The therapeutic effect of atovaquone and clindamycin on the reduction of tissue cysts of PRU strain of *Toxoplasma gondii*

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Abstract

Despite recent advances in the treatment of cerebral toxoplasmosis, monitoring parasite load and treatment response is still challenging. In the present study, the effect of atovaquone (AT) and clindamycin (CL) alone and in combination on chronic cerebral toxoplasmosis caused by PRU strain of *Toxoplasma gondii* was investigated in BALB/c mice. BALB/c mice aged 6 to 8 weeks were infected by intraperitoneal inoculation of *Toxoplasma gondii* strain PRU brain cysts. Then, the mice were divided into five groups as follows: Group 1 included mice treated with 100 mg/kg of atovaquone (AT), group 2 included mice treated with 400 mg/kg per day of clindamycin, group 3 included mice treated with combination (AT+CL), group 4 included untreated, infected mice as a positive control (PC) and group 5 included untreated uninfected mice as negative control (NC). After the completion of the treatment period, the effect of drugs in reducing or eliminating parasites in the brain was checked by counting the number of brain cysts. The results showed that although atovaquone and clindamycin did not completely remove the cysts from the brain tissue of mice, they significantly reduced the number of tissue cysts in the brain tissue of the treated mice compared to the untreated control group (PC) ($P < 0.0001$). Atovaquone had more anti-toxoplasmic effect than clindamycin and the difference between the two drugs was completely significant. In conclusion, considering the risks of infection with different strains of *T. gondii*, it is necessary to emphasize the importance of developing effective therapeutic interventions for toxoplasmosis.

Key words: *Toxoplasma gondii*, cerebral toxoplasmosis, PRU strain, atovaquone, clindamycin.

1. Introduction

Toxoplasmosis is a globally widespread infection, with particularly high rates in some areas. The demand for new treatments or drugs for toxoplasmosis arise from several key reasons. Current

therapies, mainly pyrimethamine and sulfadiazine, often cause severe side effects and require close monitoring of blood levels to prevent toxicity, particularly during prolonged use (1). Another growing issue is the potential for *Toxoplasma gondii* to develop drug resistance, which may reduce the efficacy of existing medications. Immunocompromised individuals—such as those with HIV/AIDS, undergoing cancer therapy, or receiving organ transplants—face a greater risk of severe toxoplasmosis, and available treatments may not adequately control infections in these patients. Additionally, congenital toxoplasmosis (transmitted during pregnancy) can result in fetal complications like neurological impairment, vision loss, and developmental delays. Better therapeutic approaches could significantly improve outcomes for both mothers and infants (1).

Atovaquone is an antiprotozoal drug designed to prevent and treat specific protozoal infections. It functions as a structural analog of coenzyme Q (ubiquinone), a critical element in mitochondrial electron transport. By binding to cytochrome b, atovaquone disrupts the mitochondrial membrane potential and interferes with pyrimidine synthesis in the parasite (2, 3), ultimately impairing energy production and causing cell death.

As a standalone treatment, atovaquone is effective against *Toxoplasma gondii* and Pneumocystis jiroveci infections, as well as malaria prevention (2, 3). In vitro studies demonstrate its potent activity against tachyzoites at nanogram-per-milliliter concentrations (4, 5), though higher doses are needed to eliminate bradyzoites within cysts (4). In mouse models of toxoplasmosis, atovaquone showed efficacy alone (6), but its effectiveness improved when combined with other agents, such as pyrimethamine, sulfadiazine (7), clindamycin (8), azithromycin (9), or clarithromycin (5). An experimental intravenous formulation also demonstrated high efficacy in a reactivated toxoplasmosis mouse model (10).

Clindamycin, a lincosamide-class antibiotic, targets both aerobic and anaerobic bacteria by inhibiting protein synthesis. It binds to the bacterial 50S ribosomal subunit, preventing bacterial growth. Beyond its antibacterial uses, clindamycin has also been employed—alone or in combination—for toxoplasmosis treatment (11, 12).

The need for novel toxoplasmosis therapies remains urgent to overcome current treatment limitations, safeguard high-risk populations, and reduce the global impact of this infection. Continued research is vital to advancing therapeutic options and improving patient outcomes. To address these gaps, this study systematically evaluates the anti-parasitic efficacy of atovaquone both as monotherapy and in combination with clindamycin using a well-established Balb/c mouse model infected with the clinically relevant PRU strain of *T. gondii*.

Materials and Methods

1.1 Mice

Female Balb/c mice (Razi Vaccine & Sera Institute, Karaj, Iran) weighing 20 to 25 g at the beginning of each experiment were used. Mice were housed 4 to a cage and offered drinking water ad libitum.

1.2 Parasite

The *T. gondii* PRU strain used in this study was provided by the Parasitology Department at Mazandaran University of Medical Sciences. To establish chronic infection in BALB/c mice, the parasite tachyzoites were maintained by serial intraperitoneal passage in laboratory mice. Three chronically infected donor mice were euthanized via chloroform anesthesia and surface-sterilized in 96% ethanol. Under a biosafety hood, skulls were aseptically opened, and brains were extracted. Brain tissue was rinsed in sterile distilled water, and cysts were confirmed microscopically (400× magnification). Tissue was gently homogenized to preserve cyst integrity. Homogenized brain suspension was adjusted to 2 mL with sterile water and further dispersed using a 2.5 mL syringe. Cysts were enumerated via Neubauer chamber to standardize the inoculum. Each mouse received an intraperitoneal injection containing 20–25 cysts. Infected mice were ear-marked, randomized into five groups, and housed individually.

1.3 Drugs

Clindamycin (CLI): Hydrochloride powder (Sepidaj Pharmaceutical Co., Iran) was administered at 400 mg/kg/day. Atovaquone (ATO): Micronized powder (Hubei Vanz Pharm Co., China) was given at 100 mg/kg/day. Doses were chosen based on prior studies demonstrating efficacy (13, 14) as effective doses (400 mg CLI/kg/day and 100 mg ATO/kg/day). Suboptimal doses (Lower doses of both drugs) were also tested to better evaluate combination therapy effects. Infrared (IR) spectroscopy was performed at Tarbiat Modares University's Faculty of Medical Sciences to confirm drug integrity. To control for drug side effects, separate groups of animals were given CLI (400 mg/kg/day) and ATO (100 mg/kg/day) for 3 months, as well as ATO plus CLI (100 plus 400 mg/kg/day) for 2 months.

1.4 Experimental design.

Mice were intraperitoneally inoculated with 20-25 *T. gondii* cysts and randomly divided into treatment groups (n=12 per group):1- CLI monotherapy (400 mg/kg/day); 2- ATO monotherapy (100 mg/kg/day) and 3- Combination therapy (ATO+CLI: 100+400 mg/kg/day). Two control groups were included:1- Negative control (NC): Uninfected mice and 2- Positive control (PC): Infected, untreated mice. Following inoculation, mice were housed for 8 weeks to establish chronic infection. Treatment began 24 hours post-inoculation and continued for 14 consecutive days. Survival was monitored daily throughout the experiment. The complete study was replicated three times with consistent results. Data presented represent pooled results from all replicates.

1.5 Bioassay.

Following the 14-day treatment period, mice were euthanized using chloroform anesthesia. Under sterile biosafety cabinet conditions, mice were secured on a dissection tray, skulls were aseptically opened using surgical tools (scalpel and forceps) and whole brains were extracted and rinsed with sterile distilled water. Each brain was divided into two hemispheres, left hemisphere was stored at -80°C in sterile microtubes for molecular analysis and right hemisphere was used for tissue cyst quantification. Brain hemispheres designated for cyst counting were homogenized 20-25 cysts from each sample were intraperitoneally inoculated into two naive mice per sample (1 mouse per inoculation route).

1.6 Statistical analysis.

The cyst count data were analyzed using one-way ANOVA in GraphPad Prism software (version X.X), with statistical significance set at $p < 0.05$. Post-hoc multiple comparisons were performed when ANOVA indicated significant differences between groups.

2. Results

Following the 14-day treatment regimen, quantitative analysis revealed, neither atovaquone nor clindamycin monotherapy achieved complete cyst eradication. All treatment groups showed statistically significant reductions in brain cyst counts compared to untreated controls (PC) ($p < 0.0001$). The magnitude of cyst reduction varied by treatment regimen (see Table 1 and Figure 1)

Table 1: The number of cysts counted in the brains of different groups of mice after treatment with atovaquone and clindamycin.

Groups	Drugs used	Cyst /g in brain	P- value*
1	Atovaquone (AT)	163.33 ± 29.52	2, 3, 4
2	Clindamycin (CL)	496 ± 41.85	1, 3, 4
3	AT + CL	1096.8 ± 84.82	1, 2, 4
4	No treatment for positive control (PC)	1600.8 ± 75.91	1, 2, 3

*significant differences in various groups based on One-way ANOVA test.

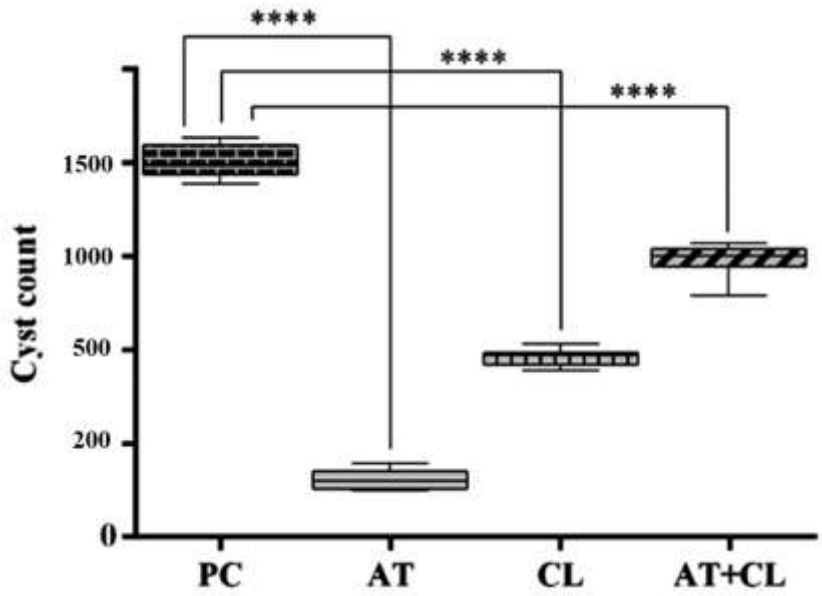

Fig. 1: The effect of treatment with Atovaquone, Clindamycin and combined drug (Atovaquone + Clindamycin) on the number of tissue cysts in the brain tissue of mice. PC: Positive Control (No treatment), AT: Atovaquone, CL: Clindamycin.

In addition, a significant variation in cyst counts was observed across all treatment groups ($p < 0.0001$). Atovaquone (ATO) monotherapy demonstrated superior efficacy, with significantly fewer cysts than Clindamycin (CLI) monotherapy ($p < 0.0001$) and combination therapy (ATO+CLI) ($p < 0.0001$). CLI monotherapy also showed reduced efficacy compared to the combination group ($p < 0.0001$) (Figure 1).

ATO exhibited significantly stronger anti-toxoplasmic activity than CLI. The ATO+CLI combination paradoxically showed lower efficacy than ATO alone, suggesting drug antagonistic interaction and interference against the PRU strain. No treatment-related toxicity (e.g., lethargy, weight loss) was observed during extended maintenance (2–3 months) in any drug-treated group.

4. Discussion

T. gondii exhibits limited species diversity but comprises three major strain types with distinct biological properties. Type I (e.g., RH, GT1) is highly virulent in mice, often lethal in acute infection models. Type II (e.g., PRU, ME49) is moderately virulent; preferentially establishes chronic infections in intermediate hosts (including humans) and type III (e.g., CEP) is attenuated virulence, primarily used for comparative studies (16). As a canonical Type II strain, PRU is widely employed in toxoplasmosis research due to its clinical relevance as a model for human chronic infection and cyst persistence. It is often used in laboratory studies to understand *T. gondii*

biology, host-parasite interactions, and the immune response. It helps researchers to investigate pathogenesis, therapeutic development and vaccine design (15).

Toxoplasmosis represents a major public health concern due to its severe consequences for pregnant women and immunocompromised individuals. As awareness of its health impacts increases, so does the need for more effective and accessible treatments. Recent advances in our understanding of *T. gondii* biology have uncovered promising new drug targets, including essential metabolic pathways, critical signaling mechanisms, and vulnerable stages of the parasite's lifecycle that could be exploited for therapeutic development. Novel drug compounds or combination therapies may provide improved treatment outcomes through enhanced efficacy, reduced toxicity, or innovative mechanisms of action. The development of such treatments could significantly reduce cases of severe toxoplasmosis, thereby decreasing healthcare burdens and improving quality of life for affected populations (16).

The management of toxoplasmosis varies based on patient age, immune status, and clinical presentation. While most available medications effectively target the tachyzoite stage, they demonstrate limited activity against persistent tissue cysts. Naphthoquinone derivatives such as atovaquone represent an exception, showing modest cysticidal activity in experimental models, though their clinical use has primarily been documented in HIV/AIDS patients (17). The structural complexity of the cyst wall presents a significant therapeutic challenge.

In clinical practice, the combination of pyrimethamine with either sulfadiazine or clindamycin remains the gold standard for both acute treatment and secondary prophylaxis (17,18). However, treatment-limiting toxicity frequently necessitates alternative approaches in a substantial proportion of patients.

Our experimental findings demonstrate that while neither atovaquone nor clindamycin monotherapy achieved complete cyst eradication in the *T. gondii* PRU strain model, both agents significantly reduced cerebral cyst burden compared to untreated controls. Notably, atovaquone exhibited superior anti-parasitic efficacy relative to clindamycin, with statistically significant differences in cyst reduction between the two treatment modalities.

In the study by Moshkani and Dalimi (2000), mice infected with *T. gondii* RH strain tachyzoites were treated with atovaquone and azithromycin, either alone or in combination. The results showed dose-dependent survival rates with atovaquone monotherapy: 8%, 17%, and 25% of mice survived at doses of 20, 50, and 100 mg/kg/day, respectively. While azithromycin failed to eradicate the parasite from either brain or visceral tissues, atovaquone demonstrated complete clearance of visceral infection at all tested doses (20-100 mg/kg/day) and eliminated brain infection at the highest dose (100 mg/kg/day). Notably, combination therapy with atovaquone and azithromycin showed neither synergistic nor additive effects and failed to achieve complete parasite eradication in any tissue compartment (9).

Djurković-Djaković et al. (1999) evaluated the efficacy of clindamycin (CLI) combined with atovaquone (ATO) in a murine model of acute toxoplasmosis. Swiss Webster mice were intraperitoneally infected with either 10^2 or 10^4 tachyzoites of the *T. gondii* RH strain and received oral treatment with each drug alone or in combination for 14 days starting from day 1 post-

infection, with survival monitored over 7 weeks. In mice infected with 10^2 parasites, the drug combination significantly improved survival compared to ATO monotherapy, though it provided no additional benefit over CLI alone, which showed strong efficacy. For the higher inoculum (10^4 parasites), the combination therapy outperformed ATO alone at both low and high doses but again showed no advantage over CLI monotherapy (8).

In a follow-up study in 2002, the same research group examined this drug combination against the ME49 strain in Swiss-Webster mice orally infected with 10 or 20 cysts. Treatment with ATO (5–100 mg/kg/day) and CLI (25–400 mg/kg/day), either alone or combined, was administered for 2–4 weeks. In acute infection, all treatments significantly enhanced survival and reduced brain cyst burden, with ATO-containing regimens (both monotherapy and combination) showing superior cyst reduction compared to CLI alone. For chronic infection, only the combination therapy achieved a significant decrease in cyst burden when assessed 2 weeks post-treatment (19).

Several studies have evaluated atovaquone's effectiveness against toxoplasmosis in various animal models. In hamsters with acute acquired toxoplasma retinochoroiditis, systemic atovaquone monotherapy demonstrated comparable efficacy to standard regimens (pyrimethamine-sulfadiazine, clindamycin, and spiramycin) in reducing Toxoplasma brain cyst burden during acute infection. Notably, atovaquone also significantly decreased cyst numbers in chronic infection (20).

Formulation advancements have enhanced atovaquone's therapeutic potential. Azami et al. (2018) developed an atovaquone nanoemulsion that showed improved bioavailability and tissue distribution in mice infected with both RH and Tehran *T. gondii* strains. This formulation increased survival time while reducing parasitemia, brain cyst count, and cyst size (21). More recently, Goudarzi et al. (2024) demonstrated that atovaquone-loaded exosomes (EXO-ATQ) achieved 97.3% cyst reduction in chronic infection (Tehran strain) and showed enhanced efficacy against tachyzoite proliferation in vitro compared to conventional atovaquone suspension (22).

Beyond treatment, atovaquone shows promise for toxoplasmosis prophylaxis in transplant recipients, though its safety and efficacy in this specific population require further investigation (4, 23).

Collective research findings position atovaquone as a promising therapeutic candidate against T. gondii, demonstrating superior cyst-reducing efficacy compared to conventional therapies. Its potential integration into future treatment protocols is further supported by evidence that combination regimens can significantly enhance therapeutic outcomes. These advances are particularly critical given toxoplasmosis' growing public health burden, especially among immunocompromised individuals and pregnant women, underscoring the urgent need for continued investigation into parasite biology and host-pathogen dynamics.

While pyrimethamine-sulfadiazine remains the current standard of care, its limitations—including treatment-limiting toxicity and incomplete cyst eradication—necessitate the development of safer, more effective alternatives. A multidisciplinary approach combining parasitology, drug development, and clinical research will be essential to advance next-generation therapies. Such innovations must address the full spectrum of toxoplasmosis management, from acute infection to chronic cyst clearance, ultimately improving therapeutic efficacy and patient quality of life.

Certificate of medical ethics

This study was approved by the ethics committee of Tarbiat Modares University with code number IR.MODARES.REC.1400.117

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Authors' contributions

Study concept and design: N.Z. and A.D.

Acquisition of data: N.Z.

Analysis and interpretation of data: A.D.

Drafting of the manuscript: A.D. and N.Z.

Critical revision of the manuscript for important intellectual content: A.D.

Statistical analysis: A.D.

Administrative, technical, and material support: A.D.

Conflict of interest

The authors have declared no conflicts of interest.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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