Department of Zoonotic Diseases,
National Research Centre, Egypt

THE EFFECT OF CLINDAMYCIN AND SULFADIAZINE ON EXPERIMENTAL MURINE MODEL WITH ACUTE TOXOPLASMOSIS

(With 3 Tables and 4 Figures)

By

A.M.A. BARAKAT and SYLVIA O. AHMED*

*Dept. of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Assiut University, Egypt.

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تأثير الكليندااميسين والسلفاديازين على فئران التجارب المعدية بداء التكسوبلازموزيس (الطور الحاد)

أشرف بركات ، سيلفيا أحمد

مرض التكسوبلازما من الأمراض المشتركة التى لها أهمية كبرى عند الانسان والحيوان. الطفيل يسبب المرض فى كل من الإنسان والحيوان من خلال ابتلاع الطور الحويصلى المفرز فى براز القطط المريضة أو عن طريق تناول الطور المعدى فى اللحوم غير المطهية جيداً. وقد صممت هذه الدراسة لتوضيح تأثير كل من دواء الكليندااميسين والسلفاديازين كل على حدا وكذلك تأثيرهما سوياً على الفئران المصابة بداء التكسوبلازما (الطور الحاد). تم عدوى ثلاثة مجموعات من فئران (نوع البينو السويسرية) بحقن التاكيزويت فى الغشاء البريتونى بعدد 10^3 من النوع الضارى ار اتش. وتركت مجموعة رابعة بدون عدوى (ضابطة). تم إعطاء المجموعات المختلفة دواء الكليندااميسين والسلفاديازين كل على حدا ومجتمعة على حد سواء بدءاً من اليوم الأول للعدوى وحتى أربعة عشرة يوماً بعد العدوى. تم عمل اختبار الفحص البيولوجى وكذلك الكشف عن الحمض النووى للطفيل. وقد تبين وجود أثر العلاج فى جميع الفئات المعالجة مقارنة مع الفئران الغير معالجة. وكان تأثير العلاج بالكليندااميسين والسلفاديازين اعطى حماية مؤثرة فى الفئران المصابة. وكان العلاج بالسلفاديازين والكليندااميسين بالجرعات 25 الآتية (25 + 25 مج/كجم من وزن الجسم) و (25+50 مج/ كجم من وزن الجسم) و(50+50 مج/كجم من وزن الجسم) وكانت النتائج ايجابية بنسب 25، 37، و81% من الفئران المصابة على التوالى، مما يدل على التأثير التأزرى الكبير للكليندااميسين والسلفاديازين ضد طفيل التكسوبلازما جوندياى خاصة فى حالات العدوى الحادة.

SUMMARY

Toxoplasmosis has zoonotic importance among human and domestic animals. It causes diseases in both man and animals through ingestion of oocysts that passed in faeces of cat or by ingestion of cysts in undercooked

meat. The present study was designed to clarify the effect of Clindamycin (CLI) combined with Sulfadiazine (SD) on a murine model of acute toxoplasmosis. Albino Swiss mice were intraperitoneally infected with 10^3 tachyzoites of the RH strain of *Toxoplasma gondii* and were per orally treated with either drug alone (SD) or (CLI) or both combined. Starting with day 1 for 14 days. Survival was monitored during 8 weeks. Residual infection was assessed by a bioassay of representative 4-week survivors and by parasite DNA detection using PCR for representative 8-week survivors. An effect of treatment was shown in all treated groups compared to untreated control mice. Among mice infected with parasite, SD and CLI at any dose combination protected more animals. However, treatment with SD plus CLI at 25 plus 25, 25 plus 50, and 50 plus 50 mg/kg/day protected 25, 37, and 81% of mice, respectively, thus demonstrating a significant synergistic effect of SD and CLI against *T. gondii* especially in cases of acute infection.

Keywords: *Toxoplasma gondii, Clindamycin, Sulfadiazine. PCR.*

INTRODUCTION

Toxoplasmosis is one of the most common & opportunistic parasite with zoonotic importance (Tenter *et al.,* 2000). *Toxoplasma gondii* is an obligate intracellular protozoon parasite capable of infecting many species of mammals (Obata 1996; Dubey1981 and Hassanain *et al.,* 1996). It causes diseases through ingestion of oocysts that passed in faeces of cat or by ingestion of cysts in undercooked meat (Johnson *et al.,* 1990) and (Tenter *et al.,* 1992) (Antonion *et al.,* 1995) (Hejlicek, and Literak 1995) (Paniker 2002). *Toxoplasma gondii* ranks among the 10 most commonly occurring opportunistic infections and emerged due to the latent infection in immunosuppressant hosts (Barakat *et al.,* 2009). Also fatal toxoplasmosis might occur after acute illness in domestic animals (Dubey *et al.,* 1992). *Toxoplasma gondii* encephalitis is an important and estimated to occur in 20.000 to 40.000 patients with acquired immunodeficiency syndrome in USA. (Daniel *et al.,* 1990).

The standard therapy for toxoplasmosis infection is pyrimethamine (PYR) and sulfonamides could adequately treat toxoplasmosis using a murine model of acute toxoplasmosis was infected intraperitoneally with 10^4 parasites of the RH strain of *Toxoplasma gondii* (Louis *et al.,* 1999). (Araujo *et al.,* 1992) stated that the effect of Azithromycin or sulfadiazine were did not provide any protection against death due to toxoplasmosis, while in combination remarkably and significantly synergistic action.

Also common used of antibiotics include pyrimethamine, trisulfapyrimidines, sulfadiazine, Clindamycin, and minocycline especially in cases of toxoplasmosis. Standard treatment with pyrimethamine-sulfadiazine is often, requiring discontinuation of the drugs (Haverkos 1987; Leport *et al.,* 1988). Clindamycin (CLI) is an alternative drug widely used as a single agent or combined with sulfa drugs (Dannemann *et al.,* 1992; Dannemann *et al.,* 1988). It has remarkable but delayed in vitro anti-*T. gondii* activity, achieved at low drug concentrations (Pfefferkorn *et al.,* 1992).

In addition to the well-established anti-*T. gondii* activity of CLI as a single agent in animal models of infection (Araujo and Remington 1974; Filice and Pomeroy 1991; McMaster *et al.,* 1973; Vuković *et al.,* 1997), doses of CLI that were ineffective when used alone was shown to afford protection in acute murine toxoplasmosis if combined with rifabutin (Araujo *et al.,* 1994). The combinations of CLI with the others drugs was provided by reports on the activity against both tachyzoites and bradyzoite-containing *T. gondii* cysts (Araujo *et al.,* 1991; Araujo *et al.,* 1992 and Gormley *et al.,* 1998). In addition, has been shown to enhance the anti-*T. gondii* activity of pyrimethamine and sulfadiazine (Araujo *et al.,* 1993), as well as that of rifabutin (Araujo *et al.,* 1994 and Romand *et al.,* 1996). The action of the sulpha and CLI acting as competitive inhibitors of *p*-amino benzoic acid (PABA) especially in the folic acid metabolism cycle. The sulfonamides are widely distributed throughout all tissues. CLI, which inhibits protein synthesis on prokaryotic ribosome's (Fitzhugh 1998). (Pfefferkorn and Borotz. 1994) stated that CLI act on the *T. gondii* prokaryote-type plastid-like organelle. Finally (Tsai *et al.,* 2002) who was successfully treated for brain abscess with CLI and sulfadiazine.

Thus, we examined in vivo experimental model of acute toxoplasmosis whether the addition of SD to CLI would be beneficial for its anti-*T. gondii* effect and, moreover, if this combination is capable of eliminating the parasite, as suggested in the present work was planned to this study.

MATERIALS and METHODS

1. Mice

Female Albino mice (Laboratory Animal House National Research Centre, Cairo Egypt) weighing 18 to 20 g at the beginning of each experiment were used. Mice were housed six to a cage and offered drinking water *ad libitum.*

2. *T. gondii*

Tachyzoites of the virulent RH strain maintained through serial intraperitoneally (I.P.) passages were used. For experimental infections, tachyzoites were harvested from mouse peritoneal fluids 72 hours post infection and purified by centrifugation, cotton wool filtration, and needle extraction. The parasites were counted in a hemocytometer, and their numbers were adjusted to 2×10^6/ml with saline. Suspensions were serially 10-fold diluted and 1-ml aliquots of 2×10^3/ml dilutions were inoculated I.P. into fresh mice.

3. Drugs

High efficacy of Clindamycin (CLI) combined with Sulfadiazine (SD) based therapies was demonstrated for treatment of experimental *Toxoplasma gondii* infection. Mice were infected intraperitoneally with 2×10^3 *T. gondii* strain RH tachyzoites.

Group: I. SD (micronized powder,) was administered at, 25, 50, and 100 mg/kg/day.

Group II: CLI was administered at 25, 50, and 100 mg per kg of body weight per day.

Group III: SD + CLI were administered at25 plus 25, 25 plus 50, 50 plus 50, or 50 plus 100 and 100 plus100 mg/kg/day.

Group IV: left without treatment (control).

Based on the observation that mice consume 4 g of food per day (Filice and Pomeroy 1991and McMaster *et al.,* 1973), per 1 g of ground mouse feed.

4. Experimental protocol

Mice injected I.P. with 10^3 parasites was arbitrarily assigned to one of the 12 treatment groups according to the treatment given, as follows: 3 doses as described previously.

To assess residual infection in mice treated by Clindamycin with sulfa, groups of two arbitrarily chosen survivors were sacrificed into two times:

(First): 4 weeks post infection, for sub-inoculation of brains into fresh mice to attempt re- isolation of *T. gondii* (bioassay), and

(Second): 8 weeks post infection, for the detection of *T. gondii* DNA in brains and lungs by PCR. Brains and lungs were chosen as likely reservoirs of parasites following treatment of murine infection (Piketty *et al.*, 1990).

Each treatment group comprised 12 animals. Treatment was initiated 24 h following parasite inoculation and was continued for 14 consecutive days. A group of untreated animals served as the negative control.

5. Bioassay

Brains were homogenized, and 0.5-ml saline suspensions were inoculated into two fresh mice per sample, one each by the I.P. and intraoesophageal routes. Mice were monitored daily over 4 weeks; peritoneal fluids of those succumbing were examined for the presence of *T. gondii*.

6. PCR assay

For DNA extraction, mouse brains and lungs were dilacerated and suspended in a lysis buffer containing 200 mM Tris-HCl, 1 mM EDTA, 0.5 M NaCl, 1% sodium dodecyl sulfate, and 5 mg of proteinase K/ml. The extraction procedure was conducted according to a classical protocol (Maniatis and Sambrook 1982).

PCR technique was carried on formalin-fixed, paraffin-embedded tissue samples showing histopathological changes. Formalin-fixed tissue samples were washed twice with phosphate buffer saline (PBS) to remove fixative, while paraffin-embedded tissue was treated with xylene followed by absolute alcohol. Then tissue samples were digested using a commercially available kit (QIAamp® DNA Mini kit, QIAGEN, INC., Valencia, CA) using the tissue protocol recommended by the manufacturer, prior to assay by PCR. 5µl of supernatant fluid was used in the first stage of a nested PCR (Burg *et al.*, 1989). The PCRs were conducted in 100 µl of reaction mixture consisting of 20mM ammonium sulphate, 75mM Tris HCL, pH 9.0, 0.01 percent Tween 20 (w/v), 2.5mM magnesium chloride, 0.1 mM d-NTPs, 0.2 µM each primer, 1 unit Taq polymerase (Promega). The primers and the amplification conditions were as described by (Wastling 1993) except that the denaturing, annealing and extension times were each one minute. In the first PCR, the primers were GGAACTGCATCCGTTCATGAG and TCTTTAAAGCGTTCGTTCGTGGTC. Following the successful amplification of a 193 base-pair product, 1 µl of each reaction was used in a second PCR, with a new primer pair, TGCATAGGTTGCAGTCACTG and GGCGACCAATCTGCGAATACACC, to produce a 94 base-pair amplification product. The products were visualized after electrophoresis of 30 µl of reaction mixture on 2 % agarose gels by staining with ethidium bromide. *T. gondii* DNA was used as positive control. Distilled water and uninfected tissues were used as negative controls and were run with tests to monitor for cross-contamination.

RESULTS

The effects of treatment with CLI and SD combined in five different doses on the survival of mice infected with 10^3 RH strains *T. gondii* tachyzoites were compared to those of the same drugs given alone.

Effect of SD & CLI alone on experimental animals was showed in the following Table (1):

Table 1: Shows effect of SD and CLI alone on experimental animals**.**

Drug	Dose		
	25 mg/kg/day	50 mg/kg/day	100 mg/kg/day
SD	0%	16%	12%
CLI	62%	69%	88%

* SD alone was effective compared with no treatment and when it was given at 50 or 100 mg/kg/day, it resulted in 8-week survival of 16 or 12% of mice, respectively.
* CLI alone at 25, 50, or 100 mg/kg/day led to survival of 62, 69, or 88% of mice, respectively.

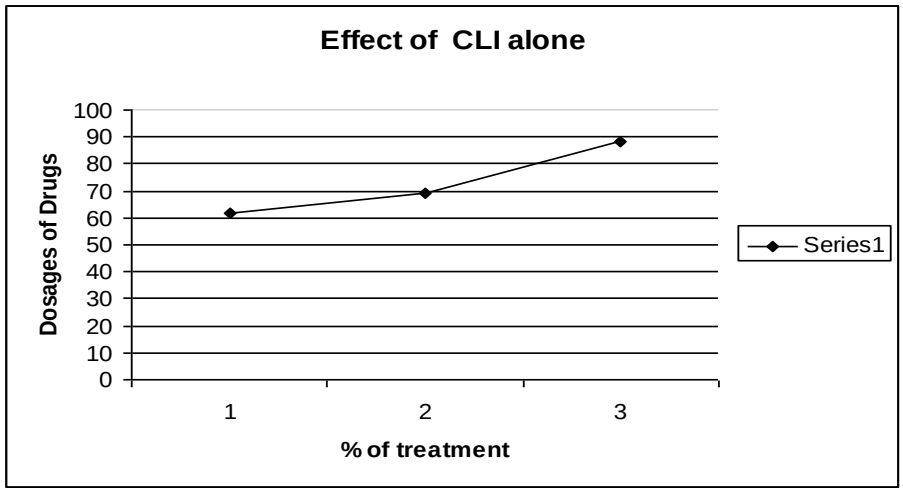


Fig. 1 Diagram of the effect of CLI

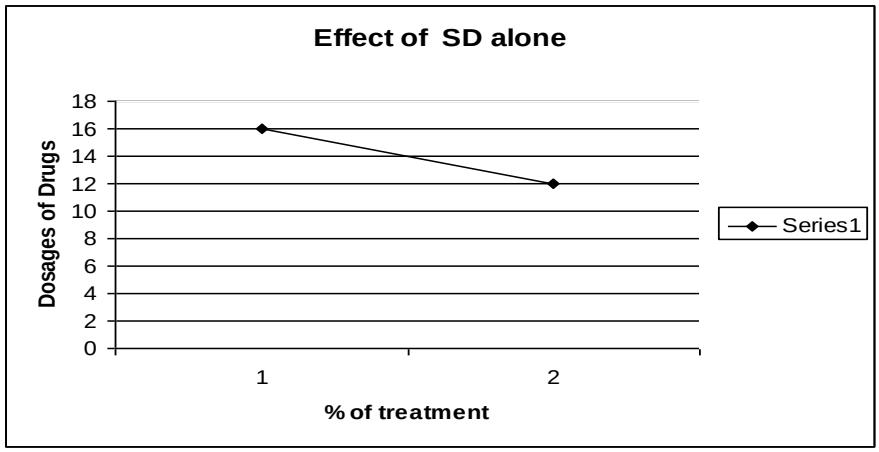


Fig. 2 Diagram oh the effect of SD:

Effect of SD and CLI combined on experimental animals was showed in the following Table (2):

Table 2: Shows effect of SD and CLI combination on experimental animals:

Drug	Dose				
	25 + 25 mg/kg/day	25+ 50 mg/kg/day	50 +50 mg/kg/day	50 +100 mg/kg/day	100 + 100 mg/kg/day
SD+CLI	62%	83%	87%	89%	90%

*Treatment with SD and CLI combined at, 25 plus 25, 25 plus 50, 50 plus 50, 50 plus 100 and 100 plus 100 mg/kg/day protected 62, 83, 87, 89, and 90% of mice, respectively, with no significant variation among particular groups.

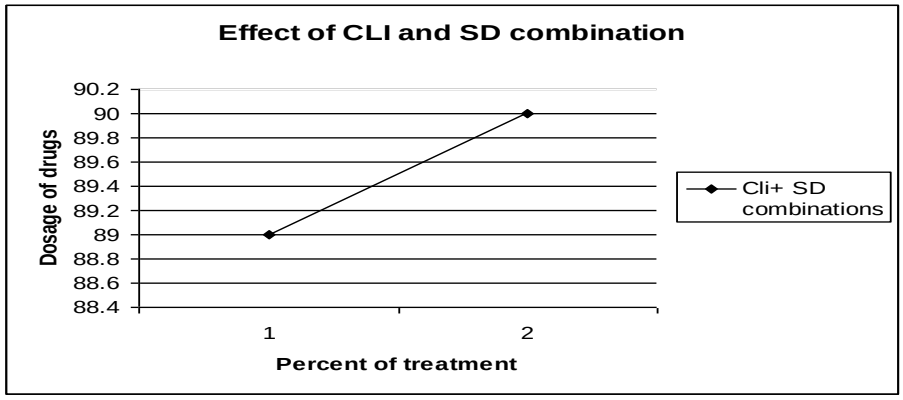


Fig. 3 Diagram of the effect of CLI&SD combination

However, all drug combinations were significantly more effective than any dose of SD alone.

The efficacy of CLI alone, the addition of SD to any given dose of CLI, including those at which the effect of the combined drugs was apparently better). Thus, the combined drugs exhibited a simple additive effect.

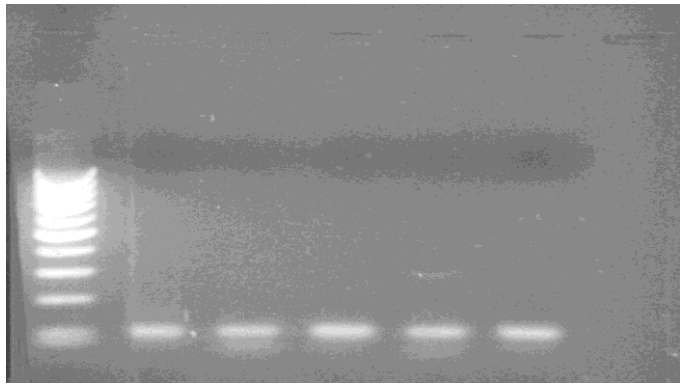
Treatment with SD and CLI combined at 25 plus 25, 25 plus 50, 50 plus 50, 50 plus 100 or 100 plus 100 mg/kg/day protected 62, 83, 87, 89, or 90% of mice,.

The drug combinations were more effective than any dose of SD alone. This was not the case when the two drugs combined were compared with CLI alone. Given the efficacy of CLI alone, the addition of SD to any given dose of CLI, including those at which the effect of the combined drugs was apparently better. The combined drugs exhibited a simple additive effect.

Table 3: Shows the residual infection in mice treated by Clindamycin with sulfa, groups

No. of parasites	SD + CLI dose (mg/kg/day)	No +ve / No. Bioassay (4 wk P.i.)	No +ve / No Bioassay (4 wk P.i.)	No +ve / No PCR (8wk P.i.)	No+ve / No. PCR (8 wk P.i.)
		P.o	I.p	Brain	Lungs
10^3	Low 25+25	ND	ND	1/2	0/2
10^3	High100+100	2/2	1/2	1/2	0/2

P.i.: post infection. P.o.: per oral infection. I.p. intrapretoneal ND: not done.



1 2 3 4 5 6 7

Fig. 4 Showing electrophoretic pattern of the second PCR product (194 bp)

Lane 1: DNA marker, Lane2: positive control (extracted DNA of *T.gondii* Spp), Lane 3, 4, 5 and 6: amplified *T.gondii* Spp DNA in r tissue samples. Lane 7: negative control.

Lethal events were recorded following both i.p. and per oral inoculation of brain tissue, suggesting the presence of both the tachyzoite and bradyzoite life stages of the parasite in some survivors at that time.

DISCUSSION

The Experiments were performed in vivo in a mouse model of acute toxoplasmosis to evaluate the effectiveness of the combination Clindamycin/Sulfadiazine. Each Clindamycin or Sulfadiazine alone provides weak protection against toxoplasmosis, while the combination of the two drugs has significantly synergistic actions. The results of the study showed the importance of using in the treatment of CLI&SD on Toxoplasmosis either alone or combination. Drug combinations were more effective than any dose of SD alone. The combination of CLI and SD acts synergistically against *T. gondii* infection.

The results presented show SD and CLI to be a promising drug combination with the potential to eliminate the parasite, therefore warranting further investigation. The synergistic effect was remarkable in infections with 10^3 parasites, in which 14-day treatment with combinations of 25 and 50 mg of CLI and SD /kg/day protected 62 to 87% of animals by week 8, whereas the same doses of either CLI or SD alone could not prevent mortality. Moreover, the effect of a combination of 50 mg of CLI/kg/day with either 25 or 50 mg of SD /kg/day was similar to the effect of 100 mg of CLI/kg/day alone. This potential is emphasized by the fact that the mere presence of *T. gondii* DNA does not necessarily indicate the presence of viable parasites able to induce infection. This agreed with the opinion of it implies immune control of infection. Thus, in the treated, apparently healthy mice in which *T. gondii* DNA was detected, drug treatment, by reducing the initial parasite burden, may have provided the time for

Protective immunity against an otherwise lethal parasite infection to develop; a delayed specific T-cell response has been associated with treatment (Murray *et al.,* 1993). (Paniker 2002, Leport *et al.,* 1988) stated that the low doses used to obtain high protection and cure rates may allow prolonged administration; 4- and 8-week courses of SD were shown to be effective in chronic murine toxoplasmosis and (Nikolić *et al.,* 1999) shown also that the effect of CLI critically depends on treatment duration.

(Dannemann *et al.*, 1992) were successfully treated for brain abscess with Clindamycin and sulfadiazine.

Furthermore, adverse side effects of CLI (Dannemann *et al.*, 1992). (Dannemann *et al.*, 1988) (Katlama *et al.*, 1996; Pfefferkorn *et al.*, 1993). May be reduced by the use of lower doses, and SD is generally well tolerated. However, to appreciate the potential of the combination of CLI and SD in the treatment of human disease, studies of the levels of the drugs achieved in serum and tissues with the regimens used, relative to the doses feasible in human therapy, are needed.

It should be bear in mind the limitations of extrapolating data from animal models to the human situation; significantly enhanced the effect of the combined drugs may offer direction for future clinical trials of the anti-*T. gondii* potential of this drug combination.

It is difficult to put these results in perspective by comparing them with the efficacy of these or other drugs, alone or in combination, obtained in other animal models, since the infection and treatment protocol characteristics vary widely.

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