

Synergistic activity of azithromycin combined with metronidazole against toxoplasmosis in experimental mice

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ABSTRACT

Objective: To evaluate the efficacy of synergistic activity of azithromycin combined with metronidazole against toxoplasmosis experimentally induced in murine model.

Material & Methods: Albino mice model (balb/c mice) acutely infected with tachyzoites, were treated for ١٠ days from day +١ postinfection with administration of azithromycin combined with metronidazole.

Results: The results showed that prolonged survival of albino mice relative to that of untreated control, and the dead mice were found only in the control group. This experiment showed that a remarkable synergistic effect on toxoplasmosis was observed when azithromycin was administered in combination with metronidazole at curative dosage i.e. (٢٥٠ and ٢٠٠ mg/kg/day), respectively. These results showed that azithromycin, which is remarkably active on toxoplasmosis, significantly potentiates the curative effect of metronidazole in the treatment. The treated mice in uncontrolled group were completely cured from toxoplasmosis as their brain were free of *T. gondii* cyst either microscopically or by bioassay.

Conclusion: Combined drugs of azithromycin and metronidazole showed a cure rate of ١٠٠% in experimental murine toxoplasmosis.

الخلاصة

الهدف: تقييم الكفاءة التعاونية لعقاري الازيثروميسين والميترونيدازول ضد داء المقوسات في الفئران المختبرية. **المواد وطرق العمل:** اتسعملت الفئران البيض من نوع (Balb/c) والتي اصيبت بالحيوانات سريعة التكاثر ولمدة عشرة ايام وتم حقنها بعقار الازيثروميسين اعتباراً من اليوم الاول للاصابة. **النتائج:** اظهرت النتائج بقاء حيوانات التجارب مدة اطول من حيوانات السيطرة ووجدت الفئران الميتة ضمن زمرة السيطرة فقط، كما اظهرت هذه التجربة بحصول تأثير تعاوني جلي ضد داء المقوسات عند اعطاء عقار الازيثروميسين مجتمعاً مع الميترونيدازول بجرعة ٢٥٠ و ٢٠٠ ملغم بالتناوب، كما اظهرت النتائج ان عقار الازيثروميسين والذي كان فعالاً بوضوح ضد داء المقوسات قد حفز التأثير العلاجي للميترونيدازول. كما شفيت فئران التجارب تماماً من داء المقوسات حيث تبين ان ادماغتها كانت خالية من طفيلي المقوسات الكوندية بعد اجراء الفحص الميكروسكوبي والاختبار الاحيائي. **الخلاصة:** اعطاء عقاري الازيثروميسين والميترونيدازول معاً اظهر نسبة شفاء تعادل ١٠٠% لدى الفئران المختبرية المصابة بداء المقوسات.

T *ondigi* is the one of the most common protozoan of mammals, birds and considered as an important human pathogens which is typically a brain infection and can also affect the eye and other organs. Only the cats play a major role in transmission of the disease because they harbor the reproductive forms of the parasite. Usually the acute infection with *T.gondii* is either asymptomatic or cause mild symptom, but in case of transmission of this parasite to the fetus during pregnancy, the development of a congenital infection that

can result in a fetopathy, but the disease in the fetus is usually more severe earlier in pregnancy that infection and transmission occurs. The most severe form of toxoplasmosis occur in immunocompromised patients includeing AIDS, malignancis, leukemia, collagen vascular diseases, organ transplant recipients and infected new born patients with ocular toxoplasmosis and women who are infected during gestation.

Treatment of toxoplasmosis has not changed essentially during the past few decades, consist of various combination of

antibiotic drugs; the most common agents used are pyrimethamine, clindamycin, and the sulphonamides. These drugs are some times given in combination with corticosteroids to alleviate the inflammatory reaction especially in treatment of ocular toxoplasmosis which is responsible for the tragic loss of eye sight in this parasitic disease.² In addition, the combination of these drugs in the standard therapy regimen has been successful in most patients but may cause bone marrow suppression, hematological toxicity and or life threatening allergic reactions.³ The relatively results in a lowering of the dosage or discontinuation of one or both drugs in the combination therapy predisposing to failure of treatment.⁴ Thus the purpose of this study is to investigate alternative drugs with lower level of toxicity and increase potencies to treat toxoplasmosis. As far as we know the present was the first to evaluate the efficacy of the combination of azithromycin and metronidazole in the treatment of experimental toxoplasmosis.

Material and Methods

Collection of samples

T.gondii was originally isolated from sixty suspected infected placentae taken from aborted and sixty normal delivery women attending Al-Batol, Al-Khansah and Al-Salam Teaching Hospitals in Mosul. All samples of placentae were examined microscopically by direct methods, and biopsy was done in normal (uninfected) mice to confirm the diagnosis.⁵ The infected one processed according to the method described by previous workers.⁶ From each placenta 0.5 g were taken and cut into small pieces mixed with equal volume of normal saline and grinded with mortar and a pestle or homogenized by electric homogenizer. The suspension was filtered through cotton to avoid large particles.

One thousand units of penicillin and 100 mg of streptomycin were added to prevent contamination.⁷ 1 ml of the filtered solution inoculated through intraperitoneal passage in laboratory bred, healthy Swiss albino mice (8-10 weeks old) to maintain the infection of intraperitoneal fluid with *T.gondii*. These infected mice were sacrificed within 3-5 days after inoculation. They were regularly inoculated intraperitoneally throughout the experiment by means of sterile tuberculin

syringe, delivering 0.1-0.2 ml of fresh peritoneal exudate taken from a mouse inoculated intraperitoneally 2 days before.¹¹ The exudates contained approximately 10⁶ organisms/ml. Dilution of heavily infected exudate was done with sterile normal saline solution.¹² The experimental white albino mice (Balb/c strain) 8-10 weeks old of both sexes were used, and these mice were examined to be sure that they were not infected with toxoplasmosis by using serological tests Latex agglutination & ELISA.¹³

Induction of toxoplasmosis in mice: For evaluation to get infection orally or intraperitoneally equal doses of tissue cysts (1 ml.) contain 10⁶ cysts were given to 20 mice. These mice were divided into two groups. The first group of mice were given orally, and in the second group, cysts were given by inoculation intraperitoneally. The peritoneum exudates were examined for each group 2-3 days after inoculation for diagnosis of *T. gondii*. One month after inoculation the mice were sacrificed, their brains were examined to find the tissue cysts. The inoculation was also done in another 20 mice by using equal doses (1 ml) contain 10⁶ tachyzoites inoculated orally and intraperitoneally. The mice were under observation for diagnosis of the parasite as mentioned before.

Combined antimicrobial chemotherapy:

1. Azithromycin. This drug is an antibiotic belongs to the macrolide group, Its action is to bind to the 50S subunit of bacterial ribosomes, blocking protein synthesis, breterostatic.¹⁴ It was also used against parasitic infection like cryptosporidiosis and angiomatosis, also has potent activity against chronic experimental toxoplasmosis.¹⁵

2. Metronidazole. (benzoyl metronidazol) It has fungicidal activity and also it has a direct action on parasitic infection like trichomoniasis, giardiasis cryptosporidiosis and amoebiasis. It is thought to enter the cells of microorganisms that contain nitroreductase. Unstable compounds are then formed that bind to DNA and inhibit its synthesis, causing cell death.¹⁶

The doses of combined drugs used in this experiment were as follows:

Azithromycin (200 mg/kg) + Metronidazole (200 mg). Ten infected mice by intraperitoneal cysts were treated with metronidazole and azithromycin which was given before. The combined treatment was given 24 hours after inoculation and continued for 14 days post-infection. The

mice were under observation for 30 days, and the experiment was terminated after 30 days post-infection. Other 10 mice was not given any treatment and served as control. At the end of the experiment all mice were sacrificed. Microscopical examination and bioassay were done for brain of mice, and the living mice were evaluated for efficacy of drugs used. Data were analyzed statistically using Z-test between two proportions to compare between methods and drugs. The results were considered significant at ($P < 0.05$). Numbers, percentages were also calculated.

Results

Synergistic activity of azithromycin and metronidazole

All ten mice were found dead in the control group. These mice had not been given any treatment from the 7th day till the 13th day after inoculation with *T. gondii*. They showed signs and symptoms of illness (weakness, piloerction, ascitis of abdomen) which were not seen in treated group. After three days from the beginning of treatment the mice became active and their activity were normal in the period of treatment (13 days) then the treatment was stopped for all mice. The observation of these mice was continued for the period of 30 days. The results revealed that the dead mice were found only in controlled group, but those mice which treated with azithromycin plus metronidazole were still living. This experiments showed that the synergistic activity of azithromycin and metronidazole had the ability to protect mice from acute toxoplasmosis, and there was a significant difference ($P < 0.05$) in the treated group in comparison with the control group (Table 1).

Discussion

Metronidazole is an antibiotic approved as a fungicidal, and act against *T. vaginalis*, *E. histolytica* and works at both intestinal

and extraintestinal amoebiasis, and this antiprotozoal drug enter into the microorganisms and bind to DNA and inhibited synthesis leading to cell death¹¹, azithromycin, a similar drug, have been approved as antitoxoplasmosis, and non-toxic, and azithromycin cannot prevent recurrent disease¹², so they might be useful in treating toxoplasmosis, and to bring attention to these treatment possibilities, so that others can investigate further and organize clinical trails to determine whether these drugs are valuable for treatment of toxoplasmosis and the possibility of preventing recurrence of ocular toxoplasmosis and for treating AIDS-related infections.

The results showed that all 10 mice in the control group died due to ascites. The parasites were detected in the peritoneal cavity and in the brain of dead mice. The result showed that all mice were alive in group which had been treated with azithromycin combined with metronidazole (Table 1). Others they showed that the combination of azithromycine with pyrimethamine resulted in a greater inhibition of *Toxoplasma* protein synthesis compared to azithromycin alone in vitro assay using mouse macrophages challenged with *T. gondii* tachyzoites¹³ thus, our results showed that using azithromycin and metronidazole in experimental infection in mice with *T. gondii* is considered as fruitful work which gave a good result in the treatment. This study showed that the combined use of the drugs yielded better results in terms of cure rates. So these results indicates that evaluation of the efficacy of azithromycin and metronidazole, preferably by means of randomized studies is needed to determine the future role of these and other drugs in the treatment of toxoplasmosis and the possibility of preventing recurrence of ocular toxoplasmosis and for treating AIDS-related infections.

Table 1: Effect of combined antimicrobial agents in mice infected with *T.gond*

Group	Antimicrobial dose (mg/kg daily)	30 days after infection			
		mice		Bioassay for living mice	
		No.	Living %	No.	Cure %
First	Azithromycin 200 + Metronidazole 200	10	100%	10	100%
Second	Control no treatment	10	.	.	.

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