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Sulfamethoxazole-trimethoprim associated with resveratrol for the treatment of toxoplasmosis in mice: Influence on the activity of enzymes involved in brain neurotransmission

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ABSTRACT

This study aimed to investigate the influence of sulfamethoxazole-trimethoprim (ST) associated with resveratrol on the enzymatic activities of acetylcholinesterase (AChE), adenylate kinase (AK), pyruvate kinase (PK), and creatine kinase (CK) in the brain of mice experimentally infected by *Toxoplasma gondii*. For that, 60 mice were divided into ten groups with 6 animals each: groups A to D composed by healthy mice and groups E to J consisting of animals infected by *T. gondii* (VEG strain). Animals started treatment 20 days post-infection for 10 consecutive days with oral doses of 0.5 mg kg^{-1} of ST (groups B and F), 100 mg kg^{-1} of free resveratrol (groups C and G) and inclusion complex of resveratrol (nanoparticles containing resveratrol) (groups D and H), as well as with an association of both drugs (groups I and J). The results showed increased ($P < 0.001$) AChE activity on infected animals (groups E-J) when compared to not-infected (A) animals, and also uninfected animals treated with ST (group B) had increased AChE activity. AK activity decreased ($P < 0.001$) in the infected and untreated (group E), differently from the other groups that did not differ. PK activity did not differ between groups ($P > 0.05$). When comparing control groups (uninfected (A) and infected (E)), we verified a significant ($P < 0.001$) increase in CK activity in the brain, and it is noteworthy that the animals treated with resveratrol associated with ST (group I and J) had similar CK activity to those animals from the group A. Treatment with the combination of ST and resveratrol was able to reduce ($P < 0.05$) the number of parasitic cysts in the brain, thus reduced inflammatory infiltrates in the liver, and prevented the occurrence of hepatocytes lesions due to toxoplasmosis in mice. Based on these results, it is possible to conclude that increased AChE and CK activities after *T. gondii* infection did not change with the treatment of ST-resveratrol association. In addition, decreased AK activity caused by *T. gondii* infection was normalized by ST-resveratrol treatment. *T. gondii* infection and treatment does not affect PK activity in brain.

1. Introduction

Toxoplasma gondii is a ubiquitous intracellular coccidian parasite of the Apicomplexa Phylum, the largest and most important group of obligate parasites with an unusual capacity to parasitize diverse

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cell types and virtually infect any warm-blooded animal [1]. In immunocompromised patients, the *T. gondii* causes congenital birth defects and many other serious complications [2]. Several studies have shown the mechanisms and immune responses against *T. gondii* infection through the production of high levels of interleukins, such as IL-12 and IFN-γ, responsible for activating the cholinergic system, and IL-10 liberation known as an anti-inflammatory cytokine able to cause resistance to the parasite as well as to control the immune responses of the host [3].

Cholinesterase enzymes are present in cholinergic and non-cholinergic tissues, as well as in the brain and some body fluids. This group of enzymes is divided into two distinct categories, according to their specific substrates, behavior in the presence of excess substrate, and susceptibility to inhibitors [4]. The acetylcholinesterase (AChE) is a specific cholinesterase, which hydrolyzes esters of acetylcholine (ACh) and has high levels in nervous tissue [5,6]. The ACh is the main neurotransmitter and anti-inflammatory molecule released, which controls the release of pro-inflammatory cytokines [7]. Recently, Tonin et al. [8] reported that AChE has an important role in the modulation of early immune responses against *T. gondii* infection. It has also been reported that infection by *T. gondii* is related with neurodegenerative diseases [9] and these disorders have been associated to changes in the brain energy metabolism.

Creatine kinase (CK), pyruvate kinase (PK), and adenylate kinase (AK) enzymes are part of a phosphoryl transfer network involved in maintaining energy homeostasis in the brain tissue [10]. There are no reports in the literature regarding the activity of these enzymes in the brain of animals infected by *T. gondii*. AK has been implicated in the processing of cellular signals associated with ATP utilization [11], and the deficiency of AK is associated to a hemolytic anemia [12], pathology previously described in cases of trypanosomosis [13]. PK is a key enzyme of the glycolysis pathway present in all tissues, responsible for catalyzing irreversibly the transphosphorylation [14]. CK, in addition to its buffering effect, might be used as a source of energy, replacing ATP, in areas with high energy demand such as the brain tissue [15]. Recently, studies have shown that ATP levels in the brain have peaks according to the stage of the disease, that is, increases in the acute phase and decreases during the chronic phase of the disease in mice experimentally infected by *T. gondii* [16].

The presence of reactive oxygen species (ROS) can be controlled by antioxidants such as resveratrol (3,5,4-trihydroxy-trans-stilbene), a polyphenol compound found primarily in grapes and red wine with diverse biological activities, such as antioxidant and anti-inflammatory functions [17,18]. Several neuroprotective properties of resveratrol have been attributed to its potent antioxidant activities, which in many studies have been shown to protect the neural tissue against a variety of neurodegenerative conditions caused by oxidative stress [19,20]. Therefore, this study aimed to investigate the influence of sulfamethoxazole-trimethoprim associated with resveratrol to treat mice experimentally infected by *T. gondii* on the enzymatic (AChE, AK, PK, and CK) activities involved in the metabolism of neurotransmitters in the brain.

2. Materials and methods

2.1. Resveratrol

2.1.1. Complex inclusion of resveratrol (CIRSV)

Resveratrol ($C_{14}H_{12}O_3$; molecular weight = 228.25 g/mol; purity > 98%) and 2-hydroxypropyl-β-cyclodextrin were obtained from Sigma Aldrich. A mixture containing 0.1 mM of HPβCD, water (8 mL at 40 °C), and an equimolar amount of RSV (0.1 mM) was prepared by vigorous stirring for 1 min in a ULTRA-TURRAX High-Speed Homogenizer T 25 (IKA, Reino Unido) at 3.200 rpm. RSV was previously suspended in ethanol (2 mL). The final mixture was filtered through 0.45 μm cellulose acetate membrane filter to removed undissolved particles. The solvent was removed using rotary evaporation at 40 °C for approximately 10 min and the water was evaporated under vacuum for 8 h.

2.1.2. Characterization of the complex inclusion

Fourier Transformed Infra-Red Spectroscopy analyses were performed using Perkin Elmer (Spectrum One). RSV, HPβCD, and the complex spectra were collected using an FT-IR in a spectral region between 4000 and 450 cm^{-1}. Samples were mixed in a mortar with potassium bromide (KBr) (1:100) and pressed in a hydraulic press (10 tons for 2 min) to from small tablets that were placed in the infrared beam. Nuclear magnetic resonance (NMR) spectra were performed using BRUKER DPX-400 (Bruker, Germany). Fifteen grams of samples were dissolved in DMSO-d_6 using TMS like internal reference standard. All of these procedures were carried out in the Chemistry Laboratory of the Federal University of Santa Maria (UFSM).

2.2. Toxoplasma gondii strain and inoculum preparation

T. gondii tachyzoites (VEG strain kept in liquid nitrogen was inoculated into one mouse (BALB/c). Thirty days later, the brain containing cysts with bradyzoites was collected, homogenated in saline solution, and inoculated orally in other five mice. This procedure was done in order to reactivate parasite's virulence. Twenty-five days after infection, all mice were euthanized and their brains collected. Brain cysts were counted and separated to be used as the inoculum.

2.3. Animal and experimental design

Sixty male Swiss albino mice, 80-day-old, average initial body weights of 25 ± 5 g were used in this study. They were kept in cages with six mice each, housed on a light/dark cycle of 12 h in an experimental room under controlled temperature and humidity (25 °C; 70%, respectively). They were fed with commercial feed and water *ad libitum*. All animals were submitted to a 10 days adaptation period.

The experimental design is shown on Table 1. Two major groups were formed: groups A to D (n = 24) consisted of healthy

Table 1
Experimental design: uninfected or infected animals by *Toxoplasma gondii*, as well as untreated or treated mice with free resveratrol, inclusion complex and the association of resveratrol and sulfamethoxazole-trimethoprim.

Group (n)	Infection by *T. gondii*[a]	Sulfamethoxazole-trimethoprim[b]	Free resveratrol[c]	Inclusion complex[d]
A (6)	α	α	α	α
B (6)	α	+	α	α
C (6)	α	α	+	α
D (6)	α	α	α	+
E (6)	+	α	α	α
F (6)	+	+	α	α
G (6)	+	α	+	α
H (6)	+		α	+
I (6)	+	+	+	α
J (6)	+	+	α	+

α Animals uninfected and/or untreated.
\+ Animals submitted to an infection and/or treatment.
n Animals per group.
[a] Infection orally performed with 50 cysts containing bradyzoites of *T. gondii* cystgenic strain (VEG).
[b] Sulfamethoxazole-trimethoprim administered orally at 0.5 mg kg^{-1} .
[c] Resveratrol (Free Resveratrol (FR)) administered orally at 100 mg kg^{-1} .
[d] Resveratrol (nanoparticles containing resveratrol (NCR)) administered orally at 100 mg kg^{-1} .