Diphenyl diselenide modulates nucleotidases, reducing inflammatory responses in the liver of *Toxoplasma gondii*-infected mice

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Abstract The aim of this study was to verify the effect of diphenyl diselenide (PhSe)₂ on hepatic nucleotidases and on the concentration of purines in mice infected by *Toxoplasma gondii*. The animals were divided into four groups: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂). The inoculation (groups C and D) was performed with 50 cysts of *T. gondii* (ME-49 strain). Mice from groups B and D were treated with 5 μmol kg⁻¹ of (PhSe)₂. Liver tissue from infected mice showed less severe inflammation, elevated ATP/ADO ratio, elevated NTPDase, 5′nucleotidase, and ADA

activities compared to the uninfected group (Group A; $P < 0.05$). However, infected and treated mice showed decreased ATP levels and elevated ADO levels, as well as higher NTPDase and 5′nucleotidase activities and decreased ADA activity in the hepatic tissue compared to the infected group ($P < 0.05$). Moreover, the (PhSe)₂ treatment of infected mice reduced the hepatic inflammation and showed an immunomodulatory effect on ectonucleotidases of hepatic lymphocytes, which it returned to basal levels. Therefore, chronic infection by *T. gondii* induces hepatic inflammation in mice, and it is possible that purine levels and nucleotidase activities in hepatic tissue are related to the pathogenesis of the infection in this tissue. The treatment with (PhSe)₂ was able to reverse the hepatic inflammation in mice chronically infected, possibly due to the modulation of purinergic enzymes that produce an anti-inflammatory profile through the purinergic system in the liver tissue.

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Introduction

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, and felines are the definitive hosts. They may release oocysts of the parasite in the environment, contaminating accidental hosts, as humans and animals [1]. Usually healthy infected hosts do not show clinical signs, but this depends on the host immune response and also the strain of the parasite. The ME-49 strain is known for causing chronic toxoplasmosis, in which the parasite causes the latency form of the disease in the brain tissue [2]. However, studies demonstrated an

association between infection and hepatic inflammation that leads to cirrhosis [3]. The liver plays an important role in controlling many metabolic routes of the body [4]. For protection, the organ has distinct immune cells such as Kupffer and stellate cells and lymphocytes responsible for the maintenance of the immune response in the tissue. A healthy liver has approximately 10^{10} lymphocytes to protect the organ by responding to innumerable inflammatory mediators that induce cytokine release and activation and recruitment of new immune cells [5]. Hepatic pathologies increase the number of lymphocytes that are activated through inflammatory signaling, which induce these cells to release pro-inflammatory cytokines, changing the immune response of this organ [6]. Besides cellular effectors, the immune system has numerous chemical mediators to perform the signaling of the immune response in the body [7].

The purinergic signaling is characterized by extracellular purine nucleotide interactions with specific cell receptors capable of modulating hepatic functions, leading to cell proliferation and vascularization, acting as an immune mediator [8]. When ATP interacts with receptor type P2, it triggers an increase in hepatocyte proliferation, causing vasodilation in sinusoidal vessels and enabling the immune response [9]. On the other hand, when the ATP degradation product adenosine (ADO) interacts with receptor type P1, it induces the release of anti-inflammatory cytokines, enhancing the collagenolytic activity in the liver. In this way, ADO exerts an anti-fibrotic effect and develops a protective cellular environment [9, 10]. Adenosine has a fundamental function in homeostasis of healthy tissue. However, low concentration of this molecule is related to the development of chronic liver diseases [11].

Healthy hepatic cells release low ATP concentrations into the extracellular medium through membrane channels; however, this release is increased after stimuli, such as the interaction with inflammatory mediators, stress, and cell damage. While low ATP levels have important physiological functions, high extracellular concentration of it can act as damage-associated molecular patterns (DAMPs), inducing pro-inflammatory events through lymphocyte activation [12]. Due to these events, the nucleotide concentration in the extracellular space is regulated by nucleotidases, enzymes expressed on a soluble form in the interstitial tissue or bound in the plasma membrane of the cells, so-called ecto-enzymes [13]. This regulation starts with NTPDase that hydrolyses ATP and ADP into AMP. The second phase occurs by 5'nucleotidase, which hydrolyses AMP into Ado. Adenosine deaminase (ADA) is the third regulatory enzyme that deaminates Ado into inosine. Nucleotidase enzymes regulate nucleotide interactions through the increase or decrease of its concentration in the medium, and countless studies have shown the importance of this enzymatic regulation in physiological and pathological processes [14, 15].

Diphenyl diselenide (PhSe_2) is an organoselenium compound with antioxidant, antiviral, and immunomodulatory activities [16]. Several in vivo experiments show that (PhSe_2) reduces the levels of pro-inflammatory cytokines and reactive species in various pathologies; however, the true mechanism for its protective and anti-inflammatory action is still not fully understood [17, 18]. Studies indicated that (PhSe_2) reacts with thiol groups in the tertiary protein structure, and this interaction modulates the enzymatic activity and affinity to substrates, causing alterations on important metabolic pathways, and this is possibly the molecular mechanism of action [19].

In the chronic phase of toxoplasmosis of immunocompetent mice, the parasite is latent in the brain, but the infection is still able to induce damage to other tissues such as the liver. It is possible that components of the purinergic system are involved in the pathology of *T. gondii* infection, whereas the damage caused to the body is due to a pro-inflammatory signaling in non-infected tissues. Therefore, the aim of this study was to investigate the concentration of purines and the activity of nucleotidases in hepatic tissue of mice infected by *T. gondii* treated with diphenyl diselenide (PhSe_2), as well as the effect of this treatment on ectonucleotidase activity in hepatic lymphocytes, which act as a biomarker of the immune response.

Materials and methods

Chemicals

The substrates ATP, ADP, AMP, adenosine, Coomassie Blue, (PhSe_2), and Ficoll-Hypaque (1.077 g/mL) were obtained from Sigma Chemical Co. (St. Luis, MO, USA). All other reagents used in the experiments were of analytical grade with high purity.

Study design

Forty 60-day-old Swiss albino mice, weighing approximately 25–30 g, were used in the experiments. The *T. gondii* cystogenic strain (ME-49) used was maintained by consecutive passages in the brain of mice every 30 days. The animals were divided into four groups with 10 mice each: Group A (uninfected), Group B (uninfected treated with (PhSe_2)), Group C (infected), and Group D (infected treated with (PhSe_2)). Twenty mice (groups C and D) were infected orally with 250 μL of brain homogenate containing 50 parasitic cysts of *T. gondii*. Mice from groups B and D were treated on days 1 and 20 post-infection (PI) with 5 $\mu\text{mol kg}^{-1}$ of (PhSe_2) by subcutaneous injection [19].

Sampling

Thirty days PI, all animals were anesthetized in an isoflurane chamber. For seric markers, blood samples were collected by cardiac puncture without anticoagulant, and sera samples were obtained after centrifugation at 3000 rpm for 15 min. Livers were removed and divided into four parts with approximately 3 g each. Two parts were used to histopathology and lymphocyte isolation. Another part was used for the determination of purine levels, and for that, the sample was gently homogenized in sterile flasks, and the resultant supernatant was mixed with 0.6 M perchloric acid and 1 M potassium hydroxide, as described previously [20]. And, the last 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 removed and used for hepatic lymphocyte isolation. Protein was measured by the Coomassie Blue method according to Bradford [21] using serum albumin as the standard.

Histopathology

At the necropsy, tissue samples were collected from the right hepatic lobes, fixed in 10% buffered formalin in 0.1 M phosphate buffer. The fixed tissue was dehydrated and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) for histopathological examination.

Seric markers of hepatic damage

Seric activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were evaluated using commercial kits (Analisa®).

Hepatic purine levels

ATP and its breakdown products were analyzed by HPLC according to Voelter et al. [21]. The proteins were denaturated using 0.6 mol/L of perchloric acid. All samples were centrifuged (16,000×g for 10 min at 4 °C); supernatants were neutralized with 4.0 N KOH, and clarified with a second centrifugation (16,000×g for 30 min at 4 °C). After the second centrifugation, the supernatants were collected and centrifuged again (16,000×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 × 5 µm, Restek, USA). The elution was carried out applying a linear gradient from 100% of solvent A (60 mM KH₂PO₄ 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). Mobile phases were filtered

through a 0.22-µm Millipore filter prior to analysis, and all the reagents were of HPLC grade. The amount of purines 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.

Isolation of hepatic lymphocytes

Hepatic lymphocytes were isolated from approximately 3 g of liver tissue carefully homogenized in PBS (phosphate buffer solution) and separated on Ficoll-Hypaque density gradients as previously described by Doleski et al. [22].

E-NTPDase activity in hepatic lymphocytes

Activity of E-NTPDase in hepatic lymphocytes was previously described by Doleski et al. [22]. Twenty microliters of intact lymphocytes were added to the reaction medium containing 50 mM Tris–HCl buffer (pH 8.0), supplemented with 0.5 mM CaCl₂, 120 mM NaCl, 5 mM KCl, and 60 mM glucose in a final volume of 200 µL. The reaction was initiated by the addition of the substrate (ATP or ADP) at a final concentration of 2.0 mM in 37 °C and stopped after 40 min of incubation with 200 µL of 10% trichloroacetic acid (TCA). The samples were chilled on ice, and the amount of Pi released was measured by the method of Chan et al. [23]. Enzymatic activities were expressed as nmol of Pi/min/mg protein.

NTPDase and 5′nucleotidase activities in liver homogenates

NTPDase and 5′nucleotidase activities in liver homogenates were determined using the method described by Rosenberg et al. [24], modified by Doleski et al. [25]. First, liver homogenates were centrifuged at 2500 rpm for 10 min to remove possible impurities. The reaction mixture for NTPDase activity contained 1 mM of ATP or ADP as substrate, 5 mM CaCl₂, and 50 mM Tris–HCl (pH 8.0). For 5′nucleotidase, the reaction mixture contained 10 mM of AMP as substrate, 5 mM MgCl₂, and 50 mM Tris–HCl (pH 7.5). The reaction mixtures were incubated with approximately 1.0 mg of homogenized protein at 37 °C for 30 min on a final volume of 0.2 mL. The reaction was stopped by the addition of 200 µL of 10% TCA. The samples were chilled on ice and the amount of Pi liberated was measured by the green malaquite method [23]. Enzymatic activities were expressed as nmol of Pi/min/mg protein.

E-ADA activity in hepatic lymphocytes and homogenates

E-ADA activities were measured spectrophotometrically, based on the direct formation of ammonia produced when the enzyme acts on adenosine [26]. The reaction initiated by the addition of the substrate (adenosine) to a final concentration of 21 mM/L and incubated for 60 min at 37 °C with 25 µL of liver homogenates or intact hepatic lymphocytes.

The reaction was stopped by the addition of 1.5 mL of 106/0.16 mM phenol–nitroprusside to the reaction mixture, which was immediately mixed with 1.5 mL of 125/11 mM alkaline-hypochlorite (sodium hypochlorite). Released ammonia reacts with alkaline-hypochlorite and phenol in the presence of a catalyst-sodium nitroprusside to produce indophenol (a blue color), and the concentration of ammonia is directly proportional to the absorbance of indophenol

Fig. 1 Mice experimentally infected by *Toxoplasma gondii*: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂). Normal architecture in the brain of mice of groups A (photomicrography—**a**) and B (Photomicrography—**b**) showing the corticomedullary region. HE, obj. $\times 10$. In the brain of mice from group C, it was observed intense multivalent mononuclear perivascular cuff (arrowhead) associated with rounded, encapsulated, and eosinophilic structure filled with small granules morphologically compatible with cysts of *T. gondii* (arrow). Note an extensive area of malacia associated with moderate mononuclear inflammatory infiltrate (asterisk) HE, obj. $\times 10$ (photomicrography—**c**). In mice of group D, a cyst containing bradyzoites was observed in the interior, morphologically compatible with *T. gondii* (arrow), associated with mononuclear inflammatory

infiltrate adjacent to the cyst, HE, obj. $\times 20$ (photomicrography—**d**). Liver with normal architecture in animals of groups A (photomicrography—**e**) and B (photomicrography—**f**), HE, obj. $\times 10$. All mice from Group C showed periportal mononuclear inflammatory infiltrate in the liver, HE, obj. $\times 10$ (photomicrography—**g**). In Group D, mice showed mononuclear inflammatory infiltrate and adjacent neutrophils (arrow) which corresponds to 40% of the animals in this group, HE, obj. $\times 10$ (Photomicrography—**h**). Liver with normal architecture in animals of groups A (photomicrography—**i**) and B (photomicrography—**j**), HE, obj. $\times 20$. Liver of animals from Group C showed periportal mononuclear inflammatory infiltrate, HE, obj. $\times 20$ (photomicrography—**k**). Liver with normal architecture in animals of group D (photomicrography—**l**), which corresponds to 60% of the animals in this group, HE, obj. $\times 10$

Table 1 Seric enzymatic activity of hepatic damage markers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) in mice infected by *Toxoplasma gondii* and treated with diphenyl diselenide (PhSe)₂^a

Markers	Group A	Group B	Group C	Group D	P
ALT (U/L)	49.20 ± 2.261	53.74 ± 3.652	70.20 ± 6.948	67.89 ± 8.237	>0.05
AST (U/L)	146.40 ± 16.78	178.70 ± 16.01	189.50 ± 25.44	177.80 ± 13.84	>0.05
LDH (mg/dL)	978.2 ± 117.5	1544 ± 188.7	1420 ± 206.4	1026 ± 95.59	>0.05
ALKP (U/L)	112.0 ± 10.41	150.3 ± 19.45	127.8 ± 26.06	107.7 ± 13.88	>0.05

Results are expressed as mean ± SEM ($n = 10$). Data were analyzed statistically by two-way analysis of variance (ANOVA). Post-hoc comparisons were made using Tukey test, and differences were considered significant at $P < 0.05$. Note: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂)

read at 620 nm. Ammonium sulfate of 75 μM was used as ammonium standard. The value of E-ADA activity was expressed as U/mg of protein.

Statistical analysis

All experiments were performed in triplicate. Data represent mean ± standard deviation. Differences were assessed by two-way analysis of variance (ANOVA), followed by Tukey post-hoc test. The values obtained in the assays were considered statically different for $P < 0.05$.

Results

Histopathology

Animals infected by the strain ME-49 of *T. gondii* showed bristling hair and weight loss (groups C and D), and the infection was confirmed by histopathology, i.e., there were parasitic cysts in the brain of all infected animals in both groups. Mice of groups A and B did not show histological lesions in the liver. All mice of Group C showed mild inflammatory foci in the liver (Fig. 1), and alterations of this kind were observed in only four mice of Group D.

Seric markers of hepatic damage

Seric AST, ALT, LDH, and ALP activities did not show significant difference between groups by two-way ANOVA (Table 1).

Hepatic purine levels

The levels of purine in liver samples are shown in Table 2. The (PhSe)₂ treatment (Group B) caused an increase of 60.8% on ATP concentrations and a decrease of 56% in ADP concentrations in the liver. Moreover, AMP levels did not show difference compared to the control group (Group A), but interestingly, there was an increase of 236% in Ado concentrations. Infected group (Group C) showed a great increase (150%) in ATP concentration and a decrease of 84.6% for ADP, 21% for AMP, and 51.2% for Ado. (PhSe)₂ treatment used in infected mice (Group D) decreased (24.3%) ATP concentration compared to infected mice (Group C), but when compared to the control group (Group A), it showed an increase of 88.8%. Group D showed an increase of 35% for AMP and 81.2% decrease of ADP levels, while Ado concentration did not show significant difference compared to the control or infected groups.

Table 2 Purine concentrations in the liver of mice infected by *Toxoplasma gondii* and treated with diphenyl diselenide (PhSe)₂^a

Purines	Group A	Group B	Group C	Group D
ATP (nmol/g)	1.43 ± 0.67c	2.30 ± 0.93b	3.57 ± 0.47a	2.70 ± 0.23b
ADP (nmol/g)	10.2 ± 3.41a	4.49 ± 1.87b	1.57 ± 0.78c	1.92 ± 1.02c
AMP (nmol/g)	136.3 ± 25.7b	132.1 ± 28.4b	107.5 ± 14.9c	184.1 ± 45.4a
Adenosine (nmol/g)	19.8 ± 4.8b	66.5 ± 14.9a	9.66 ± 3.7c	13.4 ± 5.1bc

Results are expressed as the mean ± SEM ($n = 10$). Data are analyzed statistically by two-way analysis of variance (ANOVA). Post-hoc comparisons were made using Tukey test, and differences were considered significant at a $P < 0.05$. Groups with equal letters in the same line do not differ statistically. Note: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂)

Fig. 2 Mice infected by *Toxoplasma gondii* and treated with diphenyl diselenide (PhSe)₂. Hepatic lymphocyte E-NTPDase activity to ATP (a) and ADP (b) substrates. Columns represent mean $\pm$ SEM ($n = 10$). Different letters in the same graph denote significance for $P < 0.05$ by two-way ANOVA followed by Tukey post-hoc test. Note: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂)

E-nucleotidases in hepatic lymphocytes

ATP and ADP hydrolysis by E-NTPDase in plasma membrane of hepatic lymphocyte samples are shown in Fig. 2. E-NTPDase activity for ATP substrate decreased 22%, and it did not show significant difference for ADP substrate in Group B. Animals of Group C showed an increase of 49.5% and 56.6% in E-NTPDase activity for ATP and ADP compared to uninfected animals (Group A), respectively. Mice of Group D did not show significant differences on E-NTPDase activity for

Fig. 3 Mice infected by *Toxoplasma gondii* and treated with diphenyl diselenide (PhSe)₂. Hepatic lymphocyte E-ADA activity to adenosine (Ado) substrate. Columns represent mean $\pm$ SEM ($n = 10$). Different letters in the same graph denote significance for $P < 0.05$ by two-way ANOVA followed by Tukey post-hoc test. Note: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂)

both substrates (ATP and ADP) compared to the control group (Group A).

E-ADA activity in the plasma membrane of lymphocytes is shown in Fig. 3. Mice of Group B did not show significant difference for E-ADA activity compared to Group A, but the infected group (the Group C) showed a decrease of 70.6% in this enzyme. Group D (infected and treated with (PhSe)₂) did not show a significant difference compared with Group A.

Hepatic nucleotidases

Nucleotidase activities in liver homogenates are shown in Table 3. NTPDase activities in Group B showed an increase of 46.52 and 46.27% to ATP and ADP hydrolysis, respectively. There was an increase of 26% to AMP hydrolysis by 5' nucleotidase activity and a decrease of 44.4% in ADA activity compared to the control (Group A). Infected animals (Group C) showed increased nucleotidase activities such as NTPDase (37.3 and 35.2% to ATP and ADP hydrolysis, respectively) and 5' nucleotidase (44.8% to AMP hydrolysis), and ADA activity (50.5%) compared to Group A. However, (PhSe)₂ treatment of infected mice (Group D) increased NTPDase activity (93.2 and 89.6% in ATP and ADP hydrolysis, respectively), as well as 5' nucleotidase activity (57.6%). ADA activity did not show significant differences compared to uninfected (Group A) or infected animals (Group C).

Discussion

T. gondii infection was confirmed by histopathological examinations of the brain that showed parasitic cysts with bradyzoites. These findings are common for *T. gondii* ME-49 strain, since it develops chronic toxoplasmosis during the parasitic latent phase [1, 2]. Histological examinations of the liver of infected animals revealed a slight inflammation and (PhSe)₂ treatment it in 60% of the animals. Serological analyses for markers of hepatic injury (ALT, AST, LDH, ALP) did not confirm tissue damage, possibly due to a slight inflammatory process histologically found, which did not cause cell lysis, and consequently, the release of such markers in the bloodstream.

Firstly, it is worthy to note that (PhSe)₂ is a Janus-faced molecule, so its pharmacological effect or toxicity depends on the concentration used. Treatment with (PhSe)₂ in healthy mice caused ATP increase compared to healthy untreated animals. The mechanism to explain this result is unknown; however, it is possible that the (PhSe)₂ stimulates a constant release of ATP by hepatic cells, and by a compensatory effect, it raises hepatic NTPDase activity in order to control the levels of this phosphate nucleotide. The powerful anti-inflammatory action of (PhSe)₂ was confirmed on the purinergic system by a significant increase in adenosine concentration in liver tissue, an important

Table 3 Hepatic NTPDase (nmol of Pi/min/mg protein), 5' nucleotidase (nmol of Pi/min/mg protein), and ADA (U/mg of protein) activity in infected mice by *Toxoplasma gondii* and treated with diphenyl diselenide (PhSe)₂^a

Nucleotidases	Group A	Group B	Group C	Group D
NTPDase activity				
ATP substratum	5.341 ± 0.735c	7.826 ± 0.555b	7.334 ± 0.178b	10.320 ± 0.420a
ADP substratum	5.469 ± 0.572c	8.003 ± 0.559b	7.395 ± 0.256b	10.370 ± 0.354a
5'nucleotidase activity				
AMP substratum	5.362 ± 0.203c	6.763 ± 0.428b	7.766 ± 0.127ab	8.449 ± 0.143a
ADA activity				
Ade substratum	1.285 ± 0.108b	0.714 ± 0.093c	1.934 ± 0.079a	1.641 ± 0.190ab

Results are expressed as mean ± SEM ($n = 10$). Data were analyzed statistically by two-way analysis of variance (ANOVA). Post-hoc comparisons were made using Tukey's test and difference were considered significant at $P < 0.05$. Groups with letters equal the same line not statistically different. Note: Group A (uninfected), Group B (uninfected and treated with (PhSe)₂), Group C (infected), and Group D (infected and treated with (PhSe)₂)

endogenous anti-inflammatory molecule [14], which may be associated with the removal of inflammatory infiltrates in liver found in the majority of treated animals. The mechanism for this effect was associated with an increase in 5'nucleotidase activity and decrease in the ADA activity, stimulating the production and inhibition of adenosine degradation. It is known that the (PhSe)₂ is able to interact with thiol groups, altering many metabolic processes, including enzymatic activity and its affinity for substrates, which can act as a possible pharmacological mechanism [19]. Our hypothesis is that the (PhSe)₂ acts by two ways: (1) increasing the concentration of nucleotides, such as ATP, and modulating by a compensatory manner the enzymatic activity or (2) interacting covalently with the enzymes in question, producing an anti-inflammatory effect through the purinergic signaling pathway. To evaluate the effect of (PhSe)₂ on modulating the immune response, ectonucleotidases were evaluated in hepatic lymphocytes since they act as biomarkers of the immune response, i.e., enzymatic activity in these cells depends on the immune microenvironment. Liver lymphocytes showed a low decrease in the E-NTPDase activity after (PhSe)₂ treatment of healthy mice, possibly by stimulating the interaction of ATP with P2 receptors, enhancing the immune activity in a controlled manner, which demonstrates an immunomodulatory effect of (PhSe)₂ [12].

Infected mice showed the highest concentration of ATP, and it is known that high ATP levels are usually presented as a DAMP related to the inflammatory process in the tissue [9]. It is possible that mediators present after *T. gondii* infection stimulate a cellular release of ATP by transport channels, in order to perform a local pro-inflammatory signaling. In an attempt to reduce high ATP levels, the tissue shows a large increase in NTPDase activity, although it is still unable to reduce exposure to the nucleotide. It is also possible to assure that *T. gondii* infection may increase all nucleotidases analyzed in this study, such as ADA and 5' nucleotidases, which in turn eventually reduces the concentration of AMP and Ado in the tissue. The nucleoside Ado acts to protect and reverse liver damage, but low interaction

of this molecule with the P1 receptor is associated with the development of chronic liver diseases [10]. Ectonucleotidase activity in lymphocytes helps to understand this complex mechanism, since increased E-NTPDase activity controls high ATP interaction with P2 receptors in the cell membrane, and decreased E-ADA activity stimulates the interaction of low Ado concentration with its P1 receptors. In this way, lymphocytes try to avoid an exacerbated pro-inflammatory activation triggered by ATP.

Interestingly, (PhSe)₂ treatment of infected mice showed a reduction in ATP concentration in liver homogenates. We propose that this effect is related to the high NTPDase activity found in the hepatic tissue of this group, which is enhanced by (PhSe)₂ compared to infected untreated mice and, consequently, reduced the nucleotide levels in the tissue. Furthermore, the consecutive increase in NTPDase and 5'nucleotidase activities along with decreased ADA activity in the tissue resulted in increased adenosine concentration, and possibly, increased interaction with P1 receptors, which may protect the hepatic tissue. This low-ratio ATP/adenosine helps to understand the anti-inflammatory mechanism produced by (PhSe)₂, which is known to produce many benefits in vivo [16]. (PhSe)₂ used to treat *T. gondii* infection was also able to modulate ectonucleotidase in lymphocytes to baseline levels, and thus, this fact can be interpreted as a reduction in markers of the immune response due to the re-establishment of the extracellular environment of nucleotides.

Our results demonstrated a change in some components of the purinergic signaling system in the liver of *T. gondii*-infected mice, as well as signs of healing through subcutaneous treatment with (PhSe)₂. These results help to understand that some mechanisms involved in fighting the infection can be dangerous to the body itself, where the high ratio of ATP/adenosine found in infected mice elucidates one possible mechanism that leads to chronic liver disease in patients with chronic toxoplasmosis. We emphasize that (PhSe)₂ treatment has high capacity to elevate adenosine concentration in healthy liver tissue, as well as to induce a healthy extracellular nucleotide environment in an

inflammatory process. For this, (PhSe)₂ is a potential drug that can be used to treat chronic liver diseases; however, more studies should be conducted to evaluate its clinical efficacy for chronic diseases.

Compliance with ethical standards

Conflicts of interest Pedro H. Doleski declares that he has no conflict of interest.

Daniela B.R. Leal declares that she has no conflict of interest.
Vanessa S. Machado declares that she has no conflict of interest.
Nathieli B. Bottari declares that she has no conflict of interest.
Alessandra G. Manzoni declares that she has no conflict of interest.
Emerson A. Casali declares that he has no conflict of interest.
Cesar E.J. Moritz declares that he has no conflict of interest.
Ana C.A. Rocha declares that she has no conflict of interest.
Giovana Camillo declares that she has no conflict of interest.
Fernanda F. Vogel declares that she has no conflict of interest.
Lenita M. Stefani declares that she has no conflict of interest.
Ricardo E. Mendes declares that he has no conflict of interest.
Aleksandro Schafer da Silva declares that he has no conflict of interest.

Ethical approval This study was approved by the Ethic Committee on Animal Use of the Federal University of Santa Maria (UFSM) under protocol number 7787270815.

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