGGTGCTGTGTTACTCAT R: GTAGGTTTTCTTGTTCCGGGC
COX-2	F: AAGAGCTTCAGGAGTCAGTCA R: ACATGGATTGGAACAGCAAGG

Table 2: Pre and post-training of body weight of 2 groups (Mean ± SD).

Group	Pre-training	Post-training	sig
Control	22.70 ± 3.34	17.60 ± 2.32	*0.003*
Exercise	21.90 ± 2.47	22.20 ± 2.30	*0.143*
sig	*0.518*	*0.021*	-----

Table 3: Changes in COX-2 and sPLA2 genes expression in breast tumors following aerobic exercise compared to the control group (Mean ± SD).

Variable	Control group	Exercise group	*sig*
COX-2 expression	1	0.53 ± 0.07	*0.001*
sPLA2 expression	1	0.60 ± 0.08	*0.001*

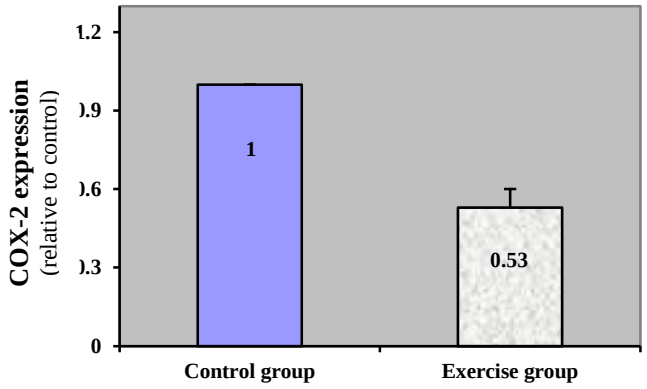

Fig 1: COX-2 expressions in breast tumors in exercise rats compare to control group.

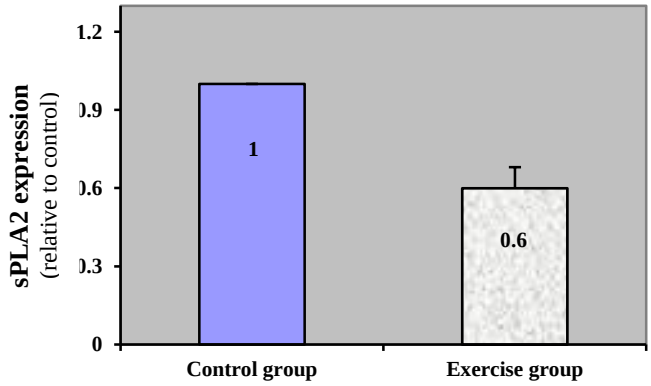

Fig 1: sPLA2 expressions in breast tumors in exercise rats compare to control group.

Discussion

The main findings of the present study are the reduction of COX-2 and sPLA2 gene expression in the breast tissue of Balb/c mice induced with breast cancer. In other words, 6 weeks of aerobic training (5 session/weekly) on a treadmill led to a reduction of COX-2 and sPLA2 expression in the breast tissue of Balb/c mice with breast cancer.

COX-2 is upregulated by several cytokine and growth factor subfamilies and its expression is highly increased in many tumors, leading to overproduction of prostaglandin E2. It plays a key role in cancer progression, apoptosis, invasion, angiogenesis, and metastasis (20). Shirali et al, (2017) showed that the mean Cox-2 level in the cancer group was significantly increased (28%) compared to the healthy control group (1). This study revealed that cancer induction in rats significantly increased Cox-2 levels compared to the healthy control group. Therefore, Cox-2 gene expression can be considered as a diagnostic factor for breast cancer (21). In support of the findings of the present study, Chan et al, (2019) reported a significant decrease in COX-2 expression in white adipose tissue of high-fat diet-induced obese mice following 8 weeks of moderate-intensity aerobic exercise on a treadmill (22). On the other hand, Lee et al, (2015) also reported that exercise training significantly inhibits COX-2 activity, which leads to suppression of proinflammatory cytokines and changes in the redox status of the cell (16).

Firozi et al, (2018) also reported an increase in COX-2 levels in response to cancer induction, as well as a decrease in its levels following 6 weeks of endurance training combined with Aloe vera (23). However, Kim et al, (2009) reported an increase in COX-2 expression in human samples after a single session of intense exercise (24). These researchers also noted that the extent of COX-2 changes depends on the intensity of exercise. In another study, it was found that ibuprofen and acetaminophen increased the prostaglandin synthesis pathway from COX-2 and muscle protein synthesis following resistance training (25). Nam et al, (2011) investigated the effects of treadmill exercise on COX-2 in diabetic mice. The results of this study showed that treadmill exercise significantly increased this factor in mice that were in the early stages of diabetes. However, it did not change in diabetic mice that had been diabetic for a longer period of time (26). On the other hand, Barrari et al, (2013) investigated the effect of aerobic exercise on the expression of this factor and coagulation factors in young inactive women. The results indicated a significant increase in Cox-2 in the exercise group (27), which was in contradiction with the study by Shirali et al (2017). Because they showed that 6 weeks of endurance exercise caused a decrease in Cox-2 levels, and this decrease was more significant in the exercise and aloe vera supplement group (1).

Another finding of the present study is the reduction in sPLA2 expression after induction of breast cancer in response to aerobic exercise. sPLA2 causes the release of arachidonic acid from membrane phospholipids, thereby effectively increasing inflammatory mediators such as thromboxane and prostaglandins. Research has also shown that sPLA2 activity is altered by exercise in human subjects and animal models. However, there is limited information available about the effects of exercise on this factor, although it has been reported that there is a

negative relationship between exercise and the expression of this factor, such that increased exercise reduces it and vice versa (28). In support of the present study, Zhang et al (2020) reported a decrease in sPLA2 following 12 weeks of aerobic exercise (29). Despite the aforementioned evidence, choosing the appropriate intensity and duration of exercise in patients with cancer is very important. Another study showed that regular low-intensity exercise favorably affects the distribution of cholesterol in serum lipoproteins in healthy middle-aged men and may have beneficial effects on circulating arachidonic acid metabolites (30). It is worth noting that sPLA2 breaks down membrane phospholipids and produces arachidonic acid, and it was observed that prostaglandin concentrations remained unchanged (30). Other findings also reported that physical activity in children and adolescents is inversely associated with phospholipase A2 activity (28).

Conclusion: Regular aerobic training is associated with a significant reduction in the expression of COX-2 and sPLA2, key inflammatory factors, in Balb/c mice with breast cancer, which supports the effectiveness of aerobic exercise as a non-pharmacological treatment to inhibit the growth or severity of breast cancer. Based on scientific evidence, the reduction in the expression of COX-2 and sPLA2 in breast tissue in response to aerobic exercise is manifested through the production of prostaglandins and related enzymes. However, further studies are required to understand the main mechanisms responsible for the effects of aerobic exercise on the factors affecting the growth and progression of breast cancer.

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