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Swimming exercise and diphenyl diselenide-supplemented diet affect the serum levels of pro- and anti-inflammatory cytokines differently depending on the age of rats

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ABSTRACT

The increase in the inflammatory process is one of the main factors that contribute to aging. The aim of this study was to investigate the effects of a diphenyl diselenide $(PhSe)_2$-supplemented diet (1 p.p.m., 4 weeks) and swimming exercise (3% of body weight, 20 min per day, 4 weeks) on the serum levels of cytokines in Wistar rats of different ages. The results demonstrated an increase in the levels of pro-inflammatory cytokines (IL-1β, IL-6, TNFα and INFγ) and a decrease in the levels of IL-10, an anti-inflammatory cytokine, with age. In middle-age rats, the swimming exercise and $(PhSe)_2$-supplemented diet decreased serum levels of pro-inflammatory cytokines and increased the levels of IL-10. By contrast, in old rats the swimming exercise protocol increased the serum levels of pro-inflammatory cytokines and decreased the levels IL-10. Diet supplemented with $(PhSe)_2$ did not alter the serum levels of cytokines in old rats. Middle-age and old rats subjected to swimming exercise and supplemented with $(PhSe)_2$ in the diet had a decrease in the serum levels of pro-inflammatory cytokines and an increase in the levels of IL-10. This study demonstrated that swimming exercise and $(PhSe)_2$-supplemented diet affect the serum levels of pro- and anti-inflammatory cytokines differently depending on the age of rats. $(PhSe)_2$ supplemented in the diet had an anti-inflammatory effect, similar to that of induced by swimming exercise, in middle-age rats and reversed the pro-inflammatory effects of swimming exercise in old rats.

1. Introduction

The immune system is a complex biological system responsible for the neutralization of harmful environmental agents which can trigger a broad spectrum of pathological mechanisms [1]. However, an immune disorder (immunodeficiency or excessive immune response) can be harmful, causing various diseases.

Evidence from healthy subjects reveals that advanced age is associated with a hyperinflammatory state, characterized by elevated circulating levels of pro-inflammatory mediators [2]. A variety of age-related pathologies; such as atherosclerosis and neurodegenerative disorders such as Parkinson's and Alzheimer's disease, diabetes, among others; are correlated with an increase in the circulatory levels of pro-inflammatory markers, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1a, IL-6 and C-reactive protein [3–5].

Cytokines are cell-signaling proteins responsible for facilitating the return of physiological homeostasis and tissue repair in response to cellular injury induced by trauma or infection, being divided into two distinct types: pro-inflammatory (e.g. TNF-α, IL-1β and IL-6) and anti-inflammatory cytokines (e.g. IL-4, IL-10 and IL-13) [6]. However, a perturbation in the anti- and pro-inflammatory balance of these molecules represents an important mechanism that contributes to age-related metabolic dysfunctions. Thus, pharmacological and non-pharmacological interventions aiming at regulating the imbalance between anti- and pro-inflammatory cytokines as well as their signaling pathways have been suggested for therapeutic purposes.

It has been shown that physical exercise is effective to reduce (or ameliorate) the risk of age-associated diseases. In fact, there is evidence supporting the involvement of inflammatory mechanisms with the beneficial effects of physical exercise, such as decrease in age-associated immune senescence [7], increase in innate immune function [8] and decrease in chronic inflammation [9]. In addition, previous studies have shown that aerobic exercise

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reduced circulatory levels of pro-inflammatory markers in both aged humans and rodents [10,11]. Nevertheless, other studies reported that aerobic exercise had no such effects [12,13], making controversial the effect of exercise intervention on circulatory markers of inflammation.

Selenium (Se) is an essential micronutrient that may be used by mammals in both inorganic and organic forms. Most of the biological functions of Se are attributed to selenoproteins. In fact, selenoproteins are involved in the protection against oxidative stress and inflammation [14]. Arnaud [15] designed a study to investigate the relationship between plasma levels of selenium and mortality in an elderly population for a period of 9 years. Increased mortality was observed in individuals with low plasma levels of Se, reinforcing the importance of this trace element for human health. Diphenyl diselenide $(PhSe)_2$, an organoselenium compound, exhibits anti-inflammatory activity in several animal models [16–18]. In addition, $(PhSe)_2$ is effective in reducing the levels of reactive species (RS) due to its antioxidant property [19,20] preventing the occurrence of oxidative stress. Since it is widely reported that such molecules can activate molecular pathways involved in the inflammatory process [21], $(PhSe)_2$ may be a potential intervention to attenuate inflammation.

The aim of this study was to investigate the effects of swimming exercise and a $(PhSe)_2$-supplemented diet on the serum cytokine profile in Wistar rats at different ages.

2. Materials and methods

2.1. Animals

Adult (4 months, 12–16% lifespan), middle-age (12 months, 36–48% lifespan) and old (24 months 72–96% lifespan) male Wistar rats were obtained from a local breeding colony and were housed in cages, with free access to food and water. They were kept in a separate air-conditioned (22 ± 2 °C) room, on a 12-h light/12-h dark cycle, with lights on at 7:00 a.m. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources (#031/2014), the Federal University of Santa Maria, Brazil.

2.2. Drugs

Diphenyl diselenide $(PhSe)_2$ was prepared in our laboratory according to the method described by Paulmier [22] and the chemical purity (99.9%) was determined by GC/MS. Analysis of ^1H and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure.

2.3. Experimental procedure

The animals were divided into four groups (five animals per group) in each age (adult, middle-age and old):

Sedentary: rats received standard diet chow and did not perform exercise.

Sedentary + $(PhSe)_2$: rats received 1 ppm of $(PhSe)_2$ supplemented diet for 30 days and did not perform exercise.

Swimming exercise: rats received standard diet chow and performed swimming training.

Swimming exercise + $(PhSe)_2$: rats received 1 ppm of $(PhSe)_2$-supplemented diet and performed the swimming training.

2.4. Dietary supplementation

Animals were fed daily with ~150 g/animal standard diet chow or chow supplemented with 1 ppm of $(PhSe)_2$ (3–4 animals per cage). The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with $(PhSe)_2$ dissolved in ethanol (1 mg/10 ml) using a spray bottle. The standard and supplemented diets were kept at room temperature for 3 h to evaporate the alcohol and then kept at 4 °C for a further 1 week [23]. The concentration of $(PhSe)_2$ found in the diet did not cause overt signals of toxicity [24,25].

2.5. Exercise training protocol

Animals were subjected to the pre-training period of 20 min/day, 5 days (swimming exercise and swimming exercise + $(PhSe)_2$ groups). After the swimming adaptation, rats performed the swimming training with a workload (3% of body weight, 20 min per day for 4 weeks) [26]. The swimming training was performed between 01:00 and 03:00 p.m in water at a temperature of 32 ± 1 °C. Rats from sedentary and sedentary + $(PhSe)_2$ groups were adapted to water, rats were placed in the bottom of a separate tank with shallow water (5 cm) at 32 ± 1 °C, without the workload. At the end of the exercise training, rats were towel-dried and returned to their respective cages.

2.6. Determination of cytokines

After training protocol, rats were anesthetized with ketamine (90 mg/kg) and xylazine (5 mg/kg), for blood collection by heart puncture. Serum (supernatant) was separated by centrifugation at 2400×*g* for 10 min and stored at −20 °C until analysis.

Measurement of serum interleukin (IL) IL-1β, IL-6, IL-10, tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) were assessed using commercial ELISA kits as described by the manufacturer (eBIOSCIENCE, San Diego, USA). Results were expressed in pg/ml for IL-1β, IL-6, IL-10, TNF-α and μg/ml for INF-γ. The sensitivity of the assays for detection of cytokines was as stated in the manufacturer's brochures.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. The statistical significance was assessed by analysis of variance (ANOVA). One-way ANOVA was used to assess the age effect (adult × middle-age × old). Two-way ANOVA was used to assess the effect of training and treatment for each age (swimming exercise × $(PhSe)_2$). Post hoc Duncan's test was carried out when appropriated. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Effect of age

Middle-age and old sedentary rats had increased levels of pro-inflammatory cytokines when compared with those of the control adult group. One-way ANOVA demonstrated a significant effect of age in serum levels of IL-1β ($p < 0.05$) (Fig. 1A), IL-6 ($p < 0.05$) (Fig. 1B) and INFγ ($p < 0.05$) (Fig. 1D). The effect of age in TNFα levels was evident only in old rats ($p < 0.05$) (Fig. 1C). Serum levels of the anti-inflammatory cytokine IL-10 were decreased depending on the age ($p < 0.05$) (Fig. 1E).

The ratio between pro- and anti-inflammatory cytokines changed with age. One-way ANOVA revealed a significant age-dependent increase in the ratio of IL1β/IL10 ($p < 0.05$), IL6/IL10 ($p < 0.05$) and TNFα/IL10 ($p < 0.05$) in both middle-age and old rats when compared with that of adult rats (Fig. 2).