

The Effect of Aerobic Training and Taxol Consumption on IL 8 and PAI-1 in Cervical Cancer

Alireza Barari, Maryam Firoozi, Maryam Ebrahimzadeh, Romina Roohani Ardeshiri, Maryam Kamarloeei

Abstract—Background: The purpose of this study was to analyze the effect of six-week aerobic training and taxol consumption on interleukin-8 and Plasminogen Activator Inhibitor-1 (PAI-1) in mice with cervical cancer. Materials and Methods: In this experimental study, 40 female C57 mice with cervical cancer, eight weeks old, were randomly divided into 4 groups including: control, taxol supplement, training, and training-taxol supplement. The implantation of cancerous tumors was performed under the skin at the upper of the pelvis. The program training was included: endurance training for six weeks, 3 sessions per week and 50 minutes per session, at the speed of 14-18 m/s. Taxol supplement at a dose of 60 mg/kg per day was injected intraperitoneally. Data analysis was performed using t-test and one-way ANOVA and if statistically significant, Bonferroni post hoc was used at the significance level $p < .05$. Results: The results showed that there was a significant difference between the levels of interleukin 8 ($P < 0.05$, $F = 12.25$) and the PAI-1 ($P < 0.05$, $P = 0.10737$ between the 4 groups). The results of this study showed a significant difference between the control group and the training - complementary group. Six weeks of aerobic training and taxol consumption have a significant effect on the level of PAI-1 and interleukin-8 mice with cervical cancer. Conclusion: Considering the effect of training on these variables, this type of exercise can be used as a complementary therapeutic approach with other therapies for cervical cancer.

Keywords—Cervical cancer, taxol, endurance training, interleukin 8, plasminogen activator inhibitor-1.

I. INTRODUCTION

CERVICAL cancer is one of the most common cancers among women in developing countries which is caused by human papillomavirus infections. Infection with this virus can lead to the cervical squamous cell carcinoma that is very fatal [1]. Various types of cytokines play different roles in developing and expanding of cancer [2]. On the one hand, they can cause developing and metastases of cancer and, on the other hand, they can inhibit the cancer progression by anti-inflammation and anti-tumor effects [2], [3]. Interleukin-8 is one of the pre-inflammation cytokines that is known as a chemokine. It specifically plays a role in chemotaxis of WBCs especially neutrophils and lymphocytes and it is a trigger and enhancer of angiogenesis. This cytokine is secreted by natural cells such as fibroblasts and monocytes [4]. Researches show that interleukin-8 can increase the growth and angiogenesis of cancer tumors and this way it can impact on the growth of tumor [5]. Also, according to several studies, PAI-1 is related to an enhanced risk of many malignancies. PAI-1 is a key element in inhibition of fibrinolysis by inactivation of tissue-type and urokinase-type plasminogen activator [6], [7]. There are several

experimental evidences that show that the plasminogen activator system plays a role in the demolition of the base membrane and extracellular matrix and leads to the attack of tumor cells and metastases [8], [9]. Expression of PAI-1 gene is highly associated with deterioration, existence and also other effective parameters such as metastasis to lymphatic nodes, depth of tumor penetration and size of tumor [10].

An increase is observed in circulating concentrations of pre-inflammation cytokines such as interleukin 8 after endurance training [11]. Another research demonstrated that contraction activities and exercising play a role in the expression of interleukin-8 [12], [13]. Findings of another research show the effects of six weeks of endurance training on a meaningful decrease in interleukin-8 levels of tumor tissue and volume of tumor [3]. It also has been shown that aerobic exercises lead to a decrease of PAI-1 gene expression [14]. In contrast, another study demonstrated that regular exercise with medium intensity does not lead to a decrease in PAI-1 gene expression but may control it [15]. Thus, the impact of aerobic exercises on increase or decrease in expression of this gene which has a crucial role in the progression of tumors in cervical cancer is not completely clear and requires more expanded studies in this field. On the other hand, using plants as a drug for preventing and treatment of diseases has been considered by traditional medicine specialists since ancient times. By the beginning of the 16th century, it has been the most esteemed way to cure diseases. Among these, the yew tree has a great medicinal value because of Paclitaxel material with the trade name of Taxol [16]. Taxol is a diterpene with a complex structure. Today, it is effectively used worldwide as a most important natural anti-tumor composition with a different mechanism in comparison with other similar drugs in this field to cure all types of cancers such as skin, lung, urinary tracts, esophagus and lymph nodes [16], [17].

According to the significance of curing cancer, implementing studies around the role of cytokines in improving patients with cancer becomes important. Considering that today most of the attempts are about preventing and treatment of diseases without using drugs, an active lifestyle is important in preventing and treatment of diseases and the use of therapeutic exercises becomes more and more significant [12], [15], [18].

Research has shown that aerobic exercise affects plasminogenesis and thus reduces inflammation, but existing results especially about cancer, is not firm. Also, there are plenty of studies about mechanisms of effects of physical activities on the health of cancer patients, but the interaction

Alireza Barari is with the Islamic Azad University, Ayatollah Amoli Branch, Iran, Islamic Republic Of (e-mail: alireza54.barari@gmail.com).

between exercise and some cytokines is not completely clear. Therefore, by considering the effect of Taxol on the treatment of cancer and importance of mentioned cytokines in increasing the function of body's immune, performing studies that represent the impact of exercising specifically aerobic exercising on levels of interleukin-8 and PAI are substantial. Considering the physical activity as an intervener along with taking Taxol extract in order to improve the health condition of patients with cervical carcinoma and change in intensity and duration of exercise and nutrition status of subjects are among matters that can make this study different from other researches. In this study, the parallel effects of six weeks of aerobic exercising and consumption of Taxol on levels of interleukin and PAI in mice with cervical cancer have been studied.

II. MATERIALS AND METHODS

This research was an experimental study in which it was possible to control factors influencing results. In this study, ethics on working with laboratory animals such as food and water availability, proper maintenance condition, and, killing the mice were noticed. In this study, 40 rats were transferred from the Iranian Pasteur Institute at Karaj to the research center. After entering and two weeks of adaptation with new environment, animals were randomly divided into four groups of cancer-Taxol supplement (S), cancer-training (T), training (C), and cancer-Taxol supplement-training (ST). For the homogenization of subjects in terms of weight, they were first weighed and then categorized in cages with weighted differences of 20 ± 2 .

During familiarizing with new environment and treadmill and also protocol implementation period animals were kept in five-member groups in transparent polycarbonate $15 \times 15 \times 30$ cages produced by Razi Rad company. Ambient temperature with 22 ± 4.1 °C and lighting cycle with 12:12 darkness hours and 55 ± 4 humidity. Rats are fed with pellets from livestock feed producing centers. Subjects of this study were fed with products of Behparvar livestock feed company. The mentioned amount of feed was put in the cages according to their weekly weighing.

At the end of 6th week of running the research, in completely equal situations, 48 hours after the last training and injection (to eliminate the acute effects of exercise and supplementation) and after 10 to 12 hours of fasting, all animals were anointed and sacrificed with intraperitoneal injection of ketamine (60 mg/kg weight) and xylosin (5 mg/kg weight) with a ratio of 5 to 2. After splitting of the abdominal cavity, the liver tissue was carefully separated. After washing with distilled water and weighing, it was frozen at 70 °C. Liver factors levels were determined by ELISA with special kits. In this study, levels of interleukin-8 were measured in milligrams with gene kit produced by China Stabiofarm Company. Levels of PAI were measured in nanogram per milliliter by ELISA with China Stabiofarm Company kit. Taxol supplement taken from Blue pure extract of Yew tree skin was injected to peritonea about 60 milligrams per 1 kilogram of mice body weight. The endurance training program was performed in 6 weeks and 5 sessions per

week. In the first two weeks, training time was about 25 minutes at a speed of 14 meters per minute. In the second two weeks, the training was enhanced to 30 minutes at a speed of 16 meters per minute. Then, in the last two weeks (week 4 and 5) training was accomplished at a speed of 18 meters per minute in 30 minutes. Considered training intensity was about 50-65% of the maximum consumed oxygen by mice.

III. RESULTS

Results of the ANOVA test showed that there is a significant difference between the mean values of the interleukin-8 variable in the four groups ($p \leq 0.05$). It also has been demonstrated with Tukey's follow-up test that meaningful level lower than 0.05 to compare "control" and "training-supplement" groups represents significant differences between the two groups. That is, 6 weeks of aerobic training along with consuming Taxol has a significant effect on interleukin-8 levels in mice with cervical cancer. According to Fig. 1, using supplement plus performing the training led to a decrease in the levels of this variable. According to results, there is a significant difference between "control" and "training" groups because of a meaningful level lower than 0.05. That is, training without supplementation led to a decrease in the level of interleukin-8 and a significant difference with the control group. There was not a significant difference between "training" and "supplement" groups. That is, taking supplemental Taxol alone or practicing alone has had a similar effect on a decrease in the level of interleukin 8. Difference between "training" and "training-supplement" was also not significant but there was a significant difference between "supplement" and "training-supplement" groups. That is, supplementation of the Taxol plus training is more effective than supplementation alone, and the effect of the training alone in reducing the level of interleukin-8 is similar to the effects of exercises with Taxol in reducing the level of this variable. But the most impact is still related to "training-supplement" group (Fig. 1).

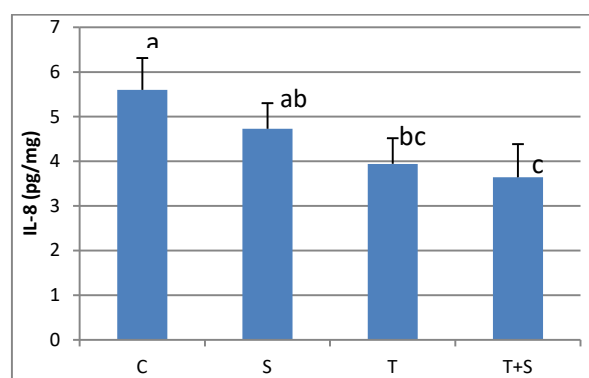


Fig. 1 Mean values of interleukine-8 in each subject groups (similar letters demonstrate lack of significant difference and different letters indicate significant difference)

Results of the ANOVA test showed that there is a significant difference between the mean values of the PAI variable in the four groups ($P < 0.05$). Also, Tukey's follow-up test showed that a meaningful level lower than 0.05 for comparison of "control"

group with all other groups indicates a significant difference. That is, six weeks of aerobic training plus consuming Taxol has a significant effect on the level of PAI in mice with cervical cancer. According to Fig. 2, consuming supplementation plus training reduces the level of this variable. Comparison of other groups did not show any significant difference, i.e., the effect of using supplementation alone or training alone was almost the same with the effect of training along with the use of the extract on the decrease in the levels of PAI in mice with cervical cancer. But the most relevant effect was still related to the "training-supplementation" group (Fig. 2).

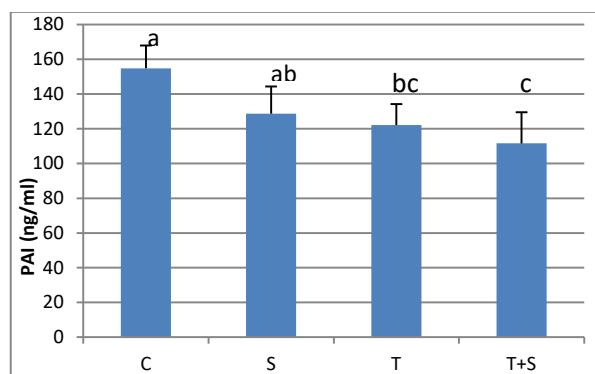


Fig. 2 Mean values of PAI in each subject groups (similar letters demonstrate lack of significant difference and different letters indicate significant difference)

IV. DISCUSSION

Results of this study showed that using Taxol and endurance training significantly decreased interleukin-8 levels in mice with cervical cancer. So, "training-supplement" group showed the lowest level of interleukin-8 compared to other groups. The results of this study about the decrease of interleukin-8 after endurance training are consistent with the findings of some other studies [3], [12], [13], [18]. Kazemi et al. [3] showed that six weeks of endurance training reduced interleukin-8 levels in mice with breast cancer. Finding of his study, similar to findings of [12] and [13], showed that exercising plays a role in the expression of interleukin-8. Results of Murphy et al. also showed a reduction in interleukin-8 in mice with cancer, after 20 weeks of aerobic training [19]. Indeed, it is possible that a decrease in some of the cytokines (especially IL-8) has an important consequence for cancer. Research has shown that chronic inflammation plays an important role in the growth, progression and survival of cancer [20]-[22]. It is believed that interleukin-8 is not only a factor in the activation of replication pathways in cancer cells, but also controls apoptotic pathways through interaction with nuclear factor kappa light-chain of activated B cells (NF-kB). That is because the reduction in expression of interleukin-8 in tumor cells can be a way for a decrease in growth and metastasis of these cells [23]. It seems that exercising can have a major role in decrease of tumor growth and in improving the cancer disease through enhancing regulation of anti-tumor factors and apoptotic pathway stimulating factors such as IFN- γ , IL-2, and reduction in factors that are effective in angiogenesis and inflammation factors. In

this study, results from the Taxol consuming group indicates a reduction in the interleukin-8 levels compared to cervical cancer group. Increase in usage of Taxol for basic researches and cancer chemotherapy requires an improvement in existing methods to produce this herbal product [24]. Results of this study demonstrated that six weeks of using Taxol reduced the interleukin-8 levels in experimental groups. Taxol is an anti-tumor complex biochemical composition which is mainly extracted from Yew herbs. This anti-tumor drug has a different mechanism compared to other common anti-tumor drugs. Various studies showed that Taxol polymerizes Tubulin and prevents its depolymerization, therefore, mitosis replication cycle is stopped [25]. Taxol causes transcription to stop in the G2/M phase of mitosis with abnormal division duct formation and thereby, it causes the death of replicating cells [24]. However, the results of this study are not consistent with [26] after the treadmill training of submaximal and [27] after aerobic power combinations. Possible reasons for this discrepancy can include the differences in the exercise protocol, type of subjects, and duration of exercise, activity intensity, and stage of cancer progression. Also, the results of this study showed that six weeks of aerobic exercise along with Taxol consumption had a significant effect on the level of plasminogen-1 activator inhibitor in mice with cervical cancer. "Training-supplement" group showed the lowest level of PAI but there were not a significant difference between "training-supplement", "training" and "supplement" groups. The results of this study are consistent with [28] which showed that physical activity reduces the PAI in the male body. The results of [14] and [29] on the effect of aerobic exercise on reducing the level of PAI are consistent with the present study. PAI-1 develops tumor growth, invasion, metastasis, and angiogenesis, and it seems that these processes take place in interaction with vitronectin, integrin, and other components of the plasminogen activation system and by affecting the extracellular matrix [30]. The incremental regulation of PAI-1 expression extends breast cancer to more aggressive stages, and this process is partially affected by angiogenesis [31]. Laboratory studies have shown that PAI-1 acts as a positive initiator for angiogenesis by promoting the migration of endothelial cells to fibronectin-rich tumor tissue and PAI-1 inhibitors prevent angiogenesis [32]. Study about PAI-1 deficient mice showed that angiogenesis was approximately 60% reduced compared to wild-type mice, while in mice with high expression of PAI-1, angiogenesis increased approximately threefold [33]. The results of studies have shown that Taxol in the presence of low picomolar concentrations inhibits angiogenesis by inhibiting the production of angiogenesis factors and preventing the protein expression of hypoxia-induced alpha-factor. The results of this study, consistent with previous studies [3], [11], [14], showed that Taxol reduces PAI-1 levels. Therefore, given the anti-angiogenic properties that Taxol has shown, its anti-tumor properties are over pre-reinforced. In addition, high expression of PAI-1 has been found in many types of cancers. High levels of PAI-1 are also associated with poor prognosis in breast and other cancers [34]. Some quantitative studies have investigated the effect of sports activities on PAI-1 in non-cancer subjects.

Similarly, higher levels of PAI-1 have been observed in inactive women compared to active women [35]. However, the results of this study are in contradiction with [36], which showed that eight weeks of exhaustive activity in the training group caused a significant increase in this gene compared to the control group. This study also contradicts the results of [15], which showed that moderate-intensity regular exercises did not reduce the activity of this gene. Probably the reduction of the levels of the studied genes is related to regular muscle contraction and activation of fibrinolysis through exercising. Decreased inflammatory factors and decreased inflammation are probably due to a reduction in the release of cytokines in response to regular muscle contraction.

V. CONCLUSION

Results of this study demonstrated that consumption of Taxol and endurance exercises significantly reduced levels of interleukin-8 and PAIs in mice with cervical cancer. According to the downturned effect of exercises on these variables, this type of exercise can be used as a complementary therapeutic approach along with other treatments for cervical cancer.

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