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Hepatic xanthine oxidase activity and purine nucleosides levels as physiological mediators to analyze a subcutaneous treatment with $(PhSe)_2$ in mice infected by *Toxoplasma gondii*

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ABSTRACT

The aim of this study was to evaluate the levels of purine nucleosides and xanthine oxidase (XO) activity in the liver of mice chronically infected by *Toxoplasma gondii* and treated with diphenyl diselenide $(PhSe)_2$. For this experiment, forty Swiss mice were used. Twenty animals were orally infected by approximately 50 bradizoites of a cystogenic ME-49 strain of *T. gondii*, and the same number of uninfected mice was used as a control group. Ten infected and ten uninfected mice were subcutaneously treated twice (days 1 and 20 post-infection (PI)) with 5 μmol kg^{-1} of $(PhSe)_2$. On day 30 PI, liver samples were collected to measure the levels of hypoxanthine (HYPO), xanthine (XAN), uric acid (UA), and XO activity. Infected animals showed increased ($P < 0.05$) levels of hepatic XAN and UA, as well as XO activity compared to uninfected animals. The use of $(PhSe)_2$ in healthy mice increased the levels of all nucleosides, but decreased XO activity compared to healthy untreated animals. The group of infected and treated animals showed increased XAN and UA levels, and XO activity compared to the healthy control group, however infected and treated mice showed a decrease in the XO activity compared to the infected untreated group. We conclude that chronic infection caused by *T. gondii* can induce hepatic changes, such as increased UA levels and XO activity, that can increase the pro-oxidative profile. The $(PhSe)_2$ treatment of healthy animals altered the levels of nucleosides, possibly due to low XO activity that decreased nucleoside degradation. Finally, $(PhSe)_2$ treatment decreased XO activity in the infected group and increased nucleoside levels; however it was unable to reduce the UA levels found during the infection.

1. Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii*, a protozoon that infects animals and humans [1,2]. Chronic toxoplasmosis is characterized by the latent infection of the brain by the parasite, which induces neurological and behavioral disorders in mice and humans [3,4]. This chronic infection is considered asymptomatic, however the latency phase can improve the pro-inflammatory profile by increasing cytokines and others immune factors [5]. Some studies showed that the latent infection can cause some physiological changes in the host, leading to chronic liver disease [1,6]. The liver is considerable the most important metabolizing organ, and its proper functioning is important to maintain homeostasis through the body [7]. Tonin et al. [8] found purine nucleosides changes in the brain of mice chronically infected by *T. gondii*, and these changes might be related to the chronic pathology of the brain. However, the level of purine nucleosides in the liver of mice chronically infected by *T. gondii* remains unknown.

Purines are important molecules to the synthesis of nucleotides,

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and consequently DNA and RNA production in the cells. Some studies revealed that these compounds play an important role maintaining the physiological status, as well as in controlling the homeostasis during some diseases [9]. Xanthine oxidase (XO) is the enzyme responsible to oxidize nucleoside hypoxanthine (HYPO) and xanthine (XAN) in hydrogen peroxide (H_2O_2) and uric acid (UA), which is excreted into urine. The XO activity can produce oxidant and anti-oxidant products [10–12], and the XO activity may also improve the immune response, and increases the anti-oxidant defense, however in some cardiac and hepatic diseases higher XO activity and elevated levels of UA can be indicators of bad prognosis of the disease [11,13]. Therefore, it is possible that the real reason for the inflammatory action showed by high XO activity might be the release of pro-inflammatory cytokines and DAMPs (damage associated molecular patterns) from leukocytes stimulated by the increase of this enzyme [14]. Anti-inflammatory compounds, and XO inhibitors are clinically administered in some diseases to avoid higher activity of this enzyme and, consequently, its pro-inflammatory action [10,11,14].

Diphenyl diselenide $(PhSe)_2$ is an organoselenium compound with antioxidant and immunomodulatory activities [15]. *In vivo* experiments have shown that $(PhSe)_2$ reduces the levels of pro-inflammatory cytokines, and reactive oxygen species in some pathologies [16,17]. In this way, Barbosa et al. [18] demonstrated that a subcutaneous treatment with 5 μmol kg^{-1} of $(PhSe)_2$ can reduce the oxidative damage and the levels of pro-inflammatory cytokines in mice acutely infected by *T. gondii*. Moreover, this low dosage was unable to decrease the immune responses against the parasite. Considering that the chronic infection caused by *T. gondii* can induce some physiological changes in the host, the aim of this study was to evaluate whether changes in purine nucleosides levels and the XO activity in the liver tissue may have occurred in mice infected by a cystogenic strain of *T. gondii*.

2. Materials and methods

2.1. Toxoplasma gondii strain

This study used standard strain (ME-49; genotype II) of *T. gondii* [8] kept in liquid nitrogen in the laboratory to inoculate one mouse (Swiss). Thirty-two days later, brain homogenate (in saline solution) containing cysts with bradyzoites was collected and inoculated orally in other three mice; this procedure was done in order to reactivate the parasite's virulence. The mice were euthanized for brain collection 30 days PI, and parasitic cysts were counted and separated in order to be used later in the experiment.

2.2. Experimental design

Forty 60-day-old female mice weighing an average of 25±5 g were used for the experiment. This study was approved by the Ethic Committee on Animal Use of the Federal University of Santa Maria (UFSM) under protocol number 7787270815. The animals were divided into four groups (A, B, C and D) with ten animals each. The groups A and B were used as controls (uninfected). Animals in groups C and D were orally infected with 0.25 mL of brain homogenate containing 50 cysts of *T. gondii* bradyzoites. For the subcutaneous treatment, $(PhSe)_2$ (SIGMA; St. Louis, MO, USA) was dissolved in 0.1% dimetilsulfoxide and administered on day 1 and 20th post-infection (PI) using doses of 5 μmol kg^{-1} [18] in mice of groups B and D. On day 30th PI, the animals were euthanized with an overdose of isoflurane.

2.3. Tissue preparation

After the euthanasia, liver samples were collected and separated into two fragments (3 g). One fragment was manually homogenized in 10 mL of physiological solution (PS) with a syringe plunger and centrifuged at 1500 rpm for 15 min. The supernatant was collected, and used to measure XO activity. The other liver fragment was used to measure the levels of purine by gently homogenization in a sterile flask, and the resultant supernatant was mixed with 0.6 M perchloric acid and 1 M potassium hydroxide, as described previously [19].

2.4. Analysis of purine levels in the liver

Purine compounds and metabolic residues were analyzed by HPLC according to Voelter et al. [19]. The proteins were denaturized using 0.6 mol/L perchloric acid. All samples were centrifuged (16000 × g for 10 min at 4 °C), supernatants were neutralized with 4.0 N KOH, and clarified with two more centrifugations (16000 × g for 30 min at 4 °C). Aliquots of 20 μL were applied to a reversed-phase HPLC (LC-20AT model, Shimadzu, Kyoto, Japan) using a C18 column (Ultra C18, 25 cm × 4.6 mm x 5 μm, Restek – USA). The elution was carried out applying a linear gradient from 100% solvent A (60 mM KH_2PO_4 and 5 mM of tetrabutylammonium chloride, pH 6.0) to 100% of solvent B (solvent A plus 30% methanol) over a 30 min period (flow rate at 1.4 mL/min) according to a method previously described [19]. Mobile phases were filtered through a 0.22 μm Millipore filter prior to analysis, and all the reagents used were HPLC grade. The amount of purine and metabolic residues were measured by absorption at 254 nm. The retention time of the standards was used as a parameter for identification and quantification by comparison of the peak area. Purine levels were expressed as nmol of different compounds per g of tissue.

2.5. Xanthine oxidase activity

Hepatic XO activity was determined using the method previously described [20]. First, liver homogenates were centrifuged at 2500 rpm for 10 min to remove impurities. The reaction mixture contained 1 mM of xanthine as substrate, and 50 mM phosphate buffer (pH 7.4). The reaction mixture was incubated with approximately 0.5 mg of homogenized protein at 37 °C for 60 min in a final volume of 0.5 mL. The rate of urate formation from xanthine degradation was determined by measuring the increased absorbance at 290 nm. The activity was expressed as UI/mg of protein.

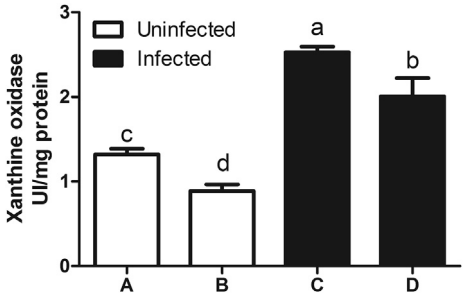

Fig. 1. Hepatic xanthine oxidase (XO) activity. Columns represent mean ± standard deviation (n = 10). Different letters (lowercase) in the same graph denote significance ($P < 0.05$) by two way ANOVA followed by Tukey's posthoc test. Note: the group A (uninfected), the group B (uninfected and treated with $(PhSe)_2$), the group C (infected), and the group D (infected and treated with $(PhSe)_2$).