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Effects of cafeteria diet on memory and hippocampal oxidative stress in a rat model of Alzheimer-like disease: Neuroprotection of green tea supplementation

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ABSTRACT

Here we investigate the neuroprotective role of green tea (GT) when cafeteria diet (CAF) is associated with a model of β-Amyloid (Aβ) injection that induces cognitive impairments related to Alzheimer disease (AD). Wistar male rats were supplemented with GT, CAF, or GT plus CAF for 8 weeks before intra-hippocampal injection of Aβ peptide (2 μL of Aβ-25–35, CA1 region). AD-like and sham rats were submitted to memory tests. Oxidative status was quantified in the bilateral hippocampus, and plasmatic triglycerides, total cholesterol, glucose, and AChE activity were determined. CAF per se does not impair object and social recognition memories. Neuroprotective role of GT was confirmed also in the conditions of CAF combined with AD-like. GT supplementation and CAF, either isolated or combined, avoid oxidative stress and damage in the hippocampus. We conclude that CAF did not influence oxidative damage and memory deficits resultant of β-Amyloid injection when GT is simultaneously ingested.

1. Introduction

The high intake of fat and sugar in the modern society (de Macedo, de Freitas, & da Silva Torres, 2016) leads to a hypercaloric condition that increases risk of metabolic diseases (Kang, Lee, & Lee, 2017; Sack et al., 2016a). A hypercaloric diet may cause peripheral insulin resistance, increases brain oxidative stress, hippocampal synaptic dysfunction, brain mitochondrial dysfunction, and brain insulin resistance (Husain et al., 2017; Kang et al., 2017; Vandal et al., 2014). Taken together, these adaptations increase risk for onset of dementias, especially the Alzheimer's disease (AD), given that this diet also increases deposition of Aβ plaques in the brain (Leffa et al., 2015; Pugazhenthi, Qin, & Reddy, 2017).

AD is characterized by declines in memory and cognition (Mendiola-Precoma, Berumen, Padilla, & Garcia-Alcocer, 2016). A condition of neurotoxicity resultant of Aβ plaques accumulated in the brain is one of AD's precursor mechanisms. These Aβ plaques increase the production of reactive oxygen species and promote oxidative stress – further increasing the β-Amyloid deposition in a cascade effect leading to neuronal losses – predominantly by apoptosis (Mendiola-Precoma et al., 2016), and cognitive deficits (Grizzanti, Lee, Camins, Pallas, & Casadesus, 2016).

Green tea (*Camellia sinensis, Theaceae*) has been shown as a potential nutritional intervention in models of brain diseases and injuries. Catechins from green tea promote neuroprotection against oxidative and inflammatory stressors in obese rats supplemented with a cafeteria diet (Macedo, Bondan, & Otton, 2017a). The antioxidant properties of green tea were also useful in protecting memory against deficits resultant of aging (Flores et al., 2014), and brain insults like ischemia reperfusion (Martins et al., 2017; Schimidt, Garcia, Martins, Mello-Carpes, & Carpes, 2017). Recently, the neuroprotective potential of green tea was also reported in a model of AD-like disease (Schimidt et al., 2017).

Whether a hypercaloric diet combined or not with green tea ingest has effects on brain functions and oxidative damage in models of neurodegenerative disease is still unknown. It would be valuable to know if an intervention based in green tea intake influences the effects of hypercaloric diet on memory and brain oxidative status. Therefore, the purpose of our study was to determine the neuroprotective potential of the green tea from *Camellia sinensis* in a rat model of Alzheimer-like

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disease associated with a cafeteria diet containing high content of fat and sugar. We hypothesized that cafeteria diet would impair the redox status in the hippocampus, and therefore negatively impact on memory, whereas the green tea would confirm its role as a neuroprotetor in the AD-like model, as previously reported (Schimidt et al., 2017).

2. Material and methods

2.1. Animals and experimental design

Male Wistar rats aged two months were bought from the Central Vivarium of Federal University of Santa Maria (RS/Brazil). They were housed three per cage under controlled light and environmental conditions (12 h light/dark cycle, at 23 ± 2 °C and 50 ± 10% air humidity). Food and water, or tea, were available *ad libitum*. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Local Institution Animal Care and Use Committee (IRB #012015). Upon arrival, rats were randomly assigned into experimental groups: (a) control: rats were treated with standard diet (STAN diet; N = 8–12); (b) rats were treated with cafeteria diet (CAF diet; N = 8–12); (c) rats were treat with standard diet and supplemented with green tea (STAN diet; N = 8–12); and (d) rats were treated with green tea plus CAF diet (GT + CAF diet; N = 8–12).

After 8 weeks, sham (saline infusion/group 1) or Aβ neurotoxicity inducing surgeries (Aβ hippocampal infusion) were performed and groups were reorganized (n = 12/group), as follow (see Fig. 1 and the details below):

- group 1 – Control (SHAM): STAN diet; sham surgery without green tea supplementation;
- group 2 – Aβ hippocampal infusion (AD-like): STAN diet; Aβ hippocampal infusion; not supplemented with green tea;
- group 3 – (AD-like + GT: STAN diet): Aβ hippocampal infusion; supplemented with green tea;
- group 4 – (AD-like + CAF diet): CAF diet; Aβ hippocampal infusion; not supplemented with green tea;
- group 5 – (AD-like + GT + CAF diet): supplemented with green tea and CAF diet; Aβ injection hippocampal infusion.

Ten days after surgery, rats were submitted to behavioral tests and euthanized (Fig. 1). Blood samples were collected to determine glucose, triglycerides and cholesterol levels. Abdominal fat was removed for quantification. Bilateral hippocampus was removed and biochemical analyses were conducted to determine the levels of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), ferric reducing/antioxidant power (FRAP), and acetylcholinesterase (AChE) activity.

2.2. Diet

Rats from group 1, 2 and 3 received STAN diet and rats from group 4 and 5 received CAF diet to resemble the modern patterns of human diet (Macedo et al., 2017a). The STAN diet consisted of commercial rat chow bought from a local supplier and achieved, on average, 22% protein, 8% fat, 41% carbohydrate and 3% fiber. The CAF diet consisted of commercial rat chow, peanuts, milk chocolate, and sweet biscuit in the proportion of 3:2:2:1, with all components powdered, mixed and pelleted. The cafeteria diet achieves, on average, 16% protein, 35% fat, 40% carbohydrate, and 8% fiber. Peanut was composed by 6% protein, 56.6% fat, 21.5% carbohydrate and 8% fiber; the chocolate was composed by 23.7% protein, 62.8% fat, 48% carbohydrate and 10.8% fiber, and the sweet biscuits were composed by 3.2% protein, 11% fat, 79% carbohydrate and 2.4% fiber. Based on these values, 100 g of the STAN diet offered 1429 kJ/g and the CAF diet 4587 kJ/g. The average daily intake per rat was determined by the total consumption from each home cage divided by the number of rats in each home cage. To compare the groups we considered the caloric densities in kJ/g. Diets were continued until the day of euthanasia.

2.3. Green tea supplementation

Rats from groups 3 and 5 received green tea mixed with drinking water (13.33 g/L), as described elsewhere (Flores et al., 2014; Schimidt et al., 2017, 2014). Green tea was purchased from a local supplier (Madrugada Alimentos LTDA, RS, Brazil) and prepared daily with water at 90 °C, brewed for 3 min, filtered and cooled down. The solution was then protected from light with aluminum foil and administered at ambient temperature (Schimidt et al., 2014). The average daily tea intake per rat was determined by the total consumption from each home cage divided by the number of rats in the home cage. Tea supplementation continued until the day of euthanasia.

To determine the compounds profile of the green tea used, the extracts were analyzed concerning the presence of Epigallocatechin (EGC; 213.68 μg/mL), Epicatechin (EC; 191.15 μg/mL), Epigallocatechin gallate (EGCG; 313.43 μg/mL) and Epicatechin gallate (ECG; 86.95 μg/mL) by high-performance liquid chromatography (HPLC system YL9100, Young Lin, with diode array detector). HPLC was performed with a Shimadzu Prominence Auto Sampler (YL9100) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator, and YL9160 diode array detector. To determine compounds profile the extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column (4.6 × 250 mm). The mobile phase was composed of water (pH = 3): acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100% acetonitrile. At 38 min water (pH = 3): acetonitrile (5:95, v/v) was used again, in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8 ml/min was used and 20 μL of

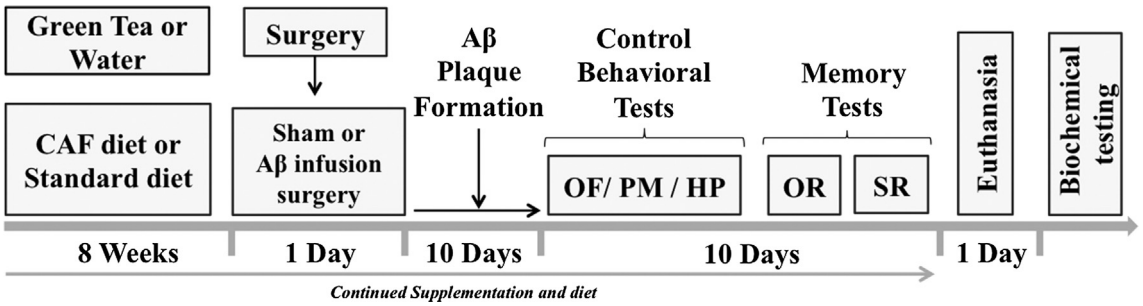

Fig. 1. Experimental design. Rats were supplemented with green tea and/or CAF diet for 8 weeks before surgeries. Behavioral testing started 10 days after surgery. Euthanasia occurred 20 days after surgery; biochemical testing was the last step of the study. OF – open field; PM – plus maze; HP – hot plate; OR – object recognition memory test; SR – social recognition memory test.