Experimental Parasitology 181 (2017) 7–13

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Toxoplasmosis treatment with diphenyl diselenide in infected mice modulates the activity of purinergic enzymes and reduces inflammation in spleen

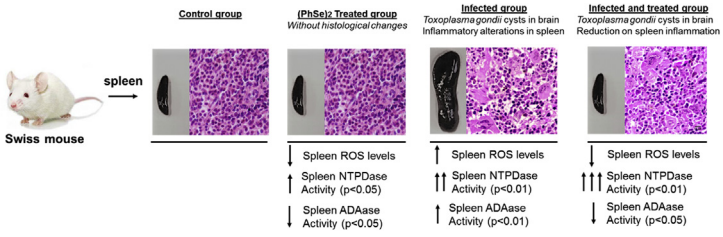
Pedro H. Doleski [a, b], Maura V. Ten Caten [a, b], Daniela F. Passos [a, b], Lívia G. Castilhos [a, b], Daniela B.R. Leal [a, b], Vanessa S. Machado [a], Nathieli B. Bottari [b], Fernanda F. Vogel [c], Ricardo E. Mendes [d], Aleksandro Schafer da Silva [b, e, *]

[a] Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Brazil
[b] Department of Biochemistry and Molecular Biology, Universidade Federal de Santa Maria (UFSM), Brazil
[c] Department of Preventive Veterinary Medicine, Universidade Federal de Santa Maria (UFSM), Brazil
[d] Instituto Federal Catarinense, Concordia, Brazil
[e] Department of Animal Science, Universidade Do Estado de Santa Catarina (UDESC), Brazil

HIGHLIGHTS

- Chronic toxoplasmosis is characterized by the presence of *Toxoplasma gondii* cystys in the brain.
- Diphenyl diselenide is an organoselenium compound with *in vivo* antioxidant and immunomodulatory activities.
- Infected mice showed splenomegaly, elevated ROS levels and nucleotidases activities in the spleen.
- The treatment with diphenyl diselenide induce an anti-inflammatory profile in the nucleotidases activities.
- Nucleotidase activities and elevated ROS levels can be related with the disease pathogenesis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form
20 April 2017
Accepted 8 July 2017
Available online 12 July 2017

Keywords:
Diphenyl diselenide
Oxidative stress
Purinergic enzymes

ABSTRACT

Toxoplasma gondii, an intracellular protozoan, may cause chronic infection in the brain tissue of the host inducing a systemic pro-inflammatory profile. Chronic infections can induce numerous physiological changes, such as alterations in the immune and oxidative profiles. Diphenyl diselenide $(PhSe)_2$, an organoselenium compound, has shown antioxidant and immunomodulatory activities in recent studies. So, the aim of this study was to investigate the activity of purinergic enzymes and reactive oxygen species (ROS) in serum and spleen of mice chronically infected by *T. gondii*, untreated and treated with $(PhSe)_2$. For this experiment, were divided into four groups: Group A (healthy mice), Group B (healthy mice treated with $(PhSe)_2$), Group C (infected mice) and Group D (infected mice treated with $(PhSe)_2$). Group C and group D were infected via oral route with ME49 *Toxoplasma gondii strain*. Groups B and D were treated subcutaneously with 5 μmol kg^{-1} of $(PhSe)_2$. Chronic *T. gondii* infection induced splenomegaly and physiological changes in the spleen and raised histologic inflammatory markers, ROS levels

* Corresponding author. Departamento de Zootecnia, Universidade do Estado de Santa Catarina (UDESC), Rua Beloni Trombeta Zanini, 680E, Bairro Santo Antônio, 89815-630, Chapecó, Santa Catarina, Brazil.
E-mail address: aleksandro_ss@yahoo.com.br (A.S. da Silva).

http://dx.doi.org/10.1016/j.exppara.2017.07.001

Spleen
Toxoplasma gondii

and the activity of purinergic enzymes activity such as NTPDase, 5ńucleotidase and ADA. In serum, the infection increased 5ńucleotidase and ADA activities. $(PhSe)_2$ *per se* has managed to decrease ROS levels and ADA activity and increase NTPDase and 5ńucleotidase in spleen. In infected mice, treatment with $(PhSe)_2$ reversed splenomegaly, reduced histological inflammatory markers, ROS levels and ADA activity in the spleen. Our results prove that chronic toxoplasmosis can induce splenomegaly, heightens ROS levels and purinergic enzyme activity in mice. These results suggest that $(PhSe)_2$ is a potential therapy for the alterations found in the spleen in chronic *T. gondii* infection.

1. Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii,, a* protozoan that infects intestinal cells of felines to complete its biological cycle. Mammals and others warm-blooded animals can also be infected; however, they display a different pathology which will depend on the parasite strain and the immune status of the host (Dubey, 2007). Immunocompetent animals, when infected with avirulent strains of *T. gondii*, experience a short acute phase where the parasite may be either eliminated from the host or quickly form small cysts in the brain tissue. This latent infection by *T. gondii* is denominated chronic toxoplasmosis, and it is considered an asymptomatic disease (Fergunson and Hutchinson, 1987). Some studies reveal that the small brain inflammation connected to the cysts can induce neurological disorders in rodents (Machado et al., 2016) and humans (Ene et al., 2016). Moreover, the infection reduces serum levels of testosterone (Eslamirad et al., 2013) and increases the levels of pro-inflammatory cytokines, as INF-γ, to prevent a reactivation of the parasites (Barbosa et al., 2014). This systemic inflammatory profile may induce physiological changes in the body, as in the liver and spleen; organs that exhibit immune function and display pro-inflammatory cells in *T. gondii* infection (Nam et al., 2011). Spleen has two different tissues; (1) a red pulp that consists in the reticuloendothelial system responsible to remove damaged erythrocytes from the bloodstream, and (2) a white pulp, that act as a peripheral lymph node, and presents important immune functions such as storing lymphocytes and producing platelets. In inflammatory processes, the tissue can induce lymphocytes to produce and release antibodies and cytokines (Kraal and den Haan, 2016). In some pathologies, the immune response can increase the size of the spleen, a condition called splenomegaly, which can result in tissue damage and dysfunction such as spleen enlargement and frailty. (Kraal and den Haan, 2016). Besides the systemic pro-inflammatory profile developed against the parasite, little is known about the status and functions of the spleen in the chronic infection by *T. gondii*.

The interaction of extracellular purines with specific cell receptors is denominated purinergic signaling, which is involved in numerous physiological functions (Ralevic and Burnstock, 1998). Stimulation of leukocytes through P2 purinergic receptors with ATP (adenosine triphosphate) may induce a pro-inflammatory profile in tissues. This interaction can lead to leukocyte activation and increased cell proliferation, reactive oxygen species (ROS) and cytokines production (Burnstock and Verkhratsky, 2010; Kuroki and Minakami, 1989). Therefore, the interaction of adenosine (ADO) with P1 receptors induces the antagonist effects of ATP. ADO is produced after tissue damage in order to reverse the pro-inflammatory profile and recover damaged tissues (Bours et al., 2006; Ward et al., 1988). Purine compounds can be released by cells through membrane channels, which can be stimulated in inflammatory processes (Junger, 2011). To regulate the concentration of purines, cells express nucleotidases, which metabolize phosphate nucleotides such as ATP in their nucleosides derivatives (Zimmermman, 2000). NTPDase is responsible for hydrolyzing ATP and ADP to AMP, while 5'nucleotidase hydrolyzes AMP to the nucleoside ADO. The enzyme responsible for metabolizing ADO is a nucleosidase named ADA (adenosine deaminase), which deaminates ADO into inosine. These enzymes control the levels of purines, playing an important role in physiological processes, and are altered in inflammatory diseases (Schetinger et al., 2007).

Chronic diseases may cause cell damage and oxidative stress, which over time can cause irreversible tissue damage, thus treatments with antioxidant compounds have been proposed to reverse the harm that may occur (Hussain et al., 2016). In this way, the organoselenium compound, Diphenyl diselenide $(PhSe)_2$, which possesses antioxidant and immunomodulatory activities, may be an effective therapy against chronic diseases (Nogueira et al., 2004). Studies reveal that $(PhSe)_2$ can induce an anti-inflammatory profile and protects tissues by reducing pro-inflammatory cytokines and ROS production (Leite et al., 2015; Nogueira and Rocha, 2010). Chronic toxoplasmosis is considered a silent infection in immunocompetent patients, which show no symptoms or signs of major damage to the body in the short term. In this study, we aimed to analyze the activities of purinergic enzymes and the levels of ROS in serum and spleen of immunocompetent mice infected by *T. gondii* strain ME49 to evaluate possible physiological changes induced by the chronic infection with this parasite. Moreover, we present the antioxidant and immunomodulatory compound $(PhSe)_2$ as a potential treatment to the changes caused by this infection.

2. Materials and methods

2.1. Chemicals

Purinergic substrates ATP, ADP, AMP, ADO as well $(PhSe)_2$ were obtained from Sigma Chemical Co. (St. Luis, MO, USA). All other reagents used in the experiments were of analytical grade and of highest purity.

2.2. Toxoplasma gondii strain

Cystogenic ME49 strains of *T. gondii* stored in liquid nitrogen were used to inoculate one single *Swiss* mouse. Thirty-two days after the infection, the mouse was euthanized the brain containing cysts of *T. gondii* were homogenized in saline solution and orally inoculated in another three mice; this procedure was done to reactivate the parasite's virulence. The mice were then euthanized for brain collection 30 days post-infection (PI), and parasitic cysts counted and separated in order to infect the other animals of the experiment.

2.3. Experimental design

For this experiment, we used forty female Swiss mice weighing