EFFICACY OF AZITHROMYCIN ON EXPERIMENTAL TOXOPLASMOsis INFECTED MICE

By

MONA E. NASR¹, ATEF H. ABD EL HAMID¹, NAGWA S. M. ALY¹, GHADA H. OMAR¹*, ASHRAF M. A. BARAKAT², KAWKAB A. AHMED³, SAFAA H. YOUSIF⁴and GEHAN A. RASHED¹

Department of Parasitology¹, Faculty of Medicine, Benha University, and Department of Zoonotic Diseases², National Research Centre, Cairo 12622, and Department of Pathology³, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

*Correspondence: ghadahelmy84@yahoo.com

Abstract

Toxoplasma gondii is an opportunistic protozoan that causes a devastating disease in immunocompromised individuals and congenitally infected neonates or children. This study evaluated the efficacy of early Azithromycin treatment of Toxoplasma gondii infected mice with cystogenic Me 49 non-virulent strain. Fifty laboratory-bred female Swiss albino mice were divided into 5 groups: G1: Non-Infected, G2: Infected non-treated, G3: Infected and treated by Azithromycin 200 mg/kg at 4th day post-infection for 3 days, G4: Infected and treated by Azithromycin 200mg/kg at 4th day post-infection for 10 days & G5: Infected and treated by Spiramycin 200mg/kg at 4th day post-infection for 2 weeks, brain cysts' number, size and histopathological changes were assessed after 3 months. Treatment with Azithromycin for 10 days decreased number and size of brain cysts by 70% & 40% respectively without significant difference with Spiramycin treated mice that caused reduction by 74% & 50% respectively. Azithromycin treatment for 3 days caused the least reduction by 51% & 21% respectively and Azithromycin treatment for 10 days decreased the histo-pathological changes in cerebral toxoplasmosis. Azithromycin treatment started at 4th day post-infection was effective than Spiramycin against T. gondii infection with a significant effect on cysts count and size and brain pathological changes. Azithromycin treatment for 10 days gave better effect than 3 days.

Keywords: Mice, Azithromycin, Spiramycin, Toxoplasma gondii, Treatment.

Introduction

Toxoplasmosis is a disease caused by Toxoplasma gondii an obligate intracellular protozoan parasite infecting many hosts, including humans (Hampton, 2015). Toxoplasmosis is categorized into acute and chronic stages. Acute or early stage correlated with the proliferative form (tachyzoite), but tissue cyst predominated during chronic infection, although tachyzoites have been found outside of cysts at this stage (Sullivan and Jeffers, 2012). Studies on T. gondii drug(s) is a complicated task and must be active against both tachyzoites and bradyzoites (Konstantinovic et al, 2019). In-vitro & in-vivo studies were carried out to reach LC₅₀, a high selectivity index in vitro, prolonged survival of infected in animal models of acute infection and decreased parasite loads in brain and muscle tissues in chronic infection (McFarland et al, 2016). Treatment of cerebral toxoplasmosis was hindered by the less-drug brain penetration to have effective dose (Chew et al, 2012). Combination of sulfadiazine & pyrimethamine were effective against acute toxoplasmosis (Israelki and Remington, 1993), but was less effective against chronic cerebral one (Faucher et al, 2011). Pyrimethamine and sulfadiazine gave some side-effects (Castro-Filice et al, 2014).

Azithromycin, a macrolide antibiotic, is a derivative of erythromycin with an anti-Toxoplasma effect (Castro-Filice et al, 2014), used to treat pneumonia, Chlamydia, and protozoa like Entamoeba histolytica, Giardia lamblia (Crouch et al, 1990; Lode et al, 1996), Plasmodium spp. (Srivastava et al, 2012), Cryptosporidium parvum (Giacometti et al, 2000) and Leishmania major (Kroliewiecki et al, 2002). In-vitro, human Azithromycin dose neither affected fertility nor the fetus (Briggs et al, 2011).

This study aimed to evaluate the efficacy of early Azithromycin® treatment of acute to
xoplasmosis in murine with cerebral infection by Me 49 non-virulent strain of *T. gondii* as compared to Spiramycin®.

### Materials and Methods

*T. gondii* Me 49 non-virulent strain was kindly supplied by Zoonosis Department, National Research Center was regularly maintained by sub-passage in Swiss Albino mice with 0.1ml of brain homogenate, as 1x10^5 tissue cysts/ml every 8 weeks to develop chronic toxoplasmosis. Brains were grinded with sterile pestle in a clean mortar and diluted to 1x10^2 cysts/ml brain cysts suspension (Djurković-Djaković *et al*, 2002).

Drugs: 1- Spiramycin tablets (control) 3 MIU (704mg) Spirex were purchased from Medical Union Pharmaceuticals, Egypt. Tablets were crushed and dissolved in distilled water for oral suspension a dose of 200mg/kg/day. 2- Azithromycin drug 200mg/5ml powder for oral suspension Zithromax manufactured by Pfizer Egypt. The drug was daily prepared as liquid suspension; after sonication, the homogenized suspensions were given orally to mice by stomach tube, in a dose of 200mg/kg day. Oral dose was 0.1ml/mouse (Grujic *et al*, 2005).

Experimental Design: Fifty clean laboratory-bred female Swiss albino mice, 10 weeks old and 20-25gm were divided into 5 groups of 10 mice in each: G1: Non-Infected, G2: Infected non-treated group, G3: Infected and treated by Azithromycin 200mg/kg at 4th day post-infection for 3 days, G4: Infected and treated by Azithromycin 200mg/kg at 4th day post-infection for 10 days, G5: Infected and treated by Spiramycin 200mg/kg at 4th day post-infection for 2 weeks.

Assessment of anti-*Toxoplasma* effects: 1- Brain cysts number: At the end of 8 weeks, all mice were sacrificed, and brains were dissected out. Each brain was divided into 2 parts, one part for counting the cysts and the second was fixed in 10% formalin for histopathological studies. They were harvested, rinsed in sterile saline solution, weighed, and 1ml of sterile saline was added, follo-

The following equation was used: Mean cyst number = cyst count in 100μl×10×2.

- Brain cyst size was measured by an ocular & stage micrometers.

Statistical analysis: Data were coded, tabulated and analyzed using (ANOVA) procedure and Post hoc test.

### Results

The brain cysts counting (Tab. 1) showed that Azithromycin and Spiramycin treated groups caused significant reduction in number as compared to infected non-treated control ones (*P*<0.05). (G4) treated by Azithromycin for 10 days gave a higher significant reduction in the number as compared with mice treated by Azithromycin for 3 days (*P*<0.05). But, mice treated by Azithromycin for 3 days significantly reduced number of brain cyst, still Spiramycin treated mice (G5), showed more reduction (*P*<0.05). (G4) treated by Azithromycin for 10 days gave more or less equal effect as Spiramycin treated ones (*P*>0.05).

Brain cysts size (Tab. 2) showed that Azithromycin and Spiramycin treated mice caused a significant reduction in size as compared with control infected non-treated mice except those with mice treated by Azithromycin for 3 days. (G3) did not cause significant reduction in size as compared to mice treated by Azithromycin for 10 days (*P*<0.05). Both Azithromycin treated mice showed lower statistical reduction in cyst size as compared with Spiramycin treated mice (G5) (*P*<0.05).
Microscopically brain sections from mice infected with Me 49 non-virulent strain of *T. gondii* showed many histopathological alterations in infected non-treated ones in the form of multiple large *Toxoplasma* cysts scattered through the brain parenchyma (Fig. 1), multiple focal necrosis associated with severe glia cells infiltration and severe perivascular cuffing with mononuclear inflammatory cells (Fig. 2). Mice treated by Azithromycin for 3 days showed histopathological alterations in form of mild meningitis, shrunken, atrophy and severe neurons necrosis (Fig. 3) with neurofibrillary tangles (Fig. 4). Moderate perivascular cuffing with mononuclear cells were noticed (Fig. 5). More improvement was in brain tissues in mice treated by Azithromycin for 10 days, as moderate degeneration, necrosis of some neurons and intracellular edema (Fig. 6), mild neuron ophagia of degenerated neurons (Fig. 7) and moderate gliosis (Fig. 8) were noticed, but, without meningitis or perivascular cuffing. The brain of mice infected with *T. gondii* and treated with Spiramycin 4 days post infection for 2 weeks mice showed congestion of some blood vessels with perivascular edema and degeneration of neurons (Fig. 9).

**Discussion**

In the present study, (G4) treated by Azithromycin for 10 days (4th day post infection) with good therapeutic efficacy among mice. This treatment regimen reduction rate in brain cysts’ number was 70%. More or less similar effect was recorded with the matching (G5) treated by Spiramycin (74%) without significant difference between both drugs, and the least reduction found in mice treated by Azithromycin for 3 days (51%). This agreed with Derouin *et al.* (1992) who reported that administration of azithromycin at 300, 150, or 75 mg/kg of body weight per day for 10 days from day +1 post-infection did not prevent *T. gondii* dissemination to the brain.

In the present study, a dose (200mg/kg) of Azithromycin administered daily for only 3 days was significant when compared with infected control group but better effect was obtained when used for 10 days, as Azithromycin is protein bound in serum and eliminated slowly due to low serum clearance and extensive distribution in the tissues. Thus, detectable levels of the drug can be found in the urine 7 to 14 days after administration of a single dose. The relatively long half-life, the affinity for tissues, and the slow elimination of azithromycin indicate potential for a once-a-day dosing regimen (Araujo *et al.*, 1988). These results were explained by the fact that Azithromycin have significant intracellular accumulation (Gladue *et al.*, 1989) and the only macrolide causing prolonged inhibitory activity on intracellular tachyzoites replication (Chamberland *et al.*, 1991).

### Table 1: Effect of Azithromycin on number of ME49 *Toxoplasma gondii* strain brain cysts in female Swiss albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose/day</th>
<th>Total dose</th>
<th>No of <em>Toxoplasma</em> cyst in mice brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>95% CI</td>
<td>% reduction</td>
</tr>
<tr>
<td>G3: Infected and treated by Azithromycin for 3 days</td>
<td>200mg/kg/day</td>
<td>600mg/kg/day</td>
<td>260±43</td>
</tr>
<tr>
<td>G4: Infected and treated by Azithromycin for 10 days</td>
<td>2000 mg/kg/day</td>
<td>164±26</td>
<td>145-183</td>
</tr>
<tr>
<td>G5: Infected and treated by Spiramycin for 2 weeks</td>
<td>200mg/kg/day</td>
<td>2800 mg/kg/day</td>
<td>138±46</td>
</tr>
<tr>
<td>G2: Infected non treated group</td>
<td>N/D</td>
<td>N/D</td>
<td>526±40</td>
</tr>
</tbody>
</table>

SD: Standard deviation. CI: convenient interval. N/D: No Data

### Table 2: Effect of Azithromycin on size of ME49 *Toxoplasma gondii* strain brain cyst in female Swiss albino infected mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose/day</th>
<th>Total dose</th>
<th>No of <em>Toxoplasma</em> cyst in mice brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>95% CI</td>
<td>% reduction</td>
</tr>
<tr>
<td>G3: Infected and treated by Azithromycin for 3 days</td>
<td>200mg/kg/day</td>
<td>600mg/kg/day</td>
<td>19±3</td>
</tr>
<tr>
<td>G4: Infected and treated by Azithromycin for 10 days</td>
<td>2000 mg/kg/day</td>
<td>15±2</td>
<td>15-17</td>
</tr>
<tr>
<td>G5: Infected and treated by Spiramycin for 2 weeks</td>
<td>200mg/kg/day</td>
<td>2800 mg/kg/day</td>
<td>12±1</td>
</tr>
<tr>
<td>G2: Infected non treated group</td>
<td>N/D</td>
<td>N/D</td>
<td>24±4</td>
</tr>
</tbody>
</table>

SD: Standard deviation. CI: convenient interval. N/D: No Data
Azithromycin was superior to both Roxithromycin and Spiramycin in terms of protecting mice against death from acute toxoplasmosis (Araujo et al, 1991). Synergistic activity of Azithromycin combined with metronidazole against experimental toxoplasmosis infected mice was reported (Al-Jader and Al-Mukhtar, 2010). Azithromycin has a good effect in vitro and more effective to control the trophoblast infection with both 2 Brazilian T. gondii genotypes, TgChBrUD1 or TgChBrUD2 when compared to conventional antibiotics Spiramycin and sulfadiazone/pyrimethamine (Ribeiro et al, 2017). Lopes et al. (2009) found that Azithromycin has high potency than different toxoplasmosis treatment option during pregnancy as it reduced the number of pathogenic cysts in brain, and none was detected in the fetuses eyeballs of infected female mice in contrast with mice treated by a combination of Azithromycin (300mg/kg), pyrimethamine (100 or 50mg/kg), sulfadiazone (100 or 75mg/kg), and folic acid (15mg/kg).

In the present study, Azithromycin treated groups effectively reduced the size of brain cysts by 21% in mice treated for 3 days and 40% in mice treated for 10 days in contrast to 50% in Spiramycin treated mice. This could be due to that Azithromycin is the only macrolide demonstrating prolonged inhibitory activity on replication of intracellular tachyzoites (Chamberland et al, 1991), and killed intra-cystic bradyzoites in vitro 6 days after incubation, and decreased the cysts’ size (Huskinson-Mark et al, 1991).

In the present study, the patterns of T. gondii cyst growth within the brains of experimentally infected mice differed among the mice groups. The tissue cysts were often spherical with well-defined cyst walls and varied in size, from small cysts with 1 or 2 bradyzoites, to large cysts with more than 50 bradyzoites. These results agreed with others found that Toxoplasma cysts in brain grow uniformly in size up to 2 to 3 months post infection and persist for many months post infection (Melzer et al, 2010).

Araujo et al (1991) found that Spiramycin was effective in acute murine infection, with an inhibitory rather than a curative effect.

In the current study, Spiramycin showed the highest effect in decreasing number and size of brain cysts. This agreed with Grujić et al. (2005) who found that a 3-week course of 100mg Spiramycin/kg/day and a 4-week course of 200mg/kg/day in mice infected by cysts of Me49 strain, significantly enhanced protection and markedly reduced brain cyst burdens at 6 months post infection.

In the present study, the brain sections of mice infected with the Me49 non-virulent T. gondii strain showed severe histopathological changes in infected non-treated mice (G2) in the form of multiple large Toxoplasma cysts scattered through the brain parenchyma, multiple focal necrosis associated with severe glia cells infiltration and severe perivascular cuffing with mononuclear inflammatory cells. This agreed with Mahmoud (2007) who found that brain lesions were recognized by peri-vascular cellular infiltration, presence of microglial nodules and necrotic foci. The result also agreed with Hermes et al. (2008) who found that toxoplasmosis infection caused a local degenerative cell loss, parasites within neurons and directly caused death of infected neurons or its atrophy and inflammation contributed by the production of nitric oxide and other toxic oxygen products causing to neighboring neurons death.

The least histopathological lesions were found in Spiramycin treated mice (G5). The most effective Azithromycin was achieved in mice treated for 10 days (G4) that showed mild to moderate neuropathological alterations as neurofibrillar tangles, neuronophagia of degenerated neurons, congestion of cerebral blood vessel and proliferation of glia cells and the worst effect was found in Azithromycin treated mice for 3 days (G3) in form of severe necrosis of neurons, neuronophagia, neurofibrillar tangles, perivascular cuffing with mononuclear cells, meningitis (inflammatory infiltrate in meninges).
The result agreed with Dumas et al. (1994) who recorded that the histological examination of untreated animals showed inflammatory infiltrates and mean encephalitis score was 2.7/3. The brain lesions were characterized by a perivascular leucocyte infiltration (mainly around intracerebral and meningeal blood vessels), presence of microglial nodules and foci of necrosis. But, in Azithromycin treated mice, histological examination showed less inflammatory infiltration and mean encephalitis score was 0.7/3.

**Conclusion**
Azithromycin treatment at 4th day post-infection was effective as Spiramycin against *T. gondii* infection with a significant effect on cysts count and size and improved brain pathological changes. Also, Azithromycin treatment for 10 days gave better effect than 3 days treatment. Further in vivo and in vitro studies are recommended to investigate the Azithromycin efficacy against *T. gondii* on a wider scale, to identify its action as anti-*T. gondii* drug and the standardized human doses.

**References**


Azithromycin reduces ocular infection during congenital transmission of toxoplasmosis in the Calomys callosus model. J. Parasitol. 95, 4:1005-10.


Explanation of figures

Fig. 1: Brain tissue from infected non treated mice (G1) showed normal histological structure (H & E stain, X200).

Fig. 2: Brain tissue from infected non treated mice (G2) showed multiple focal necrosis associated with glia cells infiltration (short arrow) and perivascular cuffing with mononuclear inflammatory cells (long arrow) (H & E, scale bar 25um).

Fig. 3: Brain tissue from mice infected and treated by Azithromycin for 3 days (G3) showed shrunken, atrophy and necrosis of neurons (arrow). (H & E, scale bar 25um).

Fig. 4: Brain tissue from mice infected and treated by Azithromycin for 3 days (G3) showed neurofibrillary tangles (arrows). (H & E, scale bar 25um).

Fig. 5: Brain tissue from mice infected and treated by Azithromycin for 3 days (G3) showed intravascular permeation with mononuclear inflammatory cells associated with perivascular cuffing with mononuclear cells (arrows) (H & E, scale bar 10um).

Fig. 6: Brain tissue from mice infected and treated by Azithromycin for 10 days (G4) showed necrosis of neurons (short arrow) and intracellular edema (long arrow) (H & E, scale bar 25um).

Fig. 7: Brain tissue from mice infected and treated by Azithromycin for 10 days (G4) showed neuronophagia of degenerated neurons (arrow) (H & E, scale bar 25um).

Fig. 8: Brain tissue from mice infected and treated by Azithromycin for 10 days (G4) showed proliferation of glia cells (diffuse gliosis) (arrow) (H & E, scale bar 25um).

Fig. 9: Brain tissue from mice infected and treated by Spiramycin for 2 weeks (G5) showed congestion of some blood vessels (arrow) with perivascular edema and degenerated neurons. (H&E stain, X200)

Fig. 10: Brain tissue from control non-infected mice (G1) showed normal histological structure (H & E stain, X200)