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Combined effect of aerobic interval training and selenium nanoparticles on expression of IL-15 and IL-10/TNF-α ratio in skeletal muscle of 4T1 breast cancer mice with cachexia

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A B S T R A C T

Cancer cachexia is characterized by inflammation, loss of skeletal muscle and adipose tissue mass, and functional impairment. Oxidative stress and inflammation are believed to regulate pathways controlling skeletal muscle wasting. The aim of this study was to determine the effects of aerobic interval training and the purported antioxidant treatment, selenium nanoparticle supplementation, on expression of IL-15 and inflammatory cytokines in 4T1 breast cancer-bearing mice with cachexia. Selenium nanoparticle supplementation accelerated cachexia symptoms in tumor-bearing mice, while exercise training prevented muscle wasting in tumor-bearing mice. Also, aerobic interval training enhanced the anti-inflammatory indices IL-10/TNF-α ratio and IL-15 expression in skeletal muscle in tumor-bearing mice. However, combining exercise training and antioxidant supplementation prevented cachexia and muscle wasting and additionally decreased tumor volume in 4T1 breast cancer mice. These finding suggested that combining exercise training and antioxidant supplementation could be a strategy for managing tumor volume and preventing cachexia in breast cancer.

1. Introduction

Caner cachexia is a multifactorial syndrome characterized by inflammation, body weight loss, muscle wasting, and functional impairment [1]. Approximately half of all patients with cancer will experience cachexia. This syndrome is observed in the majority of subjects with cancer before death [2,3]. It has been suggested that in addition to pharmacological strategies, exercise training could be an effective strategy to prevent cachexia [4]. A limited number of studies have assessed the effectiveness of exercise training on cancer cachexia [3,4].

Body composition changes and, more specifically, skeletal muscle wasting, are important components of cachexia [1,5]. It has been found that progressive loss of skeletal muscle mass and function is linked to oxidative stress and inflammatory cytokines in different conditions including disuse, aging and denervation [6,7]. In particular, inflammation is well known to impinge on muscle protein metabolism [8]. Therefore, it has been suggested that the pro- and anti-inflammatory cytokines balance is an important issue in the cancer therapy [9].

Several groups have studied the effects of aerobic exercise training on cytokines in various tissues, including skeletal muscles [10–12]. Also, it has been shown that cytokines released from skeletal muscle following exercise training exert significant effects on metabolism, insulin sensitivity, hypertrophy and atrophy in skeletal muscle [13]. However, the effects of exercise training on cytokines released from skeletal muscle in subjects with cancer cachexia symptoms have not been clarified.

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Expression of the cytokine interleukin-15 (IL-15) is robust in skeletal muscle tissue [14], and several studies have indicated IL-15 expression and/or secretion is increased following exercise [12,15,16]. Anabolic and/or anti-cachectic effects of IL-15 were observed in cultured skeletal myotubes and in muscle tissue in a rat model of cancer cachexia [17–19]. Conversely, expression of the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) is elevated in cachexia and some studies have shown it can exert catabolic effects in skeletal muscle cultures [20,21]. Interleukin-10 is considered an anti-inflammatory cytokine and the IL-10/TNF-α ratio is widely used as an index reflecting anti-inflammatory status [22,23]. In this regard, a possible antagonistic relationship between IL-15 and TNF-α has been suggested in skeletal muscle [24]. Thus, levels of these cytokines can reflect both atrophy and inflammation in skeletal muscle tissue.

Some studies have suggested that antioxidant administration could be an effective strategy for cancer prevention and therapy [25,26]. However, at least one study indicated that dietary supplementation with the antioxidant failed to maintain skeletal muscle mass and induced cachexia in tumor-bearing mice [27]. Nevertheless, anti-oxidative and anti-carcinogenic effects of dietary selenium have been reported in some studies [28,29]. Also, a more biologically potent form of selenium, selenium nanoparticles (Se NPs) exhibit significant anti-carcinogenic effects in animal models of breast cancer [30,31] and could be a promising method for cancer prevention and treatment.

Since increased inflammation and oxidative stress have an important role in the loss of skeletal muscle in cancer cachexia, combining Se NPs as an antioxidant with the anabolic action of aerobic interval training could represent a simple and promising treatment option. In this study, we tested the hypothesis that Se NP supplementation and aerobic interval training modulate skeletal muscle inflammation and thereby attenuate muscle atrophy and functional impairment in cachexia. Cachexia was induced in mice injected with the metastatic 4T1 breast cancer cell line, a model for late stage breast cancer [32] that induces a loss of skeletal muscle and fat, weakness, increasing inflammation and anorexia. Our finding suggest that combined antioxidant and exercise training could control tumor growth and cachexia symptoms in 4T1 breast cancer. Concomitant effects on IL-15 expression and IL-10/TNF-α ratios were also observed.

2. Methods

2.1. Animals and experimental design

Experimental protocols using mice were conducted following the policies of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and authorized by the Ethics Committee of the School of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran. Inbred female BALB/c mice (aged 6–8 weeks, obtained from Iran Pasteur Institute) were housed in a temperature controlled room with alternating 12-h light and dark periods and free access to water and food. Animals were maintained in the Central Animal House, School of Medical Sciences of TMU.

At start and before tumor injection, animals (16 in each group) were randomly distributed to the following groups: control (C); trained (E); selenium nanoparticles (Se); and selenium nanoparticles plus training (SeE). The four groups were subjected to oral Se NP supplementation and aerobic interval training for six weeks. After six weeks of treatment, animals in each group were divided based on body mass and strength as measured by the inverted screen and weights tests [33] in two sub-groups (8 per groups): tumor-bearing control (CT); control normal (C); tumor-bearing trained (ET); normal trained (E), tumor-bearing selenium nanoparticles (SeT); normal selenium nanoparticles (Se); tumor-bearing selenium nanoparticles plus training (SeET); normal nanoparticles plus training (SeE). Se NP supplementation and aerobic interval training continued for another six weeks. Se NP groups were supplemented with 100 μg Se NPs three times in a week. All treatments were administrated by oral gavage. Untreated or control mice were gavaged with vehicle alone. Body weight and food intake were measured three days a week during supplementation and aerobic interval training.

2.2. Selenium nanoparticle preparation

Se NPs were prepared using a method described previously [34,35]. A solution of 5.2 mM selenium dioxide (Merck, Germany) was prepared and aqueous ascorbic acid solution (5.2 mM) was added into the mixture with continuous stirring (300 rpm) with a magnetic stirrer. The resulting reaction mixture was centrifuged and washed three times with double-distilled water. A stock solution of (1 mg/ml) Se NPs was prepared and used for further oral administration in doses of 100 and 200 μl per mouse.

2.3. Tumorgenicity

4T1 cells (estrogen receptor (ER)/progesterone receptor (PR))-negative, National Cell Bank of Iran, Pasteur Institute, Tehran, Iran) were trypsinized and resuspended in 10-fold excess culture medium. After centrifugation, cells were re-suspended in PBS, and 1×10^6 cells were injected (0.1 ml, s.c) using a 21-gauge needle into the left flank of BALB/c mice under ketamine and Xylazine (10 mg/kg, IP) anesthesia. Visible tumors were observed about 2 weeks after cancer induction.

2.4. Measurement of tumor volume

Tumors were sized in two dimensions. The larger tumor dimension was considered as length (L), and the other (at 90 degrees) as width (W). After appearance of the tumor, the length and width of the tumor were sized by a digital caliper once a week. Tumor volume was then accounted with tumor volume formula of [$V = \pi/6 (w \times L^2)$].

2.5. Aerobic interval training protocol and aerobic exercise test

Training groups performed aerobic interval training on treadmill for five days/week, for 6 weeks before tumor injection and 6 weeks after it. The regimen of aerobic interval training was arrived at by performance of pilot studies and according to previous literature reports [36–38]. Mice in the training groups performed an aerobic interval training protocol comprised of a 10 min warm-up, and 10 two minutes intervals of running at 70% Vmax separated by 2 min of active recovery at 50% Vmax. Maximal exercise was tested every two weeks for determination of peak velocity of a subset of normal and tumor-bearing mice. Following a 10 min warm-up, maximal exercise tests were started at a speed of 6 m/min. The treadmill speeds were increased 3 m/min, every 3 min until exhaustion, which was defined as the point at which animals could not maintain the running speed despite a gentle tap on the tail more than 5 times [39,40].

2.6. Evaluation of skeletal muscle strength in mice

2.6.1. Weights test

This test was performed as described by Deacon (2013) to measure strength in mice [33]. The apparatus was comprised of weights, each made of a coiled wire ball for the mouse to grip, to