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Original Article

The relationship among mitochondrial dysfunction and antibiotic activities on *Toxoplasma gondii in vitro*

Laís Pessanha de Carvalho*, Hellen Jannisy Vieira Beiral and Edésio José Tenório de Melo

Laboratory of Tissue and Cell Biology, State University of North fluminense – Darcy Ribeiro, Campos dos Goytacazes, 28013-602, Rio de Janeiro, Brazil

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ABSTRACT

Toxoplasma gondii is the causative agent of toxoplasmosis, which current chemotherapies are self-limiting due to inability to eliminate the parasite and induce toxic effects. Then, new therapies are necessary and antibiotics in combinations with new drugs can be an alternative. Camptothecin, ciprofloxacin and tetracycline, known by inducing loss of mitochondrial activity, were used to verify their anti-*T. gondii* activities. Infected host cells were incubated with the antibiotics at different concentrations (1-100 μM) for 12 and 24 h. The parasites were eliminated from 5 μM, 12 h, with no toxic effect to host cell until 50 μM, 24 h. The reversibility assays confirmed that the camptothecin and tetracycline arrested the parasite replicative cycle, but not ciprofloxacin. The mitochondrial activity of parasite were gradually decreased during antibiotic treatments, although no morphological change was observed. In summary, the intracellular tachyzoites were eliminated from intracellular environment in presence of the antibiotics, possibly due to loss of its mitochondrial activity.

Key words: Camptothecin, ciprofloxacin, mitochondria, parasitophorous vacuole, Rhodamine 123, tetracycline, *Toxoplasma gondii*.

Address for Correspondence: Dr. Laís Pessanha de Carvalho, Laboratory of Tissue and Cell Biology, State University of North fluminense – Darcy Ribeiro, Campos dos Goytacazes, 28013-602, Rio de Janeiro, Brazil; **laispcarvalho1@gmail.com**

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INTRODUCTION

Toxoplasma gondii, the etiological agent of human and animal toxoplasmosis, is an obligate intracellular parasite protozoan, able to infect all type of nucleated cells of vertebrates [1]. During the intracellular development, tachyzoites of *Toxoplasma gondii* reside and multiply within a non-fusogenic vacuole called parasitophorous vacuole (PV) where that they are protected from the inimical cytoplasm of host cell [1]. The PV has a highly specialized membrane (PVM) that selects what gets the intravacuolar milieu. The PVM is mainly originated from the plasma membrane of the host cell [2], but is highly modified by secreted products of the parasite [3].

During the intracellular development of the parasites, the host cell organelles change their distribution, concentrating around the parasitophorous vacuole and forming tight associations with the PVM to benefit the parasite [4,5]. Between these organelles are the components of the endoplasmic reticulum, microtubules, cytoskeleton and mitochondria [5-7]. In case of the mitochondria, which is unique in *Toxoplasma gondii*, it failed to stain in intracellular parasites with Rhodamine 123, selective to high transmembrane potential accumulating in viable mitochondria [8].

The PV protects the tachyzoites and allowing them to replicate. After cycles of multiplication, the tachyzoites rupture the host cell and reach the blood stream to infect new ones. Successive cycles of cell rupture cause tissue damages, the main feature of toxoplasmosis [9]. Although a combination of drugs are used to treat the toxoplasmosis, they act primarily against the tachyzoites allowing to the resistance of encysted form [10]. Then, an effective therapy is need. Considering this, well-established antibiotics in combination with other chemotherapies could be an alternative.

Camptothecin was isolated in 1966 from the bark and stem of *Camptotheca acuminata* and is a quinoline alkaloide. The activity of camptothecin is associated with the binding of topoisomerase I and DNA complex, preventing the DNA ligation, leading to apoptosis [11]. Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class discovered in 1983. The mode of action of ciprofloxacin is associated with the inhibition of DNA gyrase and type II and IV topoisomerase, blocking the separation of bacterial DNA, preventing the replication [12]. Next, the tetracycline, other broad-spectrum agent, was discovered in 1940s and is able to block the protein synthesis by inhibiting the attachment of the aminoacyl-tRNA to the ribosomal acceptor site [13].

In this present work, we studied the antibiotic activities on intracellular development of *Toxoplasma gondii* and the physiological and functional role of mitochondria on parasite elimination.

MATERIALS AND METHODS

Host cell culture: Vero cells (kidney fibroblasts of the African monkey) were grown in plastic Falcon flasks (25 cm^2) containing Dulbecco's Modified Eagle's Medium (DMEM-1152, SIGMA, ST. LOUIS, USA) supplemented with 5% fetal bovine serum (FBS, NUTRICELL, CAMPINAS, BRAZIL). The cultures were treated with trypsin when the cell densities approached to monolayer. For experimental proposals, the cells were placed on Linbro 24-well plates with sterile coverslips at a density of 3 x 10^4 cells per well or on a medium flask (3 x 10^6 cells) and allowed to attach for 24 h at 37°C in a 5% CO_2 atmosphere [14].

Parasite maintenance: Tachyzoites of *T. gondii* (RH strain) were maintained in Vero cell culture. The cultures were infected at a ratio of 5 parasites per cell on semi-confluence stage. These cultures were kept at 37 °C for 48 h to allow cell lysis and release of the tachyzoites into the medium. The medium was centrifuged at 2.000 × *g* for 10 min, and the pellet was suspended in 1 mL of medium. A 0.01 mL aliquot of the suspension was transferred to a Neubauer chamber and the parasites were counted using an optical microscope (Zeiss Axioinvert 135, 20x objective). Then, the tachyzoites were transferred to a new Vero cell culture. For experimental purpose, the released parasites were transferred to the cells previously seeded on the plates in a rate of 5:1 parasite –cell, during 24 h to establishment of infection [15].

Antibiotics incubations: Stock solutions of the antibiotics were dissolved in DMSO at concentration of 2 mM (camptothecin and tetracycline chloridate) and 3 mM (ciprofloxacin). The cultures were incubated in serial concentrations (1, 5, 10, 25, 50, 100 μM) during 12 or 24 h. After that, the cultures were processed to light and transmission electron microscopy as described below. The Camptothecin, Ciprofloxacin and Tetracycline were obtained from Sigma.

Light microscopy: The cultures were washed three times with PBS (phosphate buffered saline), fixed with Bouin's solution for 5 minutes, and stained with Giemsa solution (diluted in PBS, 10 % v/v). All preparations were examined using Zeiss AXIOPLAN photomiscrocope equipped with

objective 40 x. A number of six coverslips (three fields each one) were counted for each treatment.

Confocal laser scanning microscopy (CLSM): Mitochondrial functionality: cells grown on coverslips were stained with RHODAMINE 123 stain (MOLECULAR PROBES, EUGENE, EUA) at 10 µg/ml during 30 min and visualized using a rhodamine excitation filter (546 nm) [16]. Mitochondrial structural observations: cells grown on coverslips were stained with MITOTRACKER RED (MOLECULAR PROBES, EUGENE, EUA) at 10 µg/ml during 30 min and visualized using a rhodamine excitation filter (546 nm) [17]. Mitochondrial potential membrane: cells grown on coverslips were stained with JC1 (MOLECULAR PROBES, EUGENE, EUA) at 5 µg/ml during 30 min at 37° C and visualized using a rhodamine excitation filter (546 nm) [18].

Diaphorase activity: infected Vero cells were washed in PBS, fixed in solution containing 4% Paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for 20 min at room temperature. After this fixation, the cells were washed in Phem buffer (Pipes, Hepes, EGTA, $MgCl_2$) 0.05 M, pH 7.4 and incubated in a solution containing this Phem buffer, 1mM of NADPH, 0,4 mg/ml of NBT (Nitroblue tetrazolium) diluted in DMSO and Triton x-100 at final concentration of 0,025% for 2 h and 30 min at 37ºC, protected from light. After that, the cells were washed in Phem buffer 0.05 M, pH 7.4 and the coverslips were sealed with n-propyl-galate. The black formazan deposited presents at the cell and parasites was identified using a Zeiss CLSM microscope [19].

Transmission electron microscopy: Vero cells were plated in culture flasks, cultivated as described above and infected with the parasites for 24 hours. After this time, the cultures were treated with the antibiotics for 12 or 24 h. Then, the cultures were washed with PBS and fixed for 1 h at room temperature in a solution containing 1% glutaraldehyde, 4% formaldehyde, 5mM $CaCl_2$, and 5% sucrose in 0.2 M cacodylate buffer, pH 7.2. The samples were rinsed with cacodylate buffer 0.1 M and postfixed for 1 h in a solution containing 1% OsO_4, 0,8% potassium ferrocyanide in 0.1M cacodylate buffer, pH 7.2. The samples were rinsed with 0.1 M cacodylate buffer, pH 7.2, dehydrated in graded acetone and embedded in PolyBed812 (FLUKA, ST. LOUIS, USA) and then polymerized for 2 days in 60° C. Ultra-thin sections obtained with a ultramicrotome (LEICA, WETZLAR, GERMANY) were stained with uranyl acetate and lead citrate, and observed with a JEOL 1400 Transmission Electron Microscope at 60 kV acceleration [20].

RESULTS

Effects of antibiotics on T. gondii - infected host cells: After 24 h of infection the intracellular tachyzoites were proliferating within the parasitophorous vacuole, indicating the establishment of the infection. Then, a screening of concentrations was performed to determine which ones were toxic to the parasite. The concentrations were 1, 5, 10, 25, 50 and 100 µM for 12 and 24 hours. The antibiotics at 1µM did not eliminate the parasites on the condition tested (data not shown).

All antibiotics led to a progressive decrease in infection after treatment at 5 µM, for 12 hours. The camptothecin was the most efficient drug and reduced 50 (5 µM), 67 (25 µM) and 90 % (100 µM) of the infection. In comparison, the ciprofloxacin decreased 12, 23 and 88 % of the infection while tetracycline reduced 17, 34 and 34 % at the same conditions (Figure 1 A).

The elimination of the intracellular tachyzoites in the presence of the three antibiotics decreased the infection (Figure 1 B). Again, the camptothecin was the most efficient antibiotic and eliminated around 80 (5, 25 µM) and 100 % (100 µM) of the intracellular parasites. The ciprofloxacin led to a 23, 45 and 89 % of parasite elimination, while the tetracycline led to a 37, 48 and 50 %, at the same conditions.

For 24 h of treatment, only the concentrations of 25 and 50 µM were considered (Figure 2). In untreated cultures, the percentage of infected cells was close to 80 %. Nonetheless, after both treatments with camptothecin (25 and 50 µM) this percentage dropped down to 30 % (Figure 2 A), while to 40 (25 µM) and 35 (50 µM) % with ciprofloxacin (Figure 2 C) and to 15 (25 µM) and 5 % (50 µM) with tetracycline (Figure 2 E). In the untreated cultures the mean number of intracellular parasites was about 700. However, after both treatments with camptothecin (25 and 50 µM) this number reduced to 50 (Figure 2 B), while to 280 (25 µM) and 250 (50 µM) with ciprofloxacin (Figure 2 D) and to 50 (25 µM) and 10 (50 µM) with tetracycline (Figure 2 F). The infection and number of parasites significantly decreased in all treatments, but tetracycline caused a sharp drop compared to 12 h of incubation.

In order to observe whether the parasites can revert the toxic effects of the antibiotics (camptothecin at 25 µM and ciprofloxacin or tetracycline at 50 µM/ 12 h), the infected cells were re-incubated with a drug-free medium for 24 h more (Figure 3). As showed, no difference was observed for camptothecin and tetracycline, but the parasites proliferated after the treatment with ciprofloxacin.

Morphological and ultrastructural features of toxic effect of antibiotics: The morphological analysis of the treatment with antibiotics during 12 or 24 h and the reversibility assays can be observed in figures 4-6. The untreated cultures showed proliferative intracellular tachyzoites within the parasitophorous vacuole (Figure 4, 5 and 6 A). Nonetheless, infected cells treated with camptothecin at 25 μM for 12 (Figure 4 B), 24 h (Figure 4 C) or for reversibility (Figure 5 D) showed parasites at an advanced stage of structural disorganization.

The treatment with ciprofloxacin at 50 (Figure 5 B) and 100 μM (Figure 5 C) for 12 h eliminated a great number of intracellular parasites and led to morphological alterations on the remaining ones. After 24 h of incubation, 25 μM also eliminated a high number of tachyzoites while the host cells presented normal morphological features (Figure 5 D), but at 50 μM morphological changes were observed on nuclei of host cells (Figure 5 E). Despite the ciprofloxacin decreased the infection, the parasites were able to proliferate during the reversibility assay (Figure 5 E).

The tetracycline at 50 (Figure 5 B) and 100 (Figure 5 C) μM for 12 h eliminated a high number of intracellular parasites. After 24 h of the same treatments no viable parasites were seen, but the host cells were damaged (Figure 5 D and E). The reversibility assay showed that the intracellular tachyzoites did not replicate again and part of the host cells recovered their usual morphology.

The ultrastructural analysis was performed in infected cells treated with ciprofloxacin (25 μM for 12 h, a condition that parasites were still observed) (Figure 7) to observe how the intracellular parasites are affected and eliminated in presence of the antibiotics. The untreated infected cells and their organelles were well-preserved and cytoplasmic organelles as mitochondria migrated to near to the tachyzoites-containing parasitophorous vacuole (T) (white arrow) (Figure 7 A). In comparison, treated cells had parasites with severe structural disorganization (Figure 7 B), but although mitochondrial functionality was altered during intracellular *Toxoplasma gondii* infection, its structure was not affected. These data correlate with results using the fluorescent marker Mitotracker red (Figure 9 I and J).

Mitochondrial functionality

Diaphorase activity: Untreated infected cells were labelled with the NBT cytochemistry methods to detect the formazan precipitate and to visualize mitochondrial enzymatic functionality. The results showed that the reaction product was detected in the mitochondria of the host cell (Figure 8 A), and in the single mitochondria of the parasite after 12 (Figure 8 A) and 24 hours of infection (Figure 8 B). However, the precipitate of formazan which indicates the enzymatic functionality decreased or did not occur after ciprofloxacin at 50 μM for 12 h (Figure 8 C). These results suggested that mitochondrial functionality decreased during antibiotic treatments.

Vital fluorescence staining: The mitochondria of the host cell and parasites were labelled with Rhodamine 123 and MitoTracker Red to observe the functional and structural viability, respectively (Figure 9). Untreated Vero cells infected with *T. gondii* for 12 h (Figure 9 A and B) showed mitochondria with functional features as filamentous appearance spread through the cytoplasm, while no stain was observed on the parasites. However, after 24 (Figure 9 C and D) and 48 h (Figure 9 E and F) of infection the fluorescence was weaker and discontinuous or punctual. After 72 h of infection, the moment that precedes the parasite egress, the fluorescence could be observed inside the parasitophorous vacuole (Figure 9 G and H), indicating that the dye was present in both host cytoplasm and intracellular parasites. These results indicate that *Toxoplasma gondii* influences the functionality of the mitochondria of the host cell modifying it from filamentous (onset of infection) to punctual (end of infection) form. Then, the dye MitoTracker Red was used to visualize the mitochondrial structure (Figure 9 I and J).

Mitochondrial functions after treatment with antibiotics: Infected cells treated for 12 or 24 h with antibiotics at 25 (camptothecin) or 50 μM (ciprofloxacin or tetracycline) were labelled with Rhodamine 123 to observe the mitochondrial functionality (Figures 10 and 11).

The host cells showed the fluorescence concentrated around the tachyzoite - containing parasitophorous vacuole after treatment with camptothecin for12 h (Figure 10 A and B), as untreated Vero cells infected for 12 h hours (Figures 9 A and B). However, after the ciprofloxacin (Figures 9 C and D) or tetracycline (Figures 9 A and B) for 12 h the fluorescence was weaker and punctual, as the untreated cells infected for 48 h (Figure 8 E and F). In all cases, mitochondria of parasites was slightly stained. These results suggest that the treatments with ciprofloxacin and tetracycline decrease mitochondrial functionality of the host cell and parasites. In addition, the mechanisms of action of the antibiotics may involve membrane permeability, thereby facilitating the diffusion of Rhodamine 123 to the parasitophorous vacuole,

once for the first time the mitochondria of intracellular tachyzoite was stained with Rhodamine 123.

After 24 h of incubation with camptothecin, the mitochondria started to present a punctual mark (Figure 11 A and B) as in untreated cells infected for 48 h (Figure 8 E and F), but the fluorescence also occurred inside the parasitophorous vacuole as in untreated cells infected for 72 h (Figure 8 G and H). These results indicate that 24 hours of incubation with camptothecin was sufficient to strongly alter the functionality of mitochondria of host cell and tachyzoites. No significant difference was observed after both ciprofloxacin (Figure 10 C and D) and tetracycline (Figure 10 E and F) treatments for 12 or 24 h and although the mitochondria were punctuated, they remained around the parasitophorous vacuole.

DISCUSSION

The intracellular *Toxoplasma gondii* has two morphological stages: tachyzoite, of fast replication, and bradyzoite, able to form cysts (21). The current chemotherapies of toxoplasmosis target the tachyzoites, allowing the permanence of the cysts that develop during an immunosuppression period, causing the reactivation of the disease. Other problems related to the drugs are the parasite resistance and the serious side effects (22,23). Consequently, the use of more efficient drugs able to eliminate both parasite forms without toxic effect is extremely important. In this context, the current antibiotics in combination with new drugs can be an alternative.

Antibiotics can inhibit essential metabolites, bacterial wall turnover, change selective permeability in ribosomes, change or disrupt protein synthesis, inhibit the synthesis of DNA and RNA, inhibit the synthesis of protein and DNA, induce mitochondrial dysfunction inducing production of reactive oxygen radicals, as cited in (24). Some of the antibiotics that interrupt or alter the synthesis of proteins or genetic material are included in the classes of Alkaloids, Quinolenes and Tetracyclines.

Our results show that these three antibiotics were able to eliminate the intracellular *Toxoplasma gondii*. The camptothecin drastically reduced the infection at low doses (Figure 1), and the remaining parasites were not able to proliferate after the removal of the antibiotic (Figure 3), without drastic toxic effect to host cells (Figure 4). Alkaloids such as camptothecin are natural, semi-synthetic or synthetic compound that block the synthesis of mitochondrial and nuclear DNA, through its binding to the DNA-topoisomerase I complex, causing cell death during the S phase of the cell cycle (25,26).

The ciprofloxacin decreased the number of parasite but at higher doses in 12 h of treatment (Figure 1) with little morphological damage to host cells (Figure 5). However, the parasites reverted the anti-parasitic effect of ciprofloxacin (Figure 3). The quinolenes comprise nalidixic, oxolinic and pipemidic acids, the other antibiotics of this class are analogous to these acids. Ciprofloxacin, for example, is analogous to nalidixic acid. The quinolenes, natural or semi-synthetic, inhibit the replication of bacterial and mitochondrial DNA by interfering with the synthesis of enzymes responsible for this biological event (27). The ciprofloxacin acts preferentially on the DNA of bacteria, but also induces the release of cytochrome C oxidase and mitochondrial dysfunction (27).

Tetracycline also led to a drastic decrease on the infection (Figure 1 and 2), and no parasite proliferation occurred during the reversibility assay (Figure 3). However, morphological changes and reduction on number of host cells were observed (Figure 6). Tetracycline, a semi-synthetic compound, is a known broad-spectrum antimicrobial agent extracted from the *Streptomyces* species. This antibiotic inhibits the polypeptide synthesis by the ribosomal 30S unit, and also induces the formation of ROS and consequent depolarization of the mitochondrial membrane, releasing caspase 9 and 3, the precursors of apoptosis, in the cytoplasm of eukaryotic cells (28).

All antibiotics cited above target mitochondrial activity and although their mechanism of bacterial elimination is well known, their anti-parasitic activities was unknown. In this scenario, *T. gondii* was an interesting model of study. Previous study stated that the unique mitochondria of tachyzoites did not stain with rhodamine 123, a mitochondrial functionality marker, suggesting that the mitochondria was not functional (8), although it showed enzymatic activity when observed by ultrastructural cytochemistry (29). Our results showed a progressive loss of mitochondrial functionality on treated intracellular tachyzoites (Figures 10 and 11), and this fact may be related to the posterior elimination of the parasites from the intracellular environment. Substances that have mitochondria as the primary or secondary target cause dysfunctions in mitochondrial metabolites and DNA. Some consequences of this action are related to the reduction of the functioning of the respiratory chain, thus providing a decrease in ATP production, formation of free radicals and alterations in the distribution of cellular calcium. These events may initiate mitochondrial DNA,

protein and lipid peroxidation, events related to programmed cell death or apoptosis (30).

After the early treatments with the antibiotics the mitochondria of host cells and parasites were intense stained to visualize their functional and structural features. Unlikely the control, in the presence of the antibiotics the rhodamine 123 stained the mitochondria of intravacuollar parasites, although in a punctual manner (Figures 10 and 11). Once the membrane of the replicative parasites - containing parasitophorous vacuole is a structural and functional barrier (31), our results suggest that the antibiotics changed the features of the membrane of the parasitophorous vacuole and allowed the entry of Rhodamine 123. Then, the absence of Rhodamine 123 fluorescence in mitochondria of the tachyzoite can result from its inability to penetrate the membrane of the parasitophorous vacuole instead of mitochondrial inactivation as suggested by (8).

CONCLUSIONS

The mechanisms of action of some drugs against mitochondria of intracellular tachyzoites may provide new perspectives for their application in synergy with current anti-parasitic drugs. This strategy could avoid the resistance to therapeutic treatments. The fact that these three antibiotics can alter the permeability of the membrane of the parasitophorous vacuole can be used to allow the entrance of other drugs, facilitating their activities against the intracellular parasites. This *in vitro* model may be extended to the therapeutic interest against *Toxoplasma gondii* infection *in vivo*.

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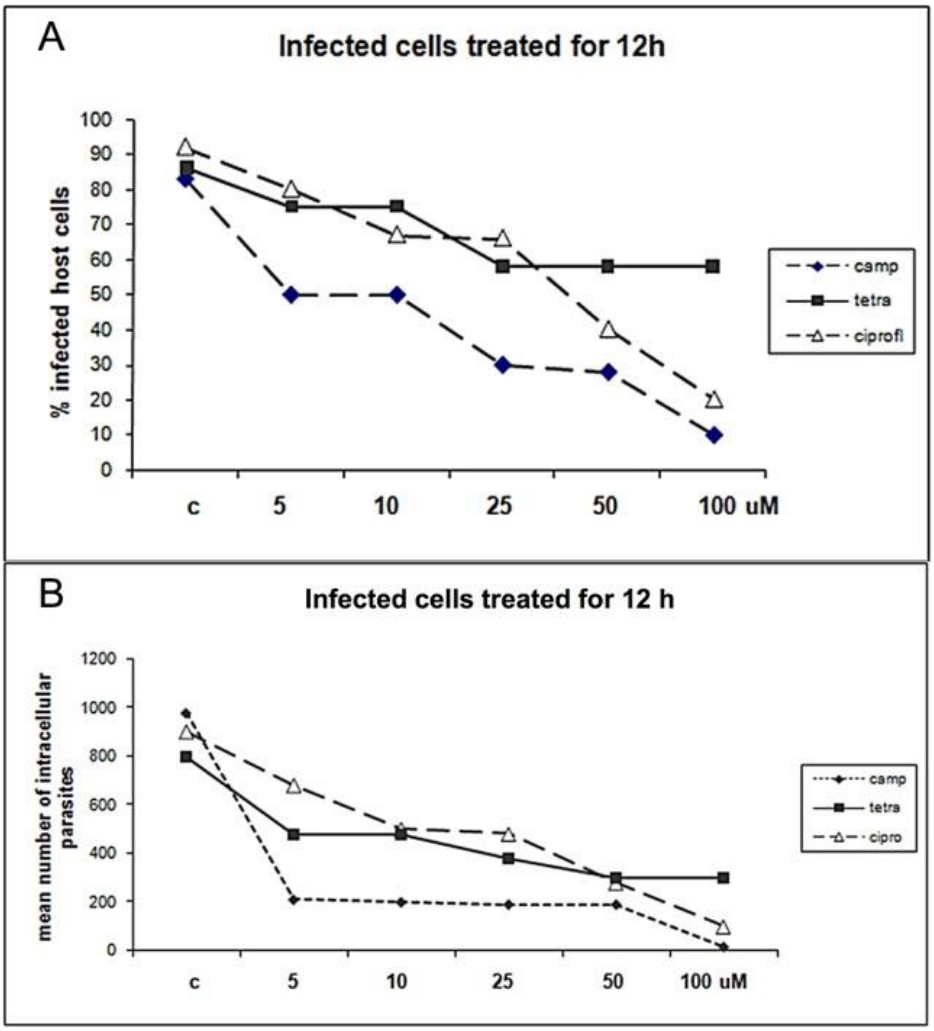

Figure 1: Infected host cells treated with camptothecin (camp), tetracycline (tetra) or ciprofloxacin (cipro) for 12 h. (A) Percentage of infected host cells. (B) Mean number of intracellular parasites.

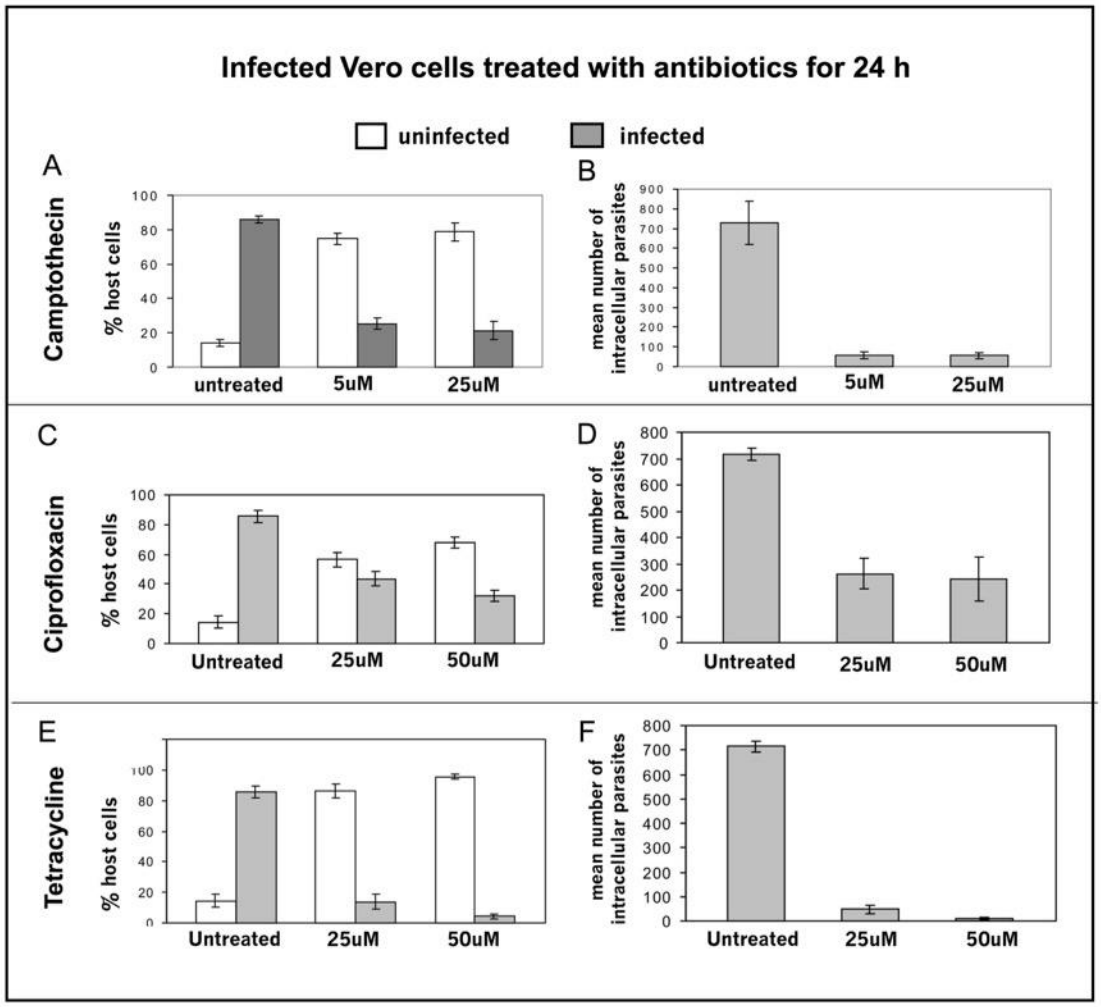

Figure 2: Infected Vero cells treated with camptothecin, ciprofloxacin and tetracycline at 25 and 50 µM during 24 h. (A, C and E): percentage of uninfected (white bars) and infected (gray bars) cells. (B, D and F): mean number of intracellular parasites.

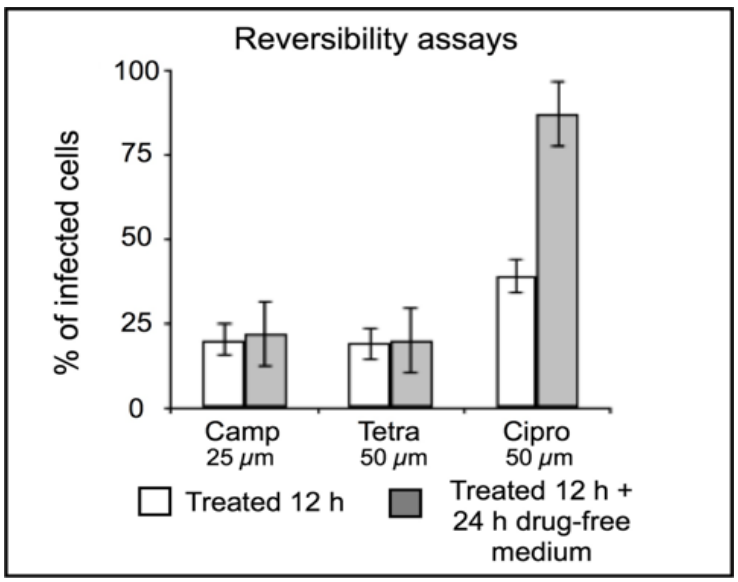

Figure 3: Percentage of infected cells after the reversibility assays. Camptothecin (25 µM), Tetracycline (50 µM) or Ciprofloxacin (50 µM) were incubated for 12 h. After, the cells were washed and incubated with a drug-free medium for 24 h more.

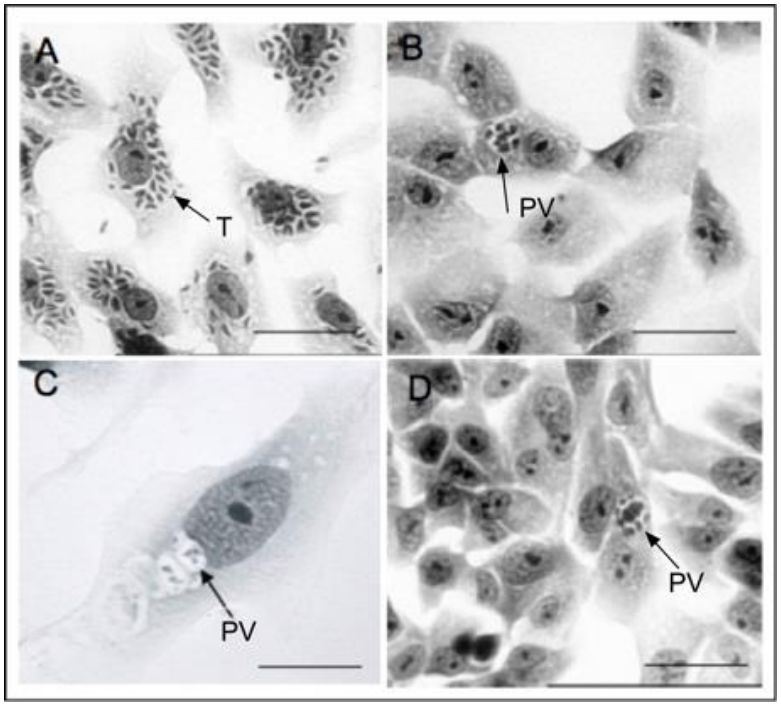

Figure 4: Infected cells incubated with camptothecin at 25 µM for 12 or 24 h. (A) untreated cells. (B) camptothecin for 12 h. (C) camptothecin for 24 h. (D) camptothecin for 12 h, 24 h more with drug-free medium. T: tachyzoites. PV: destroyed parasites-containing parasitophorous vacuole. Scale bars: A, B and D: 100 µm, C: 50 µm.

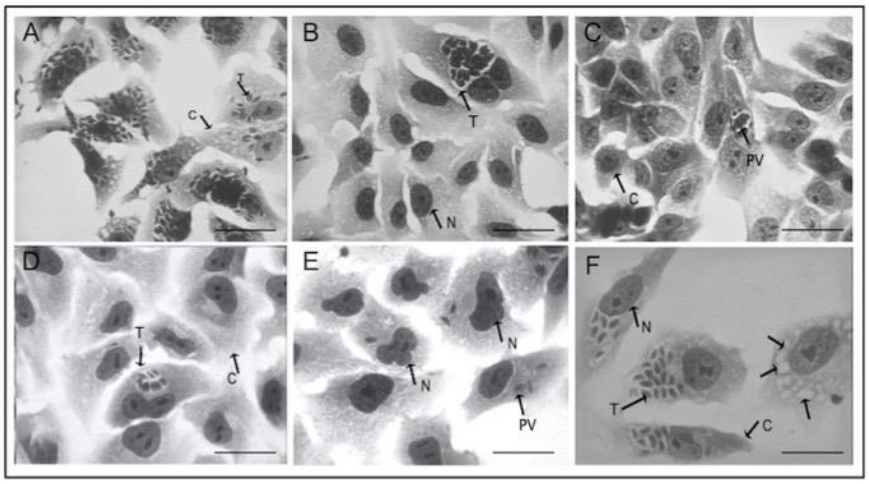

Figure 5: Infected cells incubated with ciprofloxacin at 50 or 100 µM for 12 or 24 h. (A) untreated cells. (B) ciprofloxacin at 50 µM for 12 h. (C) ciprofloxacin at 100 µM for 12 h. (D) 25 µM for 24 h. (E) 50 µM, 24 h. (F) 50 µM 12 h and 24 h more with drug-free medium. C: host cell. N: host cell nuclei. T: tachyzoites. PV: destroyed parasites-containing parasitophorous vacuole. Arrow: empty parasitophorous vacuole. Scale bars: 100 µm.

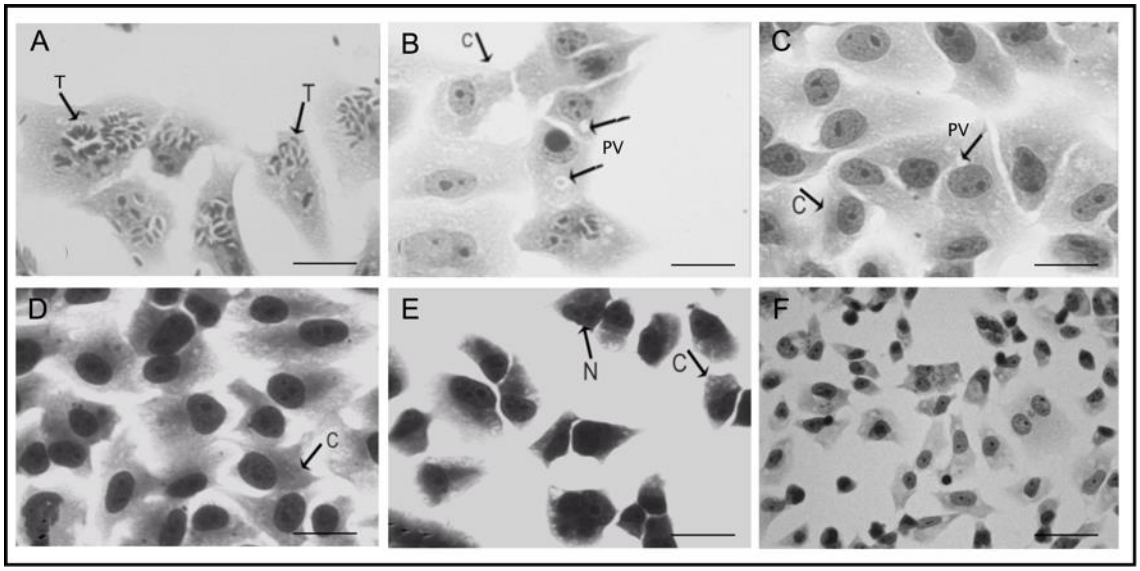

Figure 6: Infected cells incubated with tetracycline at 50 or 100 µM for 12 or 24 h. (A) untreated cells. (B) tetracycline at 50 µM for 12 h. (C) tetracycline at 100 µM for 12 h. (D) 50 µM for 24 h. (E) 100 µM, 24 h. (F) 50 µM 12 h and 24 h more with drug-free medium. Scale bars: 100 µm.

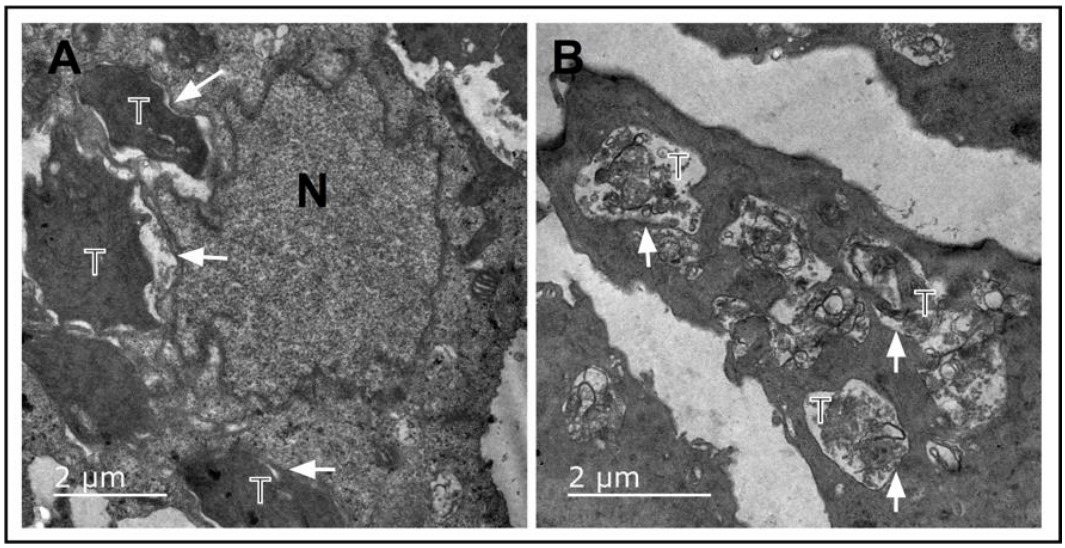

Figure 7: Transmission electron microscopy of *Toxoplasma gondii*-infected host cells treated with ciprofloxacin at 25 µM for 12 h. (A) Untreated cell. (B) Treated cells. N: host cell nucleus. T: tachyzoites. White arrows: parasites-containing parasitophorous vacuole.

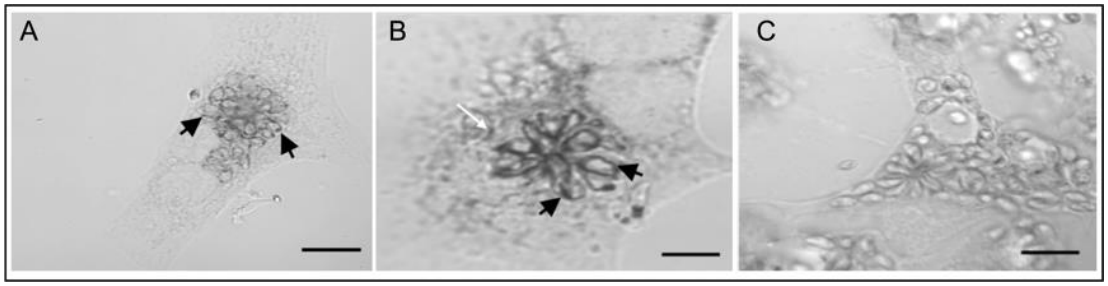

Figure 8: Infected host cells labelled with NBT-cytochemistry method. (A) untreated infected cells (12 h) (white arrow) and (B) 24 h and intracellular parasites showing mitochondrial enzymatic functionality (black arrows). (C) infected cells treated with ciprofloxacin at 50 µM for 12 h show no mitochondrial functionality. Scale bars: 20 µm

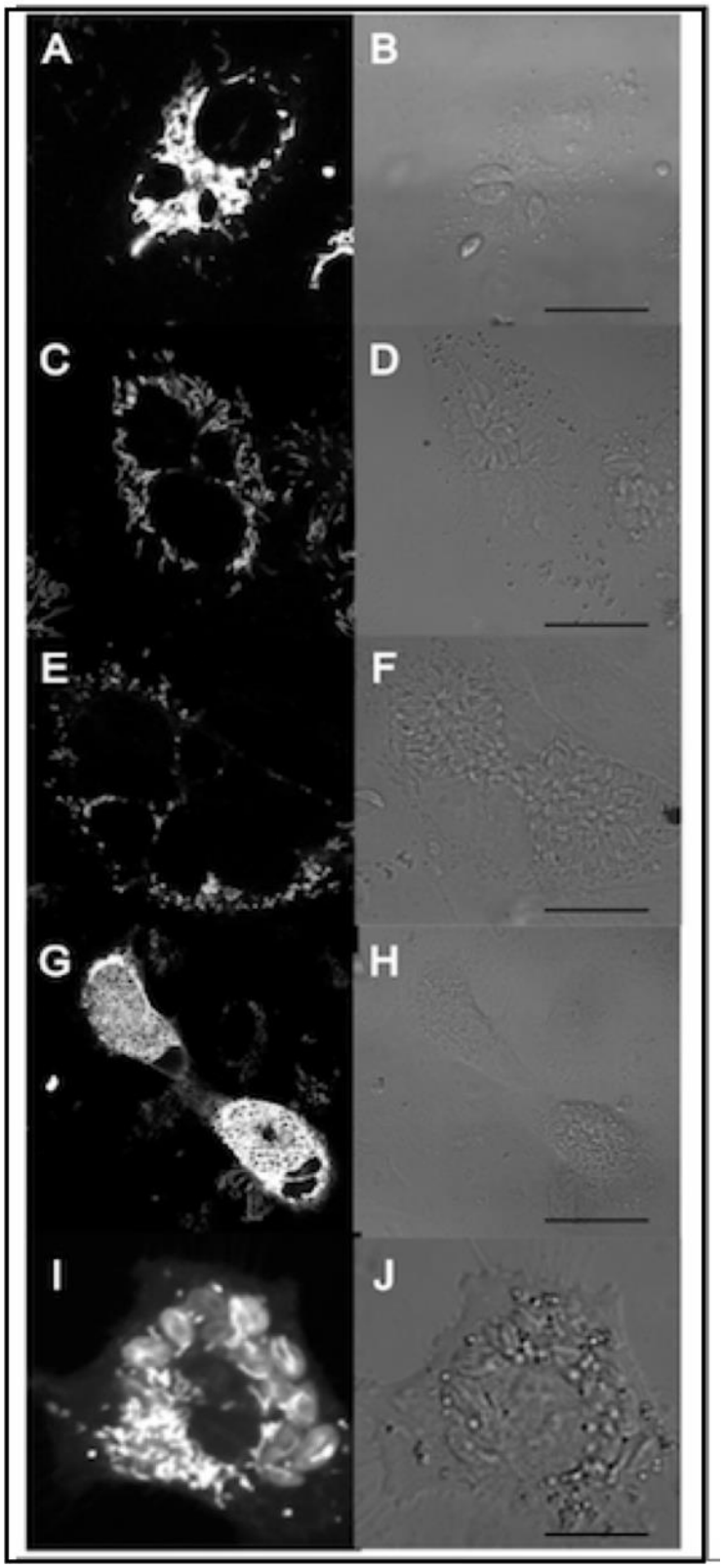

Figure 9: Untreated infected cells were labelled with Rhodamine 123 (functional dye) and MitoTracker Red (structural dye) to visualize mitochondrial behavior. (A and B) Cells infected for 12 h showed strong fluorescence around the parasites-containing parasitophorous vacuole. (C and D) Cells infected for 24 h showing lighter fluorescence around the parasites-containing parasitophorous vacuole. (E and F) Cells infected for 48 h showing punctual fluorescence around the parasites-containing parasitophorous vacuole. (G and H) Cells infected for 72 h and intracellular parasites showing functional stain. (I and J) Cells infected for 24 h and stained with MitoTracker Red. Scale bars: 50 µm.

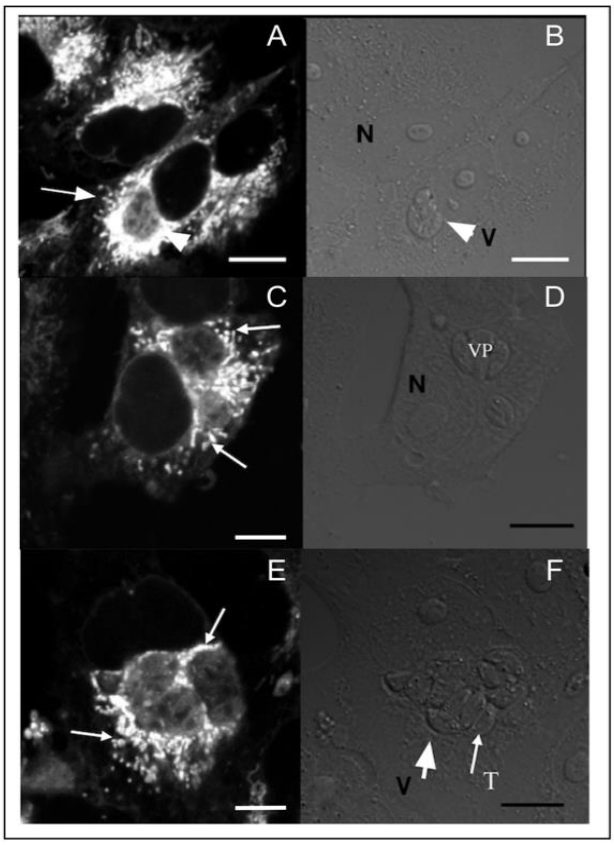

Figure 10: Infected host cells were treated with the antibiotics during 12 h and labelled with Rhodamine 123. (A and B) Camptothecin (25 µM). (C and D) Ciprofloxacin 50 µM. (E and F) Tetracycline 50 µM. White arrows: mitochondrias. White arrowhead: parasites. Scale bars: 20 µm.

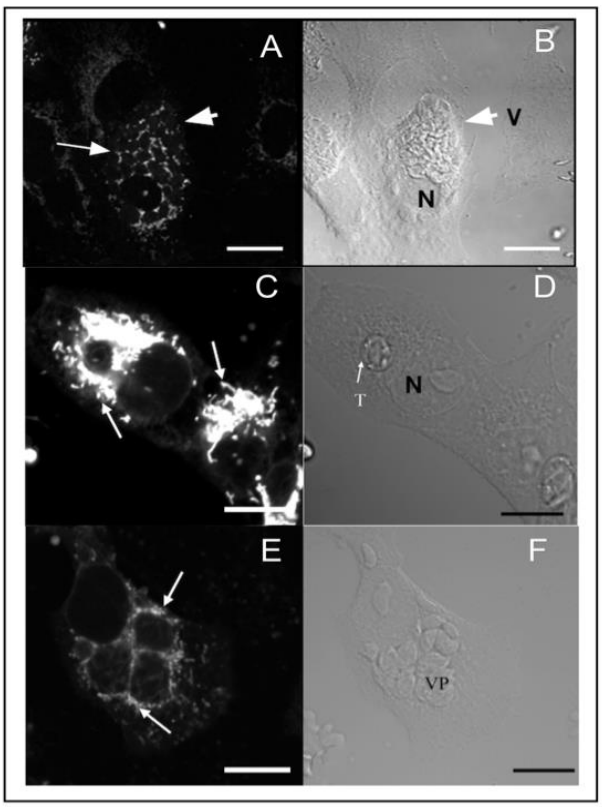

Figure 11: Infected host cells were treated with the antibiotics during 24 h and labelled with Rhodamine 123. (A and B) Camptothecin (25 µM). (C and D) Ciprofloxacin 50 µM. (E and F) Tetracycline 50 µM. White arrows: mitochondrias. White arrowhead: parasites. Scale bars: 20 µm.

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