

Azithromycin macrolids with a potent activity on chronic experimental toxoplasmosis

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ABSTRACT

Objective: To assess the efficacy of Azithromycin, a macrolids antibiotic, against experimentally induced *Toxoplasmosis* in murine model

Design: Experimental study.

Setting: Department of Microbiology, Nineveh College of Medicine, from October 2004 to November 2005.

Results: The efficacy of Azithromycin against *Toxoplasma gondii* was examined in murine models of infection with a moderately virulent strain. Forty white Albino mice inoculated by mouth with 2×10^6 cysts, 20 mice were treated with Azithromycin at dose of 250 mg/kg /day for 90 days. Drug treatment was initiated 30 days after infection (chronic infection). Treatment with Azithromycin significantly enhanced survival and reduced the brain cyst burden as compared with untreated (control) group of mice. Since 80% of treated mice were completely cured from toxoplasmosis as their brain were free of *Toxoplasma* tissue cysts either microscopically or by bioassay.

Conclusion: The results warrant clinical evaluation of Azithromycin in the treatment of toxoplasmosis in both immunocompetent and immunocompromised patients.

الخلاصة

اُختبرت كفاءة المضاد الحيوي ازيثرومايسين ضد الإصابة بالمقوسات الكوندية على أنماط الخمج في الفئران بالسلالة المعتدلة الضراوة. حقن ٤٠ فأراً عن طريق الفم بعشرين من الأكياس النسيجية لطيفلي المقوسات الكوندية وتم معالجة ٢٠ من هذه الفئران بالمضاد الحيوي ازيثرومايسين بجرعة مقدارها ٢٥٠ ملغم/كغم /يوم ولمدة ٩٠ يوماً. شرع في العلاج بعد ٣٠ يوماً من تنشئة الخمج في الفئران (خمج مزمن). اظهر العلاج بالمضاد الحيوي ازيثرومايسين انخفاضاً في معدلات الوفيات وانخفاضاً في عدد اكياس الدماغ النسيجية للمقوسات الكوندية مقارنة مجموعة الفئران المخمجة وغير المعالجة حيث تم شفاء ٨٠% من الفئران المعالجة تماماً من المقوسات الكوندية وتبين خلو أدمغتها من الاكياس النسيجية بعد فحصها مجهرياً وحيوياً. يمكن أن يستدل من النتائج الأهمية السريرية للمضاد الحيوي ازيثرومايسين في علاج داء المقوسات الكوندية في كلا من المرضى المثبتين والكفونين مناعياً.

Toxoplasma gondii is a well described ubiquitous protozoan parasite of mammals and birds that is an important human pathogen.^{1,2} Infection with this parasite result in dissemination throughout its host by the trophozoite differentiation into bradyzoites within cysts that remain latent.^{2,3} These bradyzoites can transform back into tachyzoites, and in immunocompromised individuals often results in symptomatic disease.

Infection is typically acquired by ingestion of undercooked meat, harboring tissue cysts (which contain bradyzoites). Infection can also be acquired by ingestion of food contaminated with oocysts or by exposure to cat feces containing oocysts. In immunocompromised patients, reactivation

of latent disease can cause life-threatening encephalitis.

In most individuals, acute infection with *Toxoplasma gondii* is asymptomatic or cause mild symptoms similar to a self limited mononucleosis like syndrome.^{4,5} If a seronegative woman is infected transmission of this parasite to the fetus can occur with the development of a congenital infection that can result in a fetopathy⁶, but the disease in the fetus is usually more severe earlier in pregnancy that infection and transmission occurs.

Toxoplasmosis remains a significant problem among immunocompromised patients, infected newborns and individuals with ocular *Toxoplasma* involvement and women who are infected during gestation.

In most cases, infection with *Toxoplasma* can be effectively treated with a range of drugs. The standard therapy regimen except during pregnancy includes a combination of pyrimethamine and sulfa, which cause bone marrow suppression, hematological toxicity, and or life threatening allergic reactions.⁷ Therefore, in up to 50% of cases, the standard regimen must be replaced by an alternative regimen of less effective drugs.⁸ A variety of new drugs with high in vitro activity against *Toxoplasma gondii* and fewer side effects have been developed.⁹

Treatment of acute toxoplasmosis with pyrimethamine plus sulfadiazine or pyrimethamine plus clindamycin has been successful in most patients but may be associated with considerable toxicity.^{10,11} The relatively high incidence of toxicity associated with these drug combinations frequently results in a lowering of the dosage or discontinuation of one or both drugs in the combination therapy predisposing to failure of treatment.¹¹

Antibiotic such as tetracycline's, lincosamides and macrolids like clarithromycin protect against toxoplasmosis in the murine model and are being evaluated as alternative therapies as well.^{12,13} Studies of the efficacy of drugs with activity against *Toxoplasma gondii* are commonly performed in murine models of both acute and chronic progressive infections.^{13,14}

Atovaquone has also been used with some success in people receiving increased attention for the treatment of a number of other parasitic infection.^{15,16} A number of other drugs have used experimentally but few have reached the clinic. We have previously demonstrated that ketolids, a new class macrolids are active in vivo against *Toxoplasma gondii*.^{17,18} Thus, drugs with increased potencies and lower level of toxicity are needed to treat all forms of toxoplasmosis.

The present study was aimed to evaluate the efficacy of Azithromycin in the treatment of experimental chronic toxoplasmosis.

Materials and methods

***Toxoplasma gondii*:** The moderately virulent *Toxoplasma gondii* strain which was originally isolated from infected human placenta and was maintained in laboratory by serial passage in mice as previously described¹⁷ cysts were harvested from the brains of infected mice 8 weeks previously.

Brain tissues of mice was ground through 22-gauge needle and diluted in phosphate buffered saline (PBS) before

inoculation. Infection was initiated by the inoculation of 2×10^6 cysts into the peritoneal (i.p) cavity of mice, an aliquot of brain suspension was used to determine the number of cysts in the preparation by microscopy.

Previous study in our laboratory demonstrated that this invariably led to fatal chronic toxoplasmosis in untreated animals with death occurring in 80% of cases between 10 to 24 weeks after challenge⁽¹⁷⁾. For cyst enumeration 25 ml of the brain suspension was placed on slides and microscopically counted.

Mice: Female albino mice weighting 18 to 22 g at the beginning of the experiment were used. Six mice in each cage were housed and offered drinking water ad libitum.

Antibiotic: Azithromycin (Med. Ph Indust, Damascus, MPI Syria) is an approved oral antibiotic that belongs to the macrolides class of drugs, it was also being tested as a treatment for mycobacterium avium intracellular, cryptosporidiosis and bacillary angiomatosis.⁸

A daily dose of 250 mg/kg/body weight of Azithromycin were administered orally by gavage, treatment was started 30 days after infection and continued for another 90 days.

Experimental Design: After the cystic peritoneal challenge 40 mice received sulphadiazine 100 mg/kg/per day from day 5 to 12 to prevent the development of acute toxoplasmosis. At day 30, mice were equally allocated to the treatment and control group. The experiment was carried out with a control group of untreated mice. Since murine infection with parasite is invariably lethal, survival rates and length of survival were considered parameters of drug efficacy. Mouse survival was monitored daily, the organs of mice that succumbed were removed for assessment of *Toxoplasma gondii* content and the number of organisms was counted.

The drug was measured by the prolongation of life of treated mice over controls and by the number of survivors which were crude of infection. Autopsy was done on mice that died during the experiment, at the end of the experiment the brain, spleen and liver were taken from survivors and ground with 10 ml phosphate buffer saline. One ml of the suspension was then injected intraperitoneally into a new mice, the donor mice was considered cured of the infection if all the recipient mice survived 45 days after injection.

Bioassay: If no cysts were observed in at least four samples of brain tissue

preparations, the remainder of the brain homogenate was inoculated into another two fresh mice per sample (250 ml each). Mice were killed after 6 weeks and the brain tissues were examined for the presence of cysts.

Results

Control (untreated) mice: All of the twenty (untreated) mice died within 90 days of the observation period after inoculation with ascites, the microscopic evidence of toxoplasmosis displayed evidence of severe encephalitis with brain cysts visible at microscopy.

The number of brain cysts were ranged from 36 to 105 cysts (mean 77.5) per brain (Table 1).

Histological examination of Azithromycin animals during observation period showed chronic nonspecific infiltration and cellular infiltration in both lungs, the brain lesions were characterized by perivascular cellular infiltration, the presence of microglial nodules and necrotic foci. Reactivation of latent infection in mice also resulted in inflammatory foci associated with the development of parasites in liver and lungs.

The presence of parasitic associated focal necrotic lesions in the brain parenchyma and menegial inflammation resulted in the death of untreated mice.

Treated Group: All the twenty treated mice which received Azithromycin survived and remained asymptomatic during the observation period which lasted for 90 days after challenge. At day 120 the end of the experiment all the remaining survived mice were sacrificed and their brain were tested for the presence of tissue cysts, the brains of 16 mice were found to be free from *Toxoplasma* tissue cysts either microscopically or by bioassay. In contrast, brains of the treated mice with 250 mg/kg Azithromycin showed neither inflammatory foci nor foci of parasitovorous vacuoles.

The increased survival in mice treated with Azithromycine 250 mg/kg/day regimen was associated with a significant reduction of the cyst burden. The examination of the brains of the remaining four mice revealed *Toxoplasma* cysts, their number ranged between 17 to 34 per brain with mean of 29 cysts per brain (Table 1).

Discussion

The vast majority of studies examining the therapeutic effect of antiparasitic drugs have been performed in murine models of either chronic progressive or latent disease.^{18,19,20} The results presented in this study clearly demonstrated that anti *Toxoplasma gondii* activity of Azithromycin in murine chronic Toxoplasmosis were effective in terms of both significantly increased survival rate and decreased brain cysts burdens compared with untreated control group of mice. The difference between two groups in terms of incidence of mortality was; therefore, highly significant.

In the view of known activity of Azithromycin against cysts, probably reflects the natural history of infection with the mouse strain, this strain tends continuously to give rise to new cyst formation, presumably proceeded by cyst rupture and proliferation of tachyzoites that are then converted into bradyzoites. Azithromycin is found to be active against these tachyzoites as it reduced their number and hence reducing the number of newly formed cysts.

Although *Toxoplasma gondii* was not completely eradicated, cysts counts in the brain were reduced in all treated groups compared with the control group. Extending these results experimentally, it has been shown that Azithromycine at dose of 250 mg was able to prevent the development of toxoplasmosis completely in 80% of infected mice with no parasite being detected in any tissue samples.

Table 1: The survival rate and brain cysts burden among treated and nontreated groups of mice.

Group	No of days	Infected mice		No of cysts per brain	Total number of cysts /mean
		No died	survival rate, %		
Control (untreated) group n= 20	59	2	90	36,29	1550/77.5
	65	2	85	36,31	
	79	3	70	37,61,73	
	80	1	65	79	
	85	2	55	78,85	
	86	3	50	78,81,93	
	89	2	45	103,101	
	90	1	20	105	
	120	4	0	100,110,110,120	
	Treated group n= 20	90	0	100	
120		16		No cysts	
		4		20,27,23,26	

* significant at $p < 0.001$, using nonpaired t test

The mechanism of action of Azithromycin against *Toxoplasma gondii* is not known, however, in bacteria, Azithromycin bind to the RNA of the 50S ribosomal subunit and act synergistically to inhibit protein synthesis.¹⁹ Since ribosome's encoded by prokaryotic-type ribosomal genes of *Toxoplasma gondii* are predicted to be sensitive to the macrolide class of antibiotics and may serve as the functional target to protein synthesis inhibitors in *Toxoplasma gondii* and related parasites²⁰

The results of this study demonstrated that Azithromycin had an enhanced effect against *Toxoplasma gondii*, it is possible that such enhancement in activity may be elicited through a mechanism similar to that observed in bacteria.^{21,22}

An advantage of macrolids is their fat solubility, which increases concentrations of the drugs in body tissues.²² Azithromycin in particular surpasses other macrolides in its targeted delivery. Of great value in some infections, this describes the capacity to penetrate the membranes of some immune cells and remain inside for several hours, as the cells naturally migrate to sites of infections, so does their passenger, spilling out in the attack and enhancing the cells fight against the infection.²³ Of interest are the results of experiment which evaluate the activity of Azithromycin in treatment of *Toxoplasma* encephalitis. A significant reduction in the inflammatory response in the brains of mice treated for 90 days with a dose of 250 mg of Azithromycin per kg per day was noted.

The present results suggest that Azithromycin used was effective for treatment of infection in the central nervous system in our murine model. However, since the number of *Toxoplasma gondii* cysts in the brains of treated mice were significantly reduced at the end of the therapy, suggesting that Azithromycin acts not only against the slowly replicating bradyzoites protected with the cyst wall, but also acts against free parasites or against rapidly replicating tachyzoites within cells.²⁴⁻²⁶

In vitro studies and studies in animals using Azithromycin demonstrates activity against several stages of *Toxoplasma gondii* including the cyst form.²⁷

There is clear evidence that the newer macrolids are active against *Toxoplasma gondii* both in vitro²⁸ and in vivo²⁹ although their mode of action is not clear.^{30,31} We have previously shown the efficacy of macrolids in the treatment of acute, chronic and *Toxoplasma* encephalitis in murine model. But, to be noted that is the first time for Azithromycin to be tested against this

strain of *Toxoplasma gondii* isolated from human placenta since the inflammatory responses in the brains of mice treated for 90 days with 250 mg of Azithromycin per kg per day were significantly reduced compared with those in the brains of untreated controls. These observations suggest that clinical trials with Azithromycin for treatment and prevention of human toxoplasmosis may be justified and the results warrant clinical evaluation of Azithromycin in the treatment of toxoplasmosis in both immunocompetent and immuno-compromised patients.

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