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Effects of green tea and physical exercise on memory impairments associated with aging

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A B S T R A C T

We investigated the effects of physical exercise and green tea supplementation (associated or not) on biochemical and behavioral parameters in the time course of normal aging. Male Wistar rats aged 9 month were divided into groups: control, physical exercise (treadmill running), and supplemented with green tea while either performing physical exercise or not. A young control group was also studied. Physical exercise and green tea supplementation lasted 3 months. Afterwards, behavioral and biochemical tests were performed. Biochemical measurements revealed differences in antioxidant and oxidant responses in hippocampus, prefrontal cortex and striatum. Behavioral testing showed age-related memory impairments reversed by physical exercise. The association of green tea supplementation and physical exercise did not provide aged rats with additional improvements in memory or brain oxidative markers. Green tea *per se* significantly decreased reactive oxygen species levels and improved antioxidant defenses although it did not reverse memory deficits associated with normal aging.

1. Introduction

Oxidative stress is a biological phenomenon associated with a range of degenerative diseases observed during aging, including those resulting in learning and memory deficits (Fukui et al., 2001; Serrano and Klann, 2004). Age-related deficits may be associated with structural and functional changes in macromolecules and cell membranes, which, at least in part, result from direct or indirect effect of free radicals and reactive oxygen species (ROS) (Cui et al., 2012). Regular physical exercise is an efficient strategy to protect the brain functions against deficits associated with the aging process (Asl et al., 2008).

ROS generated during sessions of aerobic exercise contribute to the long term adaptation to aerobic training, which improves both enzymatic and non-enzymatic antioxidants (Andrews, 1965). However, low adherence to physical exercise programs in the general population increases interest in behavioral or pharmacological interventions aiming to minimize ROS, especially among aged subjects not able to exercise regularly. In this regard, nutritional interventions are a frequent strategy to improve ingestion of antioxidant substances (Morillas-Ruiz et al., 2006).

A natural compound often included in the daily diet of the general population is the green tea (*Camellia sinensis*). Green tea (GT) contains a large amount of catechins (30–40% of dry weight), a polyphenol with potential antioxidant activity (Berube-Parent et al., 2005). Among elderly population, the regular ingestion of GT has been described as frequent, especially in the Mediterranean and Asian diets (Stefani and Rigacci, 2014). Specifically in the Asian countries, such as China (Tseng and Hernandez, 2005) and Japan (Hayasaka et al., 2013), which is the major tea producing country. Catechins are the major components of GT, and its intake has been associated with a variety of beneficial health effects, including neuroprotective effects in Alzheimer and Parkinson disease (Bastianetto et al., 2006; Hu et al., 2007; Kim et al., 2010; Mandel et al., 2008; Rezai-Zadeh et al., 2008, 2005; Singh et al., 2008). This effect is associated with the activity of epigallocatechin gallate (EGCG), a major constituent of GT, which has been investigated for its preventive and therapeutic potential role in cerebral aging, as well as in the progression of neurodegenerative diseases in the aging (Assuncao et al., 2011; Unno et al., 2004; Unno et al.,

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2007). A former epidemiological study (Feng et al., 2010), suggested that both black and green tea consumption were associated with better cognitive performance. Despite of this, more intervention trials and large prospective studies are needed to further assess the relation of GT ingestion and the development of Alzheimer's disease.

Considering combined interventions, regular physical exercise has been associated with ingestion of GT to reduce exercise-induced muscle damage (Haramizu et al., 2013). This was described to be dependent on the antioxidant potential of GT (Haramizu et al., 2011) after long term treatments (Haramizu et al., 2011) but not after single doses (Jowko et al., 2012). However, the biochemical and behavioral effects of GT supplementation associated with regular physical exercise on cognition have been briefly addressed (Gibbons et al., 2014; Schimidt et al., 2014). However, it has been suggested that effects of exercise on brain can be enhanced by concurrent consumption of natural products such as omega fatty acids or plant polyphenols (van Praag, 2009). These interventions could involve similar cellular pathways important for neurogenesis, cell survival, synaptic plasticity and vascular function. Therefore, any positive association of long term GT intake and physical exercise could have important implications as both constitute low cost interventions. Here we study the specific effect of exercise and GT, either combined or not, on the prevention of memory impairment and brain oxidative damage related to aging in rats.

2. Material and methods

2.1. Animals and experimental design

Male Wistar rats were purchased from Central Vivarium of Federal University of Santa Maria (RS/Brazil). During all the experimental period, they were housed three per cage (dimensions $50 \times 60 \times 22$ cm) and maintained under controlled light and environmental conditions (12 h light/12 h dark cycle; temperature of 23 ± 2 °C; humidity $50 \pm 10\%$) with food and water *ad libitum*. All experiments were conducted in accordance with the "*Principles of laboratory animal care*" (NIH publication n° 80–23, revised 1996) and in agreement with the guidelines established by the Institutional Animal Care and Use Committee of the Local Institution (IRB #0422012) to ensure that number of rats and their suffering were kept to a minimum. During the experiment, the weight of each rat and liquid consumption for each house cage were measured once a week. When rats were 9 months of age, they were divided into 4 groups: aged group ($n = 16$), exercise aged group ($n = 18$), green tea supplementation aged group ($n = 18$) and exercise and green tea supplementation aged group ($n = 21$). Moreover, at the time of memory and biochemical testing, a group of 2 month-old rats control group not submitted to any intervention was also studied ($n = 10$).

After memory testing, rats from all groups were euthanized using a rodent guillotine. Anesthesia was not used due to its effects on central nervous system (Tan et al., 2012). The brain was removed and the areas of interest were quickly dissected out on an inverted Petri dish, and homogenized in buffer solution for posterior brain tissue preparation and biochemical analyses. Biochemical analyses permitted to quantify the concentration of glutathione (GSH), reactive oxygen species levels (ROS), thiobarbituric acid reactive substances (TBARS) and glutathione peroxidase (GPx) activity.

NADPH, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and GSH reagents were purchased from Sigma (St. Louis, MO, USA). Other reagents used in this study were of analytical grades and obtained from standard commercial supplier. Experiments followed the experimental designed illustrated in Fig. 1.

During the behavioral tests, necessary to perform before biochemical analyses, interventions were sustained.

2.2. Experimental groups and conditions

2.2.1. Aged control group

Rats in the aged control group had access to food and water *ad libitum*. They were free to move and manipulated daily in parallel to rats from other groups.

2.2.2. Exercise aged group

Rats in this group had access to food and water *ad libitum*. They were submitted to the physical exercise intervention. Physical exercise was individual treadmill running performed at an intensity of 60–70% maximal oxygen uptake (VO_2) (belt velocity between 9 and 13 m/min), in sessions lasting 30 min, 5 times a week, always in the same period of day, in light time period (Andrews, 1965). In the week previous to the onset of intervention, rats performed daily treadmill running for 10 min to habituate before performing the first VO_2 test. To determine the individual intensity of exercise, we conducted an indirect VO_2 test, consisting in a treadmill running, starting with low velocity increased in 5 m/min steps every 3 min, until the rat was unable to keep running. The time to fatigue (min) and work volume (m/min) were considered as an indirect measure of maximum oxygen consumption, as described elsewhere (Cechetti et al., 2012; Costa et al., 2012). In the middle of exercise intervention (week 6), an additional indirect VO_2 test was conducted to adjust the exercise intensity for each rat. At the end of the exercise intervention (week 12), a last indirect VO_2 test was performed. The last indirect VO_2 test was performed to ensure that there was an improvement in aerobic condition of the rats after the exercise intervention.

2.2.3. Green tea supplementation aged group

Rats in this group received food and GT. They received GT mixed with drinking water (13.33 g/L), as described elsewhere (Mustata et al., 2005). Food and GT were *ad libitum* and rats were free to move and manipulated daily. GT was prepared daily in the early morning, and administered at room temperature. For tea preparation, 750 ml of water were boiled and then rest until temperature was 90 °C. Afterwards, an infusion of GT (10 g) was muffled for 3 min and allowed to rest. Finally, tea was filtered using filter paper in a container immersed in ice. Once tea was cold, it was distributed in bottles protected from light at environment temperature.

GT samples (Madrugada Co.) used in this study were purchased from standard markets and analyzed by spectrophotometry using the Folin–Ciocalteu method modified (Chandra and De Mejia Gonzalez, 2004), which measured the total polyphenols content (at a concentration of 819.5 μEq GAE/mL), and by high-performance liquid chromatography, which measured the presence of epicatechin (concentration of 83.35 μg/mL), epigallocatechin gallate (299.56 μg/mL) and epicatechin gallate (86.05 μg/mL).

2.2.4. Exercise and green tea supplementation aged group

Rats from this group exercised as described for the exercise aged group, and received green tea as described for the green tea supplementation aged group.

2.2.5. Young control group

Rats from this group were 2 months old and served as a control to verify effects of aging. They were housed as the others groups with food and water *ad libitum*. They were free to movement and daily manipulated in the same form of rats from other groups.

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