Azithromycin prophylaxis and treatment of murine toxoplasmosis

Khalid F. Tabbara, *MD,* ***Ehab Hammouda,*** *BS,* ***Abdulkader Tawfik,*** *PhD,*
Othman M. Al-Omar, *MD,* ***Ahmed M. Abu El-Asrar,*** *MD.*

ABSTRACT

Objective: To evaluate the azithromycin effects alone and in combination with other agents in the prophylaxis and treatment of murine toxoplasmosis.

Methods: A total of 280 BALB/c mice were included, and 2 x 10^3 Toxoplasma organisms of the RH strain *Toxoplasma gondii* strain ATCC50174 were given intraperitoneally to each mouse. In experiment one, 40 animals were given azithromycin 200 milligram/kilogram/daily for 3 days starting the day of inoculation, 40 mice were control. In experiment 2, the treatment was started 48 hours after inoculation and given daily for 3 days: one group received azithromycin 200 milligram/kilogram/day, the second group received pyrimethamine 25 milligram/kilogram/day, and the sulfadiazine 100 milligram/kilogram/day. The third group was control. In experiment 3, 7 groups of animals received one of the following (1) none, (2) azithromycin 200 milligram/kilogram/day, (3) pyrimethamine 25 milligram/kilogram/day and sulfadiazine 100 milligram/kilogram/day, (4) azithromycin and sulfadiazine, (5) azithromycin and pyrimethamine, (6) azithromycin with sulfadiazine and pyrimethamine, (7) sulfadiazine alone. Treatment was initiated 72 hours after inoculation for 3 days. The study was conducted at the Animal Care Facility of King Saud University, Riyadh, Kingdom of Saudi Arabia.

Results: Animals that received azithromycin simultaneously with inoculation survived, and all control animals died. All animals died in groups receiving single drug therapy. Animals treated with azithromycin and sulfadiazine showed a survival rate of 40%, sulfadiazine and pyrimethamine 40%, or azithromycin with sulfadiazine and pyrimethamine 95% ($p<0.0001$).

Conclusion: Azithromycin alone was found to be effective in the prophylaxis of murine toxoplasmosis. Combination therapy was effective in the treatment of murine toxoplasmosis.

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Toxoplasmosis is a widespread disease in nature and has a worldwide distribution. The disease affects man and animals and is caused by the organism *Toxoplasma gondii*. Toxoplasmosis can be congenital or acquired with a variety of clinical manifestations.[1-4] In congenital toxoplasmosis, the disease may be asymptomatic or may be disseminated with fatal outcome. Neonatal toxoplasmosis may lead to encephalitis with hydrocephalus, mental retardation, hepatosplenomegaly and ocular lesions.[5-10] Toxoplasmic retinochoroiditis may cause blindness in the newborn infants. Man can acquire the disease by inhalation or ingestion of Toxoplasma oocysts which are dished in cat feces, by the ingestion of the Toxoplasma bradyzoites in undercooked infected meat and milk, by skin penetration with Toxoplasma infected needles, or rarely by allograft organ transplantations.[11-13] The prevalence of toxoplasmosis may vary from one region to another. In most patients, the systemic disease is subclinical in nature and produce minimal or no symptoms in

From the Department of Ophthalmology (Tabbara, Al-Omar, Abu El-Asrar), College of Medicine, College of Pharmacy (Tawfik), King Saud University and The Eye Center and The Eye Foundation for Research in Ophthalmology (Tabbara, Hammouda), Riyadh, *Kingdom of Saudi Arabia.*

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Address correspondence and reprint request to: Dr. Khalid F. Tabbara, The Eye Center and The Eye Foundation for Research in Ophthalmology, PO Box 55307, Riyadh 11534, *Kingdom of Saudi Arabia.* Tel. +966 (1) 4649614. Fax. +966 (1) 4629675. E-mail: k.tabbara@nesma.net.sa

the immunocompetent host. Toxoplasmosis in immunosuppressed individuals or patients with Acquired Immune Deficiency syndrome (AIDS) may lead to severe retinitis and encephalitis.[5,10,14] The disease in these patients may represent de-novo infection or recrudescence of latent infection. Toxoplasma organisms may be harbored inside cysts for many years following the initial episode of infection. Toxoplasmic retinitis may follow congenital or acquired systemic infections after weeks or years. The ocular manifestations of toxoplasmosis consist of a localized area of necrotizing retinitis with subjacent choroiditis. The retina sustains the primary insult and major damage. The retinitis consists of fluffy soft whitish infiltrates surrounded by edema.

Despite the availability of antimicrobial agents that can eradicate the infection in experimentally infected rodents and in tissue cultures, such agents may have limited value in the clinical disease and in patients with severe retinochoroiditis. Agents such as pyrimethamine and sulfadiazine may fail to effect a cure of human ocular toxoplasmosis due to inadequate levels of these drugs when delivered to the site of active ocular lesion or owing to local conditions in the retinal lesions that favor the growth of the parasite. Search for new drugs that are safe and effective against toxoplasmosis is highly desirable. Several animal models for toxoplasmosis have been used.[15-17] The extreme susceptibility and rapid death of the mouse makes it suitable for the study of the efficacy of antimicrobial agents. The purpose of this study was to evaluate the effects of azithromycin alone and in combination with other antimicrobial agents in the prophylaxis and treatment of murine toxoplasmosis.

Methods.

Animals. Male BALB/c mice weighing 25-30 grams were used to evaluate the activity of azithromycin in disseminated acute systemic infection by *Toxoplasma gondii*. All mice were obtained from the vivarium of King Saud University, College of Medicine, Riyadh, Kingdom of Saudi Arabia. The mice were inbred and examined by a veterinarian. Healthy mice were used for this experiment. A total of 280 BALB/c mice were included. The maintenance and care of experimental animals comply with the National Institutes of Health guidelines for the humane use of laboratory animals.

Toxoplasma organisms. The RH strain of *Toxoplasma gondii* strain ATCC50174 was used for the induction of acute toxoplasmosis in mice. Fifty sub-cultures in both groups from the initial ATCC50174 stock samples were carried out without any notable change in virulence. The mean survival rate following acute systemic toxoplasmosis in mice was 4 days and varied from 1-6 days. A total of 2 x 10^3 Toxoplasma organisms of the RH strain were given intraperitoneally in each mouse. Intraperitoneal wash of the infected mice showed tachyzoites. The mortality rate was 100% in repeated experiments 7 days following the intraperitoneal injection of *Toxoplasma gondii* indicating that this mode of inoculation was effective in inducing disseminated infection and death in all animals. All animals were infected by intraperitoneal injection of 2 x 10^3 Toxoplasma organisms of the RH strain (ATCC50174).

Antimicrobial treatment. The medications used for treatment included azithromycin, pyrimethamine, and sulfadiazine. Animals were divided into 2 groups in experiment 1, 3 groups in experiment 2, and 7 groups in experiment 3. There were 40 animals in each group in experiment 1 and 20 animals in each group in experiments 2 and 3. The therapy was initiated at the time of inoculation in experiment 1 and 48 hours and 72 hours after inoculation in experiments 2 and 3.

Experiment 1 subjects consisted of 2 groups of mice 40 animals in each group. Each group was given intraperitoneal injection of 2 x 10^3 Toxoplasma organisms of the RH strain. One group served as control and was given no medication, and group 2 was given azithromycin 200 mg/kg by nasogastric (NG) tube feeding daily for 3 days starting on the day of inoculation as a prophylaxis for toxoplasmosis.

In experiment 2, we included 3 groups of animals consisting of 20 animals in each group - one group served as control, and received no medications. Each animal in the second group received azithromycin 200 mg/kg per day for 3 days by NG tube feeding, and each animal in the third group received pyrimethamine 25 mg/kg per day and sulfadiazine 100 mg/kg per day as given by NG feeding in combination for 3 days dose. Treatment was started 48 hours after inoculation daily for 3 days.

In experiment 3, we included 7 groups of mice 20 in each group. Animals in each group received one of the following regimens: (1) none, (2) azithromycin 200 mg/kg per day, (3) pyrimethamine 25 mg/kg per day, and sulfadiazine 100 mg/kg per day, (4) azithromycin and sulfadiazine, (5) azithromycin and pyrimethamine, (6) azithromycin and sulfadiazine and pyrimethamine, (7) sulfadiazine. Treatment was initiated 72 hours after inoculation daily for 3 days.

Statistical analysis. A chi-square test was used to assess the survival differences among different groups. In addition, multiple group comparison was carried out. The level of significance was 0.05.

Results.

Table 1 shows the result of prophylaxis of murine toxoplasmosis in experiment 1. When azithromycin was given in a single dose simultaneously with toxoplasma inoculation, all mice survived the infection and all control animals, on the other hand, died ($p<0.0001$). In experiment

Table 1 - Effects of azithromycin prophylaxis in murine toxoplasmosis. Azithromycin administered simultaneously with inoculation of Toxoplasma.

Group	Dosage (mg/kg/d)	Duration of treatment	N of mice infected	Survival at day 7	Survival at day 28
Control	-	-	40	0	0
Azithromycin	200	3 days	40	40	40
		$p<0.0001$			

2, treatment was initiated 48 hours after inoculation of Toxoplasma organisms (**Table 2**). Twelve (60%) out of 20 mice survived in the azithromycin treatment compared to 16 (80%) in the group treated with pyrimethamine and sulfadiazine ($p<0.0001$). A multiple comparison showed a non-significant difference in survival between azithromycin and the pyrimethamine and sulfadiazine combination ($p=0.1675$). In experiment 3, treatment was initiated 72 hours after inoculation. Six treatment groups and one control group were included. All animals died in the control, azithromycin, sulfadiazine, and azithromycin and pyrimethamine groups (**Table 3**). On the other hand, animals treated

Table 2 - Effects of azithromycin compared to pyrimethamine and sulfadiazine in murine toxoplasmosis. The therapy was initiated 48 hours after inoculation of Toxoplasma.

Group	Dosage (mg/kg/d)	Duration of treatment	N of infected	N of mice survived on day 28	Survival %	*P* value
Control	-	-	20	0	0	-
Azithromycin	200	Once daily for 3 days	20	12	60	<0.0001
Pyrimethamine	25	Once daily for 3 days				
Sulfadiazine	100	Once daily for 3 days	20	16	60	<0.0001

Table 3 - Effects of various treatment regimens on murine toxoplasmosis. Treatment was initiated 72 hours after inoculation of Toxoplasma organisms.

Group	Dosage (mg/kg/d)	N of infected	N of mice survived on day 28	Survival %	*P* value*
Control	-	20	-	-	-
Azithromycin	200	20	0	0	< 0.0001
Sulfadiazine	100	20	0	0	< 0.0001
Pyrimethamine Sulfadiazine	25 100	20	8	40	< 0.008
Azithromycin Sulfadiazine	200 100	20	8	40	< 0.008
Azithromycin Pyrimethamine	200 25	20	0	0	< 0.0001
Azithromycin Sulfadiazine Pyrimethamine	200 100 25	20	19	95	-

*Comparing the combination therapy of azithromycin, sulfadiazine and pyrimethamine with other groups.

with azithromycin and sulfadiazine showed survival rate of 40%, sulfadiazine and pyrimethamine 40%, and azithromycin, sulfadiazine and pyrimethamine 95%.

The combination of azithromycin, sulfadiazine, and pyrimethamine was more effective than any single therapy ($p<0.0001$), combination of azithromycin and sulfadiazine ($p<0.008$), or sulfadiazine and pyrimethamine ($p<0.008$).

Discussion. This study has shown that the combination of azithromycin with sulfadiazine and pyrimethamine showed a synergistic effect and marked increase in the survival rate of mice following intraperitoneal inoculation of *Toxoplasma gondii*. All mice in the control group died within 7 days after inoculation. The RH strain of *Toxoplasma gondii* is a highly virulent strain. The organism tends to cause parasitemia and animals die of disseminated toxoplasmosis and encephalitis. Furthermore, the use of azithromycin alone appeared to be safe and effective in the prophylactic treatment of systemic murine Toxoplasmosis. When azithromycin was given in a single dose simultaneously with toxoplasma inoculation all mice survived the infection. All control animals, on the other hand, died. Azithromycin has been shown to be effective in the prophylaxis of murine toxoplasmosis with a highly virulent strain of *Toxoplasma gondii*.[18] Despite the availability of several antimicrobial agents for the treatment of Toxoplasmosis including clindamycin, spiramycin, sulfadiazine, pyrimethamine, minocycline, atovaquone, these agents appear not to be able to eradicate the cyst from tissues, and single drug may have limited value in the clinical disease and in patients with toxoplasmic retinochoroiditis.[19]

Agents such as pyrimethamine and sulfadiazine may fail to effectively cure human ocular toxoplasmosis due to inadequate levels at the site of the ocular lesion or owing to the local conditions in the retinal lesions that favor the growth of parasites in the retina. The severe inflammatory reaction may also lead to bystander injury of the retina and choroid. It is, therefore, conceded that search for new, effective, and safe antimicrobial agents for the treatment of toxoplasmosis is highly desirable. While several workers in the field of toxoplasmosis are continuing a highly justifiable search for new and better antimicrobial agents, consideration must be given to other factors such as the attachment and penetration of the organism to the host cell. The penetration of the antimicrobial agents into the cyst of toxoplasma and tissue may be limited. Therefore, the use of a combination of antimicrobial agents may be more effective than single agent in the eradication of the disease. In this experimental study, it is clear that the combination of sulfadiazine with azithromycin and pyrimethamine afforded protection against the ravages of the parasite. The combination was given 72 hours after the infection and for 3 days only. Therapy was initiated after establishing the disease process in mice.

Atovaquone and azithromycin have been shown to be effective against the bradyzoites *Toxoplasma gondii*.[20] Since clindamycin does not penetrate the blood brain barrier it is important to find drugs that are safe and effective in the treatment of toxoplasmic encephalitis in immunocompromised patients such as patients with AIDS. In patients with AIDS who are immunosuppressed may develop Toxoplasmic encephalitis, drugs such as clindamycin may not be effective in the treatment of the disease. Azithromycin has good penetration into the cerebrospinal fluid and may afford better protection against *Toxoplasma gondii* . The clinical trials for the assessment of the efficacy of azithromycin in the treatment of toxoplasmosis had been conducted in several centers. Azithromycin appears to be safe and effective in the treatment of toxoplasmosis and has good ocular penetration. The drug may be considered in pregnant women with toxoplasmosis who cannot receive pyrimethamine.

In conclusion, azithromycin alone or in combination with other antimicrobial agents has been found to be effective in the prophylaxis and treatment of murine toxoplasmosis. Azithromycin in combination with sulfadiazine and pyrimethamine may be considered as an alternative therapy for the management of Toxoplasmic retinochoroiditis.

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