Original Article

The Effect of Six Week Resistance Training on Muscle Regeneration in Tumor-Bearing BALB-C Mice

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Received: 15 Jan 2019
Accepted: 14 May 2019

Abstract

Background & Objective: Many cancer patients suffer from cachexia or cancer-induced muscle atrophy. Cachexia can have various causes one of which is the reduction of muscle regeneration. Resistance training has been suggested as one of the proper stimulator of increasing muscular regeneration. The present study aimed at evaluating the effect of resistance training on two factors of regeneration including PAX7 and eMHC, tumor-free weight and tumor weight of mice.

Materials & Methods: This study was a kind of experimental intervention. Subjects of the study included 10 BALB-C mice (age: 6 weeks) which CT-26 tumor was transplanted to them. Mice were divided into two groups of resistance training (n=5) and control (n=5) randomly. Training group performed six-week progressive resistance training and control groups were kept in cages without any exercise intervention. At the end of the experiment, gastrocnemius muscle was taken for evaluating related factors. Data were analyzed using the independent t-test.

Results: There was no significant difference in PAX7 between two groups of training and control, but eMHC reduced significantly in training compared to the control group (P=0.038). Tumor-free bodyweight of training group increased significantly compared to the control group (P=0.0004) and there was no significant difference in tumor weight between two groups of training and control.

Conclusion: Although resistance training does not increase tumor growth but probably reduce some muscle regeneration factors in cancer-bearing mice. So, for improving muscular regeneration in cachexia bearing patients, probably resistance training is not a good choice. However, more future researches are required.

Keywords: Cachexia, muscular regeneration, resistance training, PAX7, eMHC

Introduction

Cachexia or muscle wasting syndrome is a disorder associated with cancer. Cachexia is specifically related to weight loss in cancer patients (1). Approximately 50% of cancer patients suffer from cachexia, and its highest incidence been reported among patients with gastrointestinal and pancreatic cancers (2). Cachexia is responsible for the death of 22-40% of cancer patients, which accounts for a high mortality rate in the world (3). In addition to increasing mortality, exacerbation of chemotherapy side effects and diminished quality of life are other complications of cachexia. Several methods have been proposed for the treatment of cachexia; however, none of them has managed to completely inhibit or treat muscular atrophy (4, 5).

Several causes have been suggested for cachexia, including cellular microenvironment changes of myocytes, inflammation (6), increased degradation and decreased synthesis of...
the protein (7). Nevertheless, reduced muscle regeneration is among the contributing factors to cachexia (8). Skeletal muscle has a high capacity to regenerate in response to a variety of chemical and mechanical damages (9). Differentiation of stem cells (satellite cells) present in the muscle to muscle cells is known as muscle regeneration (10). In the skeletal muscle after birth, stem cells are called satellite cells, which comprise 3-9% of submembranous nuclei associated with normal adult muscle fibers (11). The proliferation and differentiation of these cells are reduced during cachexia. Factors stimulating satellite cells and the resulting muscle regeneration are used in the treatment of cachexia (12). Several stimulants, including a variety of drugs are known to affect muscle regeneration (13, 14). Resistance training is a non-pharmacological stimulus that plays a major role in muscular hypertrophy and increasing muscle regeneration in normal and non-infectious conditions (15). For example, the results of Nadron et al. study (2016) showed that 16 weeks of resistance training by young men increased muscle regeneration as well as paired box protein (Pax7) and myogenic differentiation 1 (MyoD) levels, which are markers of stem cell differentiation (16). Dumas et al. research (2018) indicated that some muscle fibers in young men are damaged after the first session of resistance training, but the rise in satellite muscle cells (i.e. increased Pax7 expression) helps repair this injury, which is likely to cause muscle hypertrophy in the long run (17). In this regard, Codi et al. (1999) showed that compared to endurance and control groups, a 10-week course of strength training would increase the heavy chain of fetal myosin as an indicator of muscle regeneration (18). In the case of satellite cells, Calti et al. (2016) examined the effect of voluntary physical activity on a mouse rotary wheel on muscle regeneration of C-26 tumor-bearing rats. The findings of this study showed that C-26 tumor impaired the differentiation of satellite cells. However, voluntary physical activity can increase the differentiation of satellite cells (19).

Cachexia is a mortality factor for many cancer patients (3) and several papers have described impaired muscle regeneration as a factor of cancer-related cachexia (8, 19, 20). Since we found no study to assess the effect of resistance training (as one of the best methods for increasing muscle regeneration) on the rate of muscle regeneration in tumor-bearing mice, the goal of this research was to investigate the impact of a course of resistance training on Pax7 and heavy chain of fetal myosin as indices of muscle regeneration among C-26 tumor-bearing mice.

Materials & Methods

In this experimental research, 10 syngeneic male BALB/C mice with an average weight of 20±3 g were purchased from Pasteur Institute of Tehran and randomly divided into resistance training (n=5) and control (n=5) groups. Subjects were kept in polycarbonate cages (4 mice per cage) under controlled environmental conditions with an average temperature of 21±4°C, the humidity of 34-42%, 12:12 hour light/dark cycle with free access to water and food.

To induce the tumor, CT-26 tumor cell line was purchased from Pasteur Institute of Tehran and cultured in Roswell Park Memorial Institute (RPMI, Biosera, France) with 10% fetal bovine serum (FBS, Gibco, England). Then, 3x10⁶ cells were solved in 100 µL phosphate buffer salt (PBS) and injected into the left side of mice. After four weeks, the tumor of one of these mice was grafted to all the mice (21). For this purpose, the hair of the left flank of mice was shaved. Afterward, 1.5 mm² of the tumor was separated, placed into an excision created on the skin of the mouse and was then stitched. After 10 days, the mice were randomly divided into training (intervention) and control groups. The control group was kept in cages without any intervention. The resistance training involved climbing a ladder with a height of 1 m with 2 cm clearance between steps. The exercise protocol consisted of three sessions per week for 6 weeks following a two-week warm-up training period. For warm-up, the mice climbed the ladder without weights at the beginning of each session. At the beginning of each week, the maximum weight tolerable by mice was measured, 70% of that weight was calculated and the weights were attached to their tails. Each training session consisted of 10 repetitions with two minutes of rest interval (22). 24 hours after the last training session, the mice were anesthetized through intraperitoneal injection of a combination of ketamine (30-50 mg/kg body weight; Boxtel Co., Netherlands) and xylazine (3-5 mg/kg of body weight; Alfasan Co., the Netherlands) by observing ethical principles and the gastrocnemius muscle of their left leg was separated for further experiments. The quantity of myosin heavy chain was measured by an
ELISA kit of Maybiosarus Co. (USA) with a sensitivity of 0.1 ng/ml and that of Pax7 with a sensitivity of 0.06 ng/ml. Shapiro-Wilk test and independent t-test were used to verify the normality of data and to compare the data, respectively. The significance level in statistical tests was considered ≤0.05. All statistical calculations and drawing of diagrams were done using Prism software version 7.

Results
The findings of this study showed that Pax7 levels were not significantly different between resistance training (19.86±9.1) and control groups (19.15±5.8) (p=0.51) (Figure 1). However, eMCH levels in the resistance training group (3.46±0.64) was significantly reduced (18%) compared to the control group (4.22±0.24), (p= 0.038, Figure 2). Tumor-free bodyweight of mice in the resistance training group (21.5±1.3) had a significant increase (22%) compared to the control group (17.6 ± 0.9%) (p=0.004, Figure 3); however, there was no significant change in the tumor weight of mice in the resistance training group (15.4±6.4) compared to the control group (16.2±6.4) (p=0.02, figure 4) (4).
Discussion

The results of this research indicated that although resistance training did not significantly change Pax7 levels, it decreased eMHC levels of tumor-bearing mice. In contrast, although the tumor weight did not change significantly, the tumor-free weight of mice was increased.

Pax7 is one of the most important molecules in the process of muscle regeneration disorder in tumor-bearing mice, and several papers have investigated Pax7 in various types of cancer (23, 18). Paena et al. (2010) reported that Pax7 expression in C-26 tumor-bearing mice was increased, which was a major factor of reduced muscle regeneration (23). In the field of exercise activity, no study was found to evaluate the effects of resistance training on muscle regeneration of tumor-bearing mice. The only study was that of Calti et al. (2016) that investigated the impact of voluntary exercises on a rotating wheel on muscle regeneration rate of tumor-bearing mice after inducing damage to the muscle of these mice (18). Contrary to the present study, the findings of that research showed that medium intensity voluntary running on a rotating wheel increased muscle regeneration rate. In the mentioned study, the researchers concluded that exercise training decreased the activity of Kappa chain-enhancing nuclear factor (NF-KP) via decreasing Pax7 levels, which augmented regeneration because Pax7 expression should cease for the differentiation of satellite cells into myocytes (18). These results have been obtained while most studies have reported strength training as a stronger stimulant than low-intensity aerobic exercise for muscle regeneration in normal conditions (15, 16, 17). Nevertheless, different factors may have caused this difference. For example, tumor necrosis factor-α (TNF-α) is an important inflammatory factor, the increase of which both in vitro and in vivo enhances the expression of Pax7 and decreases muscle regeneration (24,25), and this factor has been shown to increase in muscles of mice (26). Consequently, it seems that cancer decreases muscle regeneration by increasing Pax7 levels. On the other hand, because resistance training probably plays a less pronounced anti-inflammatory role than aerobic exercises (27) and the effect of resistance training on TNF-α levels has not been elucidated (28), a number of studies have reported an increase (29, 30) and others a decrease in its levels (31, 32). Given the association between inflammatory factors with Pax7, resistance training is not likely to be a sufficient stimulant for Pax7. In another study, the reduction of muscle regeneration was related to ERK protein (23), and a majority of investigations have reported increased activity of this protein after a period or even a single session of resistance training (33), which may be another mechanism of reduced muscle regeneration by resistance exercises. However, resistance training is a strong stimulant for the secretion of various growth factors such as insulin-like growth factor (IGF-1) (34), which can contribute to tumor malignancy and more inflammation in the tumor microenvironment (35). Nevertheless, in the present study, the growth factor levels were not investigated and resistance training did not increase the growth of tumors. Therefore, in cancer conditions, either exercise training has no significant effect on some growth factors or growth factor inhibitors, especially in tumor tissue, are more effective than growth a stimulus, which requires future research.

Figure 4 Comparison of CT-26 tumor weight (g) in training and control groups. Independent t-test results showed no significant difference in tumor weight between the two groups. Columns represent mean and standard error.
Another finding of this study was the reduction of eMHC level as an indicator of muscle regeneration rate (8,19) in the resistance training group, while the results of studies indicate that this factor is decreased in muscles of tumor-bearing mice relative to control group in which resistance exercises increase eMHC levels (36). Although this result is unexpected and divergent with the assumption of the present study, it is partly justifiable given the lack of significant changes in Pax7 levels after resistance training. Resistance training, especially at the onset of training, causes negligible damage to muscle fibers (37). Several studies have examined the extent of muscle regeneration in mice after injury and reported disruption in the repair of injury by musculoskeletal regeneration. Perhaps in the present research, minor injuries caused by resistance training, which stimulates and enhances muscle regeneration in healthy subjects, has led to a reduction in muscle regeneration index (eMHC) of tumor-bearing mice and may have damaged the newly differentiated fibers expressing eMHC; in contrast, the differentiation of satellite cells does not occur because of cancer, which leads to decreased eMHC levels.

In the present study, resistance training increased tumor-free weight but did not significantly change tumor weight. There are few studies in this respect. The only research in this regard by Kamuyi et al. (2016) investigated the effect of resistance training on C-26 tumor-bearing rats. Consistent with our findings, no significant increase in tumor weight was observed after resistance training in their study; however, unlike our research, there was no significant change in tumor-free weight (20). The type of exercise protocol in their research was based on the weight of mice in contrast to ours that was based on the maximum power of mice and could have caused this inconsistency. Overall, the weight gain resulting from resistance training can be due to increased protein balance and muscle weight or because of the increasing weight of spleen and liver as an indication of enhanced inflammation (20) or even appetite. As indicated by several studies, exercise intensity determines the effect of training on appetite (38). Some training exercises increase appetite (39), which is recommended to be investigated in future research according to the results of this study.

**Conclusions**
Due to the lack of change in tumor weight of tumor-bearing mouse, the resistance training program has no adverse effect on tumor growth; nonetheless, according to other studied variables, this method of resistance training is not likely to be appropriate to increase muscle regeneration as one of the problems of cancer patients afflicted with cachexia. Therefore, future studies are recommended to examine other variables related to muscle regeneration of patients with cachexia as well as investigating the effect of resistance training with different intensities or with anti-inflammatory drugs.

**Acknowledgments**
This paper is part of a research project of Cancer Research Center of Shiraz University of Medical Sciences with ethics code IR.SUMS.REC.1395.S1072. We appreciate all the members of the center who fully cooperated in this research.

**Conflict of Interests**
The authors announce no conflict of interest.

**Reference**
اثر شش هفته تمرینات مقاومتی بر باززایی عضلانی موش های حامل تومور CT-26

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تاریخ دریافت مقاله: 25/10/1397
تاریخ پذیرش مقاله: 24/2/1398

چکیده
زمینه و هدف: بسیاری از بیماران سرطانی از کاشکسی یا تحلیل عضله ناشی از سرطان رنج می‌برند. کاشکسی می‌تواند علل متفاوتی داشته باشد که یکی از آن‌ها کاهش باززایی عضلانی می‌باشد. تمرینات مقاومتی به عنوان یک محرک مناسب برای افزایش باززایی عضلانی پیشنهاد شده است. هدف این مطالعه بررسی اثر تمرینات مقاومتی بر میزان دو عامل باززایی عضلانی شامل Pax7، eMHC و وزن بدون تومور و وزن تومور موش‌ها بود.

مواد و روش‌ها: مطالعه از نوع تجربی مداخله‌ای بود. موش‌ها به‌طور به‌طور تصادفی به دو گروه تقسیم شدند. گروه تمرینات مقاومتی 6 هفته تمرینات فازاینده مقاومتی را انجام دادند و گروه کنترل بدون هیچ مداخله تمرینی در قفس اندامگاهی شدند. بعد از دوره آزمایش، عضله دوقلوی موش‌ها جهت بررسی فاکتورهای مورد نظر جمع آوری شد. از آزمون آماری تحلیل آماری یافته‌ها استفاده شد.

نتایج: در بین دو گروه تفاوت معنی‌داری در میزان Pax7 مشاهده نگردید اما در میزان eMHC مشاهده نگردید. در گروه تمرینات مقاومتی با گروه کنترل کاهش معنی‌داری در میزان eMHC مشاهده نگردید. در گروه تمرینات مقاومتی با گروه کنترل کاهش معنی‌داری در میزان Pax7 مشاهده نگردید.

نتایج گزارشی: اگرچه تمرینات مقاومتی رشد تومور را افزایش نمی‌نماید اما می‌تواند عواملی در پیشگیری از سرطان را کاهش می‌دهد.

کلمات کلیدی: کاشکسی، باززایی عضلانی، تمرینات مقاومتی

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