



Azithromycin and spiramycin induce anti-inflammatory response in human trophoblastic (BeWo) cells infected by *Toxoplasma gondii* but are able to control infection

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ARTICLE INFO

Article history:

Accepted 24 August 2011

Keywords:

Azithromycin
Spiramycin
BeWo cells
Cytokines
Toxoplasma gondii

ABSTRACT

Toxoplasma gondii is an important pathogen which may cause fetal infection if primary infection. Our previous studies have used human choriocarcinoma trophoblastic cells (BeWo cell line) as experimental model of *T. gondii* infection involving placental microenvironment. This study aimed to examine the effects of azithromycin and spiramycin against *T. gondii* infection in BeWo cells. Cells were treated with different concentrations of the macrolide antibiotics and analyzed first for cell viability using thiazolyl blue tetrazole (MTT) assay. As cell viability was significantly decreased with drug concentrations higher than 400 µg/mL, the concentration range used in further experiments was from 50 to 400 µg/mL. The number of infected cells and intracellular replication of *T. gondii* decreased after treatment with each drug. The infection induced up-regulation of the macrophage migration inhibitory factor (MIF), which was also enhanced in infected cells after treatment with azithromycin, but not with spiramycin. Analysis of the cytokine profile showed increase TNF- α , IL-10 and IL-4 production, but decreased IFN- γ levels, were detected in infected cells and treated with each drug. In conclusion, treatment of human trophoblastic BeWo cells with azithromycin or spiramycin is able to control the infection and replication of *T. gondii*. In addition, treatment with these macrolides, especially with azithromycin induces an anti-inflammatory response and high MIF production, which can be important for the establishment and maintenance of a viable pregnancy during *T. gondii* infection.

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1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects a wide range of hosts. In humans, toxoplasmosis is associated with severe congenital defects when primary infection is acquired during the first trimester of pregnancy [1].

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Infection induces a Th1 response with production of proinflammatory cytokines, such as IL-12 and IFN- γ [2]. During pregnancy, however, there is an induction for type-2 immune response. While a type-2 response is compatible with successful pregnancy, a parasite-induced type-1 response may cause abortion by altering the cytokine profile of maternal-fetal interface. On the other hand, the ability of pregnant host to downmodulate immune response may interfere with mechanisms that normally control parasite multiplication and can result in congenital infection [3].

Current toxoplasmosis in pregnant is based on the administration of spiramycin, which reaches high concentrations in placental tissue, thus decreasing the risk of fetal transmission [3]. However, if fetal infection occurs, a combination of pyrimethamine with sulphadiazine is usually indicated [3]. Disadvantages of these drugs are related to limited effectiveness in parasite clearance and low penetration in fetal tissues for spiramycin and numerous toxicity problems for sulphadiazine-pyrimethamine, in

addition to the potential teratogenic effect of pyrimethamine in pregnancy first trimester [4]. Azithromycin is also a macrolide, with advantages such as high oral bioavailability, rapid cell absorption, and administration only once a day [5]. Azithromycin has better pharmacokinetics and reaches greater tissue concentrations than spiramycin, and has also shown a lower incidence of side effects, especially hepatotoxic effects [4]. Our previous studies have shown that azithromycin treatment in pregnant *Calomys callosus* females was effective in inhibiting vertical transmission of *T. gondii*, suggesting that it may be an alternative drug for the prevention of congenital infection [6]. Macrolide antibiotics have anti-inflammatory properties by modulating the production of proinflammatory cytokines [7] that are preferentially produced in *T. gondii* infection.

Clearly, placenta plays a major role in fetal protection, and understanding this function is crucial for congenital toxoplasmosis prevention. Previous studies have shown that placenta trophoblasts are directly involved in the pathogenesis of congenital toxoplasmosis [8]. The role of trophoblast cells in the immunology of pregnancy, especially in the presence of infection by intracellular parasites, has been studied using well-established cell lines, such as human BeWo choriocarcinoma cells [9]. These cells have morphological properties common to normal trophoblasts, including secretion of cytokines as IL-4, IL-6, IL-8, IL-10, TNF- α and macrophage migration inhibitory factor (MIF) [10].

Even though macrolide treatment has been effective against *T. gondii* in vitro and in vivo models [4–6], there are few data involving its use in maternal-fetal interface and congenital toxoplasmosis. In this context, the present study aimed to verify the effect of azithromycin and spiramycin against *T. gondii* infection in human trophoblastic BeWo cells, by analyzing cell viability, rates of infection and parasite replication, and cytokine profiles.

2. Materials and methods

2.1. Cell culture

BeWo cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in RPMI-1640 medium (GIBCO, Paisley, UK), supplemented with 25 mM HEPES, 2 mM L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin (all reagents from Sigma Chemical Co., St Louis, MO, USA) and 10% heat-inactivated fetal calf serum (complete medium) (Cultilab, Campinas, Brazil). Cells were grown at 37 °C with 5%CO₂ in fully humidified air.

2.2. Parasite

Tachyzoites of *T. gondii* RH strain were obtained initially from peritoneal exudates of previously infected Swiss mice [11] and maintained by serial passages in BeWo cells in order to obtain in vitro tachyzoites for further infection experiments as previously described [9]. Briefly, tachyzoites were harvested by scraping off the cell monolayer after 2–3 days of infection, passed through a 26-gauge needle to lyse any remaining intact host cells, washed (720 g, 5 min) in RPMI medium and the resulting pellet was resuspended in complete medium. Parasites were stained with 0.4% Trypan blue and counted in a hemocytometric chamber for infection experiments.

2.3. Antibiotics

Macrolides azithromycin (Biofarma, Uberlândia, Brazil) and spiramycin (Sigma Chemical Co.) were dissolved in sterile water to a concentration of 3000 μ g/mL (stock solution). Stock solution was freshly reconstituted and different macrolide concentrations were used for cell treatment.

2.4. MTT assay

Cell viability was evaluated by colorimetric assay using thiazolyl blue tetrazole (MTT) assay (Sigma Chemical Co.) as previously described [12]. BeWo cells were cultured in 96-well plates (5×10^4 cells/200 μ L/well) in complete medium for 24 h at 37 °C and 5%CO₂. Cells were treated with different concentrations of azithromycin or spiramycin, and after 24 h of treatment, cells were pulsed with MTT at 0.5 mg/mL 4 h prior to the end of the culture. Formazan particles were solubilized in 10% sodium dodecyl sulfate (SDS) and 50% N, N-dimethyl formamide. The optical density was determined at 570 nm in a plate reader (Titertek Multiskan Plus, Flow Laboratories,

Mc Lean, USA). Results were expressed as percentage of viable cells in relation to controls (100% of cell viability).

2.5. Infection and intracellular replication of parasite

BeWo cells were cultured on 13-mm round glass coverslips into 24-well plates (5×10^4 cells/200 μ L/well) in complete medium for 24 h at 37 °C and 5%CO₂. Cells were washed with medium and infected with *T. gondii* tachyzoites (host cell:tachyzoites, 1:3) in complete medium. After 24 h, cells were washed again with medium and incubated with 200 μ L/well of an antibiotic solution containing azithromycin or spiramycin in different concentrations (50, 100, 200 and 400 μ g/mL) at 37 °C and 5%CO₂ for 24 h. As controls, non-infected non-treated cells (control) or infected non-treated cells (Tg control) were kept with medium alone. After 24 h, cell-free supernatants were collected and stored at –70 °C for further cytokine assay. Cells were then washed with sterile phosphate-buffered saline (PBS), fixed in 10% phosphate-buffered formalin for 2 h and stained with 1% toluidine blue (Sigma Chemical Co.) for 3 s. Coverslips were mounted on glass slides and cells were examined under a light microscope with regards to the index of infection (percentage of infected cells per 200 examined cells) and parasite intracellular replication (number of parasites per cell in 200 infected cells) [9]. Three independent experiments were performed in triplicate for each condition.

2.6. Cytokine measurement in *T. gondii*-infected BeWo cells

2.6.1. Measurement of MIF by enzyme-linked immunosorbent assay (ELISA)

MIF was measured using a sandwich ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Briefly, plates were coated overnight with capture monoclonal anti-human MIF antibody, blocked and incubated with samples in duplicate for 2 h at room temperature. After washing, plates were incubated with biotinylated polyclonal anti-human MIF antibody for 2 h at room temperature. The assay was developed with streptavidin-horseradish peroxidase (Zymed, San Francisco, CA, USA) and revealed with 3,3',5,5'-tetramethylbenzidine (TMB, Zymed). MIF concentration was determined by extrapolation from a standard curve obtained from known concentrations of rMIF cytokine standard. The assay sensitivity was 18 pg/mL. Intra- and inter-assay coefficients of variation were 3.86% and 9.14%, respectively.

2.6.2. Measurement of Th1/Th2 cytokines by cytometric bead array (CBA)

Human cytokines (IL-2, IL-4, IL-5, IL-10, IFN- γ and TNF- α) were measured using cytometric bead array™ (CBA; BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, samples were mixed with mix of each human cytokine capture bead and incubated with PE-conjugated anti-human IL-2, IL-4, IL-5, IL-10, IFN- γ and TNF- α antibodies for 3 h at room temperature, protected from light. After centrifugation, supernatants were carefully aspirated and discarded. The bead pellets were resuspended and samples were analyzed under BD™ flow cytometry (FACSCalibur, BD Company, San Diego, CA, USA). Data were analyzed using specialized software BD™ Cell Quest and CBA software. Results are presented as mean fluorescence intensity (MFI) of all cytokines.

2.7. Data analysis

Statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, USA). Data were expressed as mean \pm standard deviation of three independent experiments performed in triplicate. Data comparisons in relation to control were performed by one-way ANOVA and Dunnett post hoc test. Comparisons of data between treated cells with azithromycin and spiramycin were performed using the Student's *t* test. Differences were considered statistically significant if a *P* value < 0.05 was achieved.

3. Results

3.1. BeWo cells maintain viability after treatment with azithromycin or spiramycin

The number of viable BeWo cells after treatment with different concentrations of azithromycin or spiramycin was evaluated using the MTT assay (Fig. 1). No significant difference in cell viability was observed when BeWo cells were treated with azithromycin in concentrations from 50 to 400 μ g/mL compared to non-treated controls, whereas higher concentrations (600 and 800 μ g/mL) exhibited significant reduction in cell viability (Fig. 1A). For spiramycin, viability of BeWo cells decreased only at the highest concentration (800 μ g/mL) compared to non-treated controls (*P* < 0.05) (Fig. 1B). Based on these observations, the subsequent

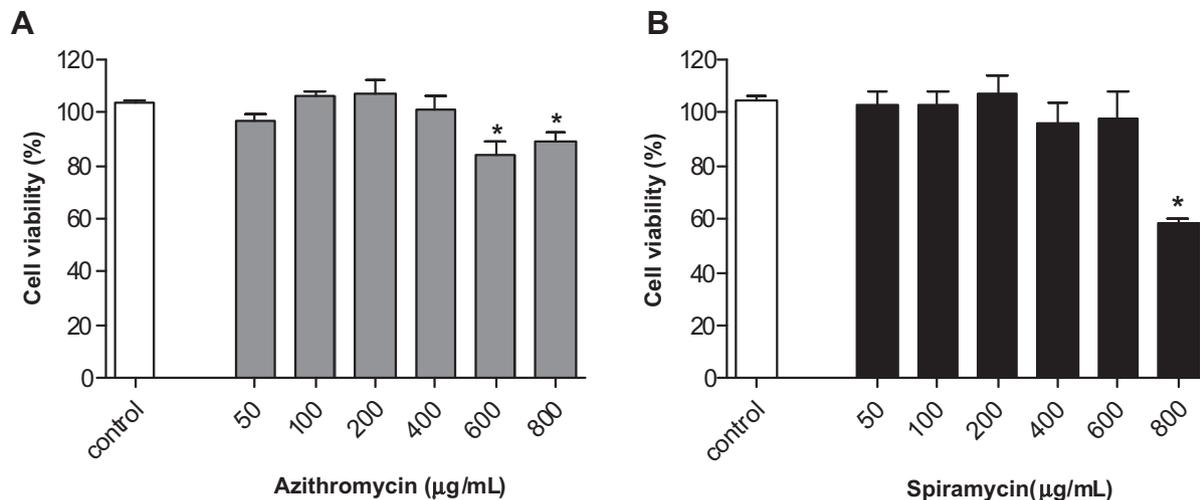


Fig. 1. Cell viability of BeWo cells after incubation with increasing concentrations of azithromycin (A) and spiramycin (B). Cells were cultured in 96-well plates (5×10^4 cells/200 µL/well) and after 24 h were treated with different concentrations of azithromycin or spiramycin or kept with medium (control) at 37 °C and 5%CO₂. Viable cell numbers were analyzed using thiazolyl blue tetrazole (MTT) after 24 h treatment. Data are expressed as the percentage of viable cells in relation to the control (mean ± S.D.) and are representative of two independent experiments in triplicate. * $P < 0.05$ (ANOVA and Dunnett multiple comparison post-test).

experiments were performed using macrolide antibiotics in the range from 50 to 400 µg/mL.

3.2. Macrolides decrease infection with *T. gondii* in BeWo cells

In order to verify if the drugs had effect on *T. gondii* control in BeWo cells, indexes of infection and parasite intracellular replication were evaluated (Fig. 2). Cell treatment with either azithromycin or spiramycin significantly reduced the infection index in BeWo cells, showing a dose-response effect rather than the other way round, with the higher reduction seen for the highest drug concentration (Fig. 2A). Comparison of the treatments showed a higher infection index in cells treated with spiramycin at concentrations of 50 and 400 µg/mL ($P < 0.05$) (Fig. 2A). Regarding the index of parasite replication, cell treatment with either dose of azithromycin significantly decreased the number of intracellular parasites compared with non-treated cells (Fig. 2B). Comparison between the drugs showed a higher number of intracellular parasites when BeWo cells were treated with spiramycin in the lowest concentrations (50 and 100 µg/mL) ($P < 0.05$) (Fig. 2B).

The effect of drugs in parasite replication was also evaluated by *T. gondii* growth inhibition as shown in Table 1. The treatment with increasing concentrations of azithromycin resulted in significant inhibition of tachyzoite growth from 13 to 41% ($P < 0.05$). The inhibitory effect of spiramycin on *T. gondii* growth was observed only at concentrations of 200 and 400 µg/mL, with inhibition of 24% and 31%, respectively. Photomicrographs of BeWo cells showed a greater number of *T. gondii* infected cells in those that were not treated (Fig. 3A) as compared to those treated with azithromycin (Fig. 3B,C) or spiramycin (Fig. 3D).

3.3. MIF production is enhanced in *T. gondii*-infected BeWo cells after azitromycin treatment

Non-infected BeWo cells showed significant increase in MIF production after treatment with azithromycin (100 and 200 µg/mL) and with any concentration of spiramycin as compared with non-treated control (Fig. 4A). Comparison of the treatments showed higher MIF production for spiramycin than azitromycin only in the highest concentration (400 µg/mL) ($P < 0.05$) (Fig. 4A). MIF secretion was increased after *T. gondii* infection and treatment with

azithromycin in any concentration analyzed ($P < 0.05$) (Fig. 4B). In contrast, no significant differences were observed when cells were infected and treated with spiramycin for any concentration (Fig. 4B). Also, *T. gondii* infection increased MIF production in BeWo cells (Tg control) compared with non-infected control ($P < 0.05$).

3.4. Macrolide treatment in *T. gondii*-infected BeWo cells induces decreased IFN-γ production

Cytokine profile was evaluated in culture supernatants from BeWo cells infected or not with *T. gondii* after treatment with azithromycin or spiramycin in different concentrations (Fig. 5). Treatment of non-infected BeWo cells with any concentrations of azithromycin or spiramycin (50–400 µg/mL) did not alter the TNF-α or IFN-γ production when compared to non-treated control cells (Fig. 5A,C). In contrast, treatment of infected cells with azithromycin (100 and 200 µg/mL) or spiramycin (400 µg/mL) slightly increased TNF-α production compared with non-treated infected cells ($P < 0.05$) (Fig. 5B). Comparison of the treatments showed a significantly higher production of TNF-α in cells treated with 200 µg/mL of azithromycin or 400 µg/mL of spiramycin (Fig. 5B). Interestingly, both macrolides induced decreased IFN-γ production in infected BeWo cells compared to non-treated infected cells, irrespective of the drug concentration used ($P < 0.05$) (Fig. 5D), and the comparison of the treatments showed no significant differences. In addition, no change was observed in IL-2 production from BeWo cells treated with either drug compared with non-treated control or from infected treated BeWo cells compared with non-treated infected control (data not shown).

3.5. Macrolide treatment in *T. gondii*-infected BeWo cells induces an anti-inflammatory response

Analyses of the anti-inflammatory cytokine levels showed that IL-10 levels decreased in non-infected cells and treated with azithromycin at 200 and 400 µg/mL, as well as in cells treated with spiramycin at any concentration ($P < 0.05$) (Fig. 5E). After infection, azithromycin at 50 and 400 µg/mL and spiramycin at 100 µg/mL significantly increased the IL-10 production compared to non-treated infected control (Fig. 5F), and no significant differences were found between the treatments. IL-4 production was not

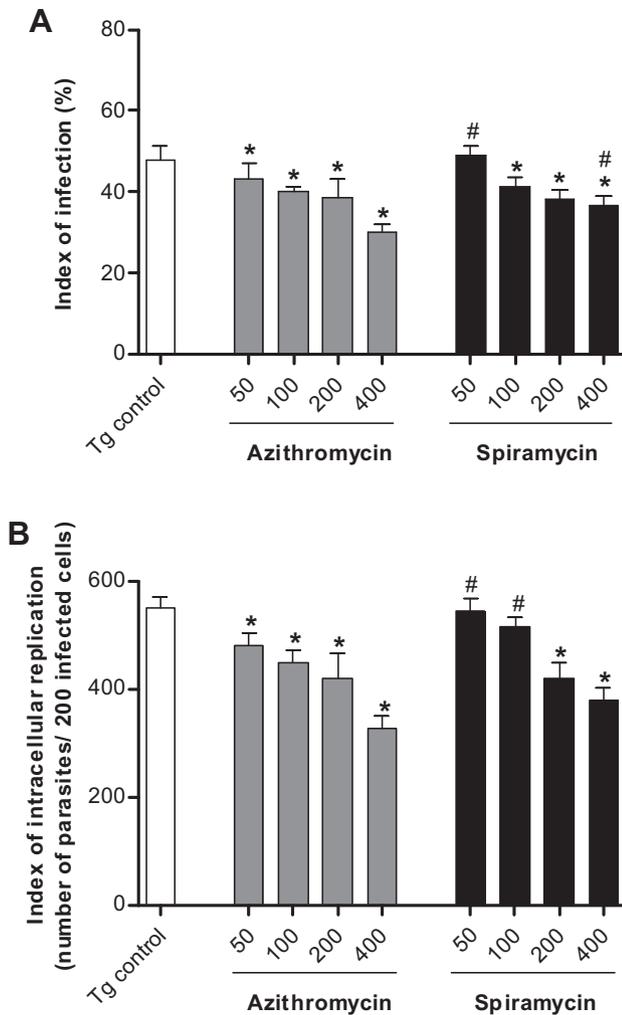


Fig. 2. *Toxoplasma gondii* infection and intracellular replication in BeWo cells without and with macrolide treatments. Cells were cultured on coverslips into 24-well plates and infected with *T. gondii* (host cell:tachyzoites, 1:3) for 24 h. Infected cells were treated with increasing concentrations of azithromycin or spiramycin or medium alone (Tg control). After 24 h, cells were washed, fixed, stained with toluidine blue and analyzed. (A), percentage of infected cells per 200 examined cells; (B), number of parasites per cell in 200 infected cells. Data are expressed as mean ± S.D and are representative of three independent experiments in triplicate. *Comparison between infected/treated cells and control for each treatment condition (ANOVA and Dunnett multiple comparison post-test, $P < 0.05$). [#]Comparison between BeWo cells treated with azithromycin and spiramycin in each drug concentration (Student's *t* test, $P < 0.05$).

changed when non-infected cells were treated with either drug (Fig. 5G), but when infected cells were treated, it was observed an increase of IL-4 at 100, 200 and 400 µg/mL of azithromycin and in all concentrations of spiramycin ($P < 0.05$) (Fig. 5H). In addition, higher IL-4 levels were detected when infected cells were treated with spiramycin at 50 µg/mL compared to ones treated with azithromycin at the same concentration (Fig. 5H). No significant difference in IL-5 production was observed when cells were treated with each drug irrespective of whether the cells were infected or not (data not shown).

4. Discussion

Although the biology and physiology of *T. gondii* is widely investigated, the current therapies for toxoplasmosis present still limited efficacy due to their substantial side effects as high toxicity and/or drug resistance [5]. Macrolides are a well-

Table 1
T. gondii tachyzoite growth inhibition in BeWo cells infected and treated with increasing concentrations of azithromycin or spiramycin.

Drugs	Intracellular replication index ^a	Inhibition of tachyzoite growth (%)
Control (without treatment)	551.2 ± 19.7	–
Azithromycin (µg/mL)		
50	479.4 ± 24.4*	13.0
100	418.8 ± 46.1*	24.0
200	447.6 ± 22.9*	18.9
400	326.5 ± 22.1*	40.8
Spiramycin (µg/mL)		
50	544.8 ± 22.3 [#]	1.2
100	514.2 ± 19.2 [#]	6.7
200	419.3 ± 28.5*	23.9
400	378.0 ± 25.0*	31.4

*Statistically significant differences in relation to control ($P < 0.05$); [#]Statistically significant differences when comparing azithromycin and spiramycin concentrations ($P < 0.05$).

^a Index of intracellular replication was calculated as the number of parasites/200 infected cells and expressed in mean ± SEM from three independent experiments in triplicate.

established class of antimicrobial agents that play an important role in the management of infectious diseases [13]. However, previous studies comparing azithromycin and spiramycin toxicity showed that they displayed inhibitory activity against *T. gondii* only at concentrations that were toxic to host cells and therefore were considered unsuitable for the therapy [14]. Hepatotoxic effects of macrolides were compared and demonstrated that azithromycin was found to be less toxic for a cultured human liver cell line [15]. In endothelial cell line and fibroblasts, azithromycin had toxic effect for endothelial cell in concentration above of 40 mg/L and for fibroblasts above of 80 mg/L [16], showing that cytotoxicity is related to cell types. In the present study, BeWo cells showed high viability even in the presence of 5–10-fold increased concentrations of azithromycin, making possible to use this trophoblast cell line as in vitro models to evaluate therapies for *T. gondii* infection.

Treatment with azithromycin and spiramycin reduced *T. gondii* infection and parasite replication in BeWo cells, especially when azithromycin was used. In vitro assays showed that azithromycin was the most active macrolide against *T. gondii* and was the only that demonstrated a prolonged inhibitory activity against the parasite after removal of drug [14]. It was also demonstrated that the anti-*T. gondii* effect of azithromycin is due to its ability to effectively inhibit the replication of the parasite [17]. It is known that some macrolides, such as azithromycin, penetrate and accumulate more readily than others in phagocytes [18] and human fibroblasts [19]. This may also be true for trophoblast cell types like BeWo cells and may account for the greater inhibitory activity of azithromycin against intracellular *T. gondii* growth.

Proinflammatory cytokines and mediators play a central role in initiating and propagating the acute and chronic inflammatory processes. There is evidence that macrolide antibiotics exerted their anti-inflammatory effects through modulation of inflammatory cascade [20]. In the present study, we showed that MIF was spontaneously secreted by BeWo cells, and when cells were treated with azithromycin or spiramycin, MIF production was increased. After *T. gondii* infection, however, only treatment with azithromycin was able to upregulate MIF production, indicating a possible interaction between azithromycin and MIF in the control of *T. gondii* infection. MIF is considered a major regulator of inflammatory responses, since it is critical for host defense against

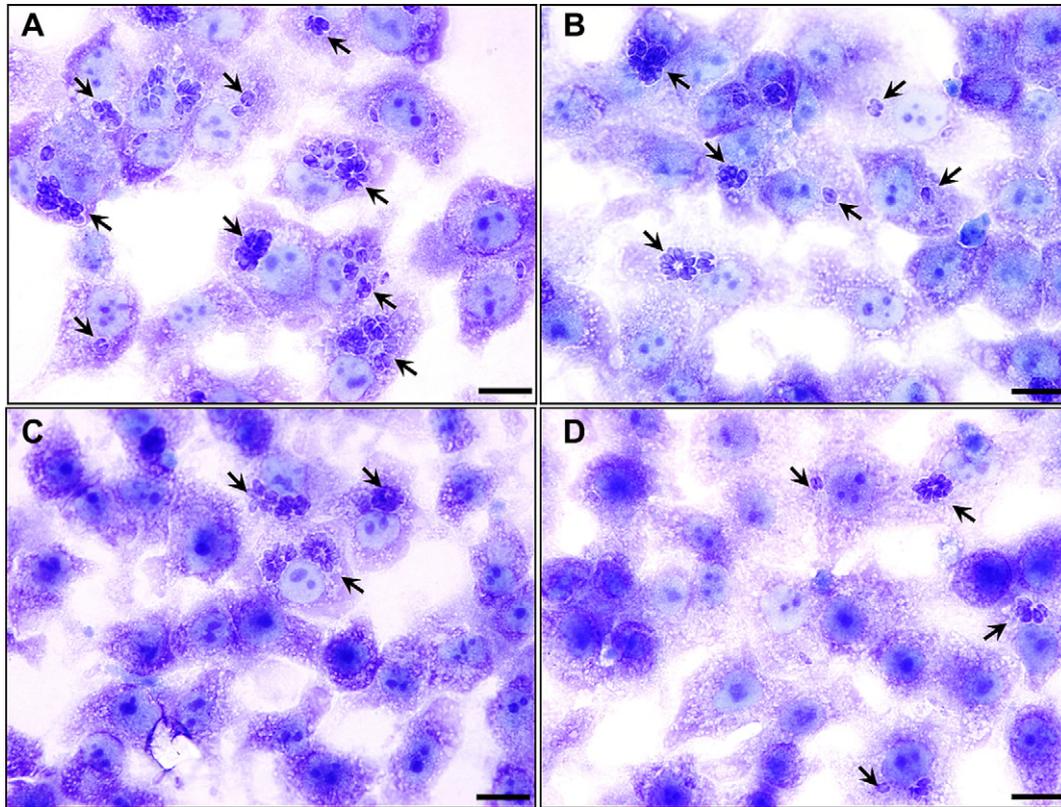


Fig. 3. Photomicrographs of BeWo cells after infection *Toxoplasma gondii* and treatment with azithromycin or spiramycin. Arrows indicate parasites inside the parasitophorous vacuoles. Note higher number of infected cells in non-treated cells (A) and the lower number of infected cells after treatment with 100 µg/mL (B) or 400 µg/mL (C) of azithromycin, and 400 µg/mL (D) of spiramycin. Toluidine blue staining. Bar scale: 33 µm.

intracellular parasites as *T. gondii* [21]. In a previous study, MIF production and secretion by human villous explants were strongly associated with stimulation by soluble *Toxoplasma* antigen (STAg) [22]. Recently, our group demonstrated that MIF was up-regulated in first-trimester explants, and is important to *T. gondii* infection control; whereas lack of MIF up-regulation in third-trimester placentas may be involved in higher susceptibility to infection at this gestational age [23].

Protective immune responses against intracellular microorganisms during pregnancy are downregulated by induction of tolerance against the semi-allogeneic fetus present in the uterus [1,2]. In this study, BeWo cells infected with *T. gondii* and treated with increasing concentrations of azithromycin or spiramycin showed a biased anti-inflammatory immune response, since both drugs were able to increase production of IL-4 and decreased IFN-γ, in addition to a slight increase in IL-10 levels. Since IL-10 and IL-4 can

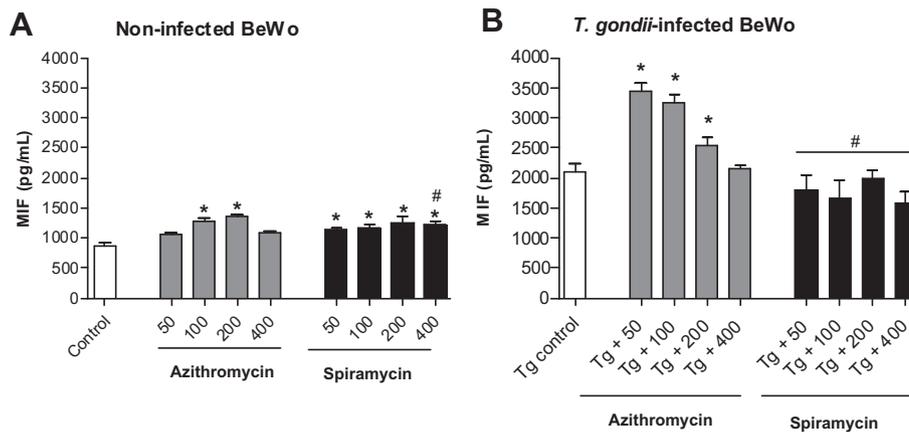


Fig. 4. MIF production by BeWo cells after *T. gondii* infection and treatment with different concentrations of azithromycin or spiramycin. Cell-free supernatants were collected for cytokine measurement by ELISA. (A) BeWo cells treated with increasing concentrations of azithromycin or spiramycin; (B) BeWo cells infected with *Toxoplasma gondii* (Tg) and treated with increasing concentrations of azithromycin or spiramycin. Data are expressed as mean ± S.D. and are representative of two independent experiments in triplicate. *Comparison between infected/treated cells and Tg control for each treatment condition (ANOVA and Dunnett multiple comparison post-test, $P < 0.05$). #Comparison between BeWo cells treated with azithromycin and spiramycin in each drug concentration (Student's *t* test, $P < 0.05$).

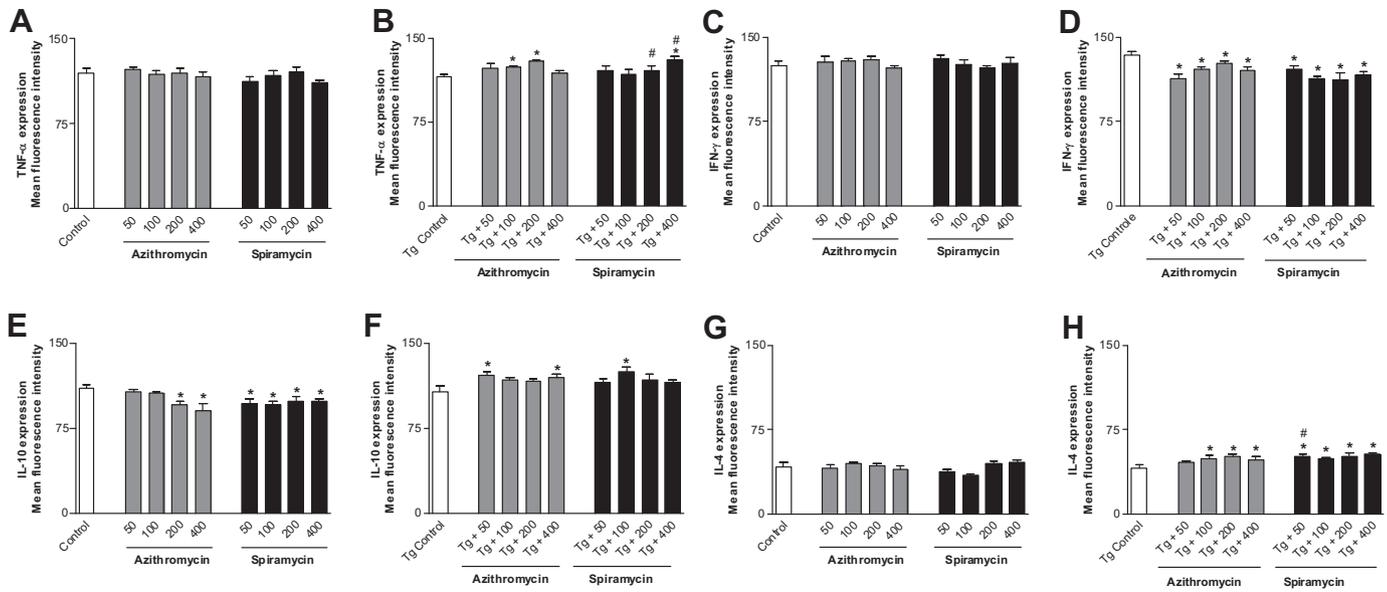


Fig. 5. Cytokine production by BeWo cells infected or not with *Toxoplasma gondii* (Tg) after treatment with different concentrations of azithromycin or spiramycin. Cell-free supernatants were collected after 24 h of treatment and the production of Th1 and Th2 cytokines was measured by Cytometric Beads Array (CBA). Production of TNF- α (A, B), IFN- γ (C, D), IL-10 (E, F) and IL-4 (G, H) by BeWo cells non-infected and treated with azithromycin or spiramycin (A, C, E, G) or infected and treated with each drug (B, D, F, H). Data are expressed as mean \pm S.D. and representative of two independent experiments in triplicate. *Comparison between treated and control or infected/treated cells and Tg control for each treatment condition (ANOVA and Dunnett multiple comparison post-test $P < 0.05$). #Comparison between BeWo cells treated with azithromycin and spiramycin in each drug concentration (Student's t test, $P < 0.05$).

directly modulate the production of proinflammatory cytokines, they are important modulators of innate and adaptive elements of cell mediated immunity [24]. IL-4, IL-10 and IL-13 have been frequently shown to display biological activities opposed to those induced by IFN- γ and that IL-10 antagonizes the expression of various genes and functions induced by IFN- γ [25]. Thus, the increased levels of IL-4 and IL-10 which we detected in infected BeWo cells treated with azithromycin or spiramycin may be involved in the decrease IFN- γ production.

Acute *T. gondii* infection during gestation may disrupt the maternal-fetal immunological balance in favor of anti-parasitic proinflammatory abortogenic cytokines, such as IFN- γ and TNF- α , which are reported to be potentially deleterious for conception [26]. However, in *T. gondii*-infected pregnant mice, TNF- α did not function as an abortogenic cytokine [26]. Furthermore, it was demonstrated that TNF- α stimulates the indoleamine 2,3-dioxygenase expression, an enzyme that catalyzes the initial and rate-limiting step of tryptophan catabolism, thus inhibiting tachyzoite replication within human fibroblast lineage cells [27]. Our results showed not only a decrease in IFN- γ levels but also a slightly increased on TNF- α production, which could be crucial to maintain pregnancy.

Despite of IFN- γ is the pivotal mediator that induces anti-*T. gondii* effector mechanisms [28], BeWo cells were unable to control replication of *T. gondii*, even in the presence of exogenous IFN- γ [9]. Moreover, IL-10 plays an important role in the balance between protective immunity and the development of immune pathology. Treatment with exogenous IL-10 induced considerable augmentation in both *T. gondii* intracellular replication and index of infection in BeWo cells, showing that the protector immune response profile seen typically in several host organisms involves the presence of inflammatory cytokines, such as IFN- γ and IL-12 [29]. BeWo cells were susceptible to *T. gondii* infection when anti-inflammatory cytokines, such as IL-10 and TGF- β 1 are involved, supporting the hypothesis that these cytokines may also facilitate parasite transmission for fetal tissues [9]. In this study, increased IL-10 levels after macrolide treatment did not influenced in the *T. gondii* intracellular replication and index of infection in BeWo

cells, indicating that *T. gondii* seems to be controlled by macrolide treatment and possibly by TNF- α .

In conclusion, the present study demonstrated that treatment of human trophoblastic (BeWo) cells with azithromycin or spiramycin is able to control *T. gondii* infection and replication. The macrolide treatment induces an anti-inflammatory immune response, with increased MIF, IL-4 and IL-10 levels, which could be important for the maintenance of a viable pregnancy as well as to control the infection. Further studies should be conducted to compare the effect of both drugs in human placental explants, as well as in congenital toxoplasmosis experimental models in vivo using cytokine knock-out mice.

Acknowledgments

This work was supported by Brazilian Research Funding Agencies (FAPEMIG, CNPq and CAPES).

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