ORIGINAL ARTICLE

Effect of clindamycin in a model of acute murine toxoplasmosis

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Objective: To characterize the antitoxoplasma activity of clindamycin in a murine model of acute toxoplasmosis.

Methods: Rates of survival and mean survival times of Swiss Webster mice infected intraperitoneally with 10^6–10^2 tachyzoites of the RH strain of *Toxoplasma gondii* treated with clindamycin or sulfamethoxazole (positive control) or untreated (negative control) were compared. Survivors were submitted to examination of untreated brain tissue preparations, intraperitoneal and peroral subinoculations of brain tissue homogenates into fresh mice, and to pathohistology, including immunohistochemistry, of brain and lungs.

Results: The effect of clindamycin treatment (400 mg/kg/day) on infected Swiss Webster mice was inoculum size dependent, ranging from no survivals in animals infected with 10^6 parasites, to 100% survivals with an inoculum of 10^2. Treatment initiated 24 h before and at time of infection prolonged mean survival times comparably to sulfamethoxazole, and significantly when compared to untreated controls. In contrast, treatment initiated 48 h postinfection with an inoculum of 10^6 did not postpone death. In the clindamycin-treated survivors, there was no biological or histologic evidence for the persistence of toxoplasma.

Conclusions: The results obtained show that at an appropriate parasite dose/drug dose ratio, clindamycin is strongly toxoplasmacidal in a murine model of acute toxoplasmosis.

Key words: *Toxoplasma gondii*, murine model, clindamycin

INTRODUCTION

Toxoplasmic encephalitis (TE) has emerged as one of the major causes of death and the leading cause of focal cerebral lesions in AIDS patients [1]. It is generally considered to develop as a consequence of reactivation of latent (chronic) infection, i.e. cyst rupture and tachyzoite proliferation. A TE episode will eventually advance to death unless promptly treated. As at present there is no drug regimen efficacious against toxoplasma cysts, treatment of TE must be followed by lifelong maintenance therapy.

Standard therapy comprises a combination of pyrimethamine and sulfonamide drugs. However, 60% of the patients receiving this regimen experienced toxicity, the severity of which led to treatment discontinuation in up to 45% of patients [2]. Newer drugs with anti-tachyzoite activity, such as clarithromycin [3] and roxithromycin [4], and others with presumed antibradyzoite activity, such as atovaquone [5] and azithromycin [6], are still being evaluated. On the other hand, the long-known lincosamide antibiotic clindamycin is often included in treatment protocols. Its efficacy in the treatment of toxoplasmic chorioretinitis has been established both experimentally [7] and clinically [8,9]. In AIDS patients, pyrimethamine

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combined with clindamycin was shown to be an acceptable alternative to the pyrimethamine–sulfadiazine combination in terms of efficacy, with lower toxicity [10–12].

Contradictory reports on the antitoxoplasma activity of clindamycin in animal models [13–16] were followed by recent observations of strong but delayed in vitro parasiticidal capacity [17,18]. This provided the impetus to evaluate and further characterize the antitoxoplasma activity of clindamycin in an in vivo experimental model.

MATERIALS AND METHODS

Mice

Female Swiss Webster mice (5–6 weeks old, weighing 18–20g at the beginning of experiments), purchased from the Animal Research Facility of the Medical Military Academy (Belgrade, Yugoslavia), were used in all experiments.

Parasites

Tachyzoites of the virulent RH strain of *Toxoplasma gondii* maintained through serial intraperitoneal passages were used. The parasite numbers were adjusted to 2×10^6/mL with saline. Such suspensions were serially ten-fold diluted up to 2×10^2/mL. For experimental procedures, 0.5-mL aliquots of different dilutions, depending on the particular experiment, were inoculated intraperitoneally into mice.

Drugs

Clindamycin

Powdered clindamycin hydrochloride (lot 353YS, Upjohn Co., supplied by Yusapharm, Belgrade) was administered at a dose of 400 mg/kg/day. Based on the observation that mice consume 4 g of food daily [19], the desired dose was obtained by adding 2 mg of clindamycin per 1 g of ground mouse food.

Sulfamethoxazole

Sulfamethoxazole (lot SR723, ICN Galenika, Belgrade) was used as a representative sulfonamide drug of good efficacy that is well tolerated by mice [3]. A concentration of 700 mg/L in drinking water offered *ad libitum* provided a dose of 375 mg/kg/day.

Neither drug administered for 3 weeks at the above doses had an effect on the survival of normal mice during an observation period of at least 3 months, and no clinically significant toxicity was observed.

Experimental design

Mice injected intraperitoneally with 10^6–10^2 parasites were divided into three treatment groups: group I, clindamycin-treated animals; group II, sulfamethoxazole-treated animals (positive control); and group III, untreated animals (negative control). Treatment groups were further divided into subgroups according to whether the drug was introduced 24 h prior to infection, at the time of infection, or 48 h post-infection. Each treatment subgroup consisted of 6–12 animals. Both drugs were applied for 3 weeks. The observation period following the discontinuation of treatment was 5 weeks. Based on the fact that murine infection with RH strain parasites is invariably lethal, the parameters for the evaluation of drug efficacy were survival rates and length of survival. Mouse survival was monitored daily. The experiments were performed two to five times each, and the data shown represent their cumulative results.

To determine the outcome of the infection in clindamycin-treated animals, groups of two arbitrarily chosen survivors infected with 10^2 parasites were each killed 3, 4, 6 and 8 weeks postinfection and the lungs and brains removed. Exact halves of all brains were homogenized and subinoculated into two fresh mice per brain sample. While the RH strain is generally considered non-cystogenic for rodents, cyst development may occur following early treatment [20,21]. Therefore, in addition to intraperitoneal subinoculation for the detection of (pepsin-sensitive) tachyzoites, the intraesophageal route was used to determine the presence of (pepsin-resistant) cysts. Survival was monitored over a period of 4 weeks. In addition, cysts were microscopically searched for in untreated preparations of the homogenized brain tissue from mice killed 6 weeks postinfection.

Pathohistology

Five-micrometer sections of formalin-fixed, paraffin-embedded brains and lungs of experimental and control mice were hematoxylin–eosin stained for visualization of the pathologic processes involved. Detection of cysts was attempted by the periodic acid–Schiff (PAS) reaction (for cyst contents), the Grocott modification of the methenamine silver technique (for the cyst wall), and by immunoperoxidase staining. The peroxidase–antiperoxidase complex (PAP) technique [22] (PAP complex purchased from Dakopatts, Germany) was performed using a rabbit polyclonal antitoxoplasma serum (kindly provided by Dr Jean Francois Dubremetz) diluted 1:100 as the primary antibody.

Statistics

The correlation between survival and inoculum size was analyzed by multiple regression. Differences in the rates of survival were analyzed by the Kaplan–Meier product limit method. Differences in the survival times

between experimental and control mice were evaluated by one-way analysis of variance (ANOVA). The level of statistical significance was considered to be 0.05.

RESULTS

The results of clindamycin treatment initiated at the time of infection with a range of doses from 10^6 to 10^2 parasites are presented in Figure 1. Survival of mice treated with clindamycin was inoculum size dependent ($y=3.01+0.86x$, $r=0.862$, df=95, $p<0.0001$), ranging from zero (0%) in the group infected with 10^6, to 100% in the group infected with 10^2 (Figure 1A). With the exception of the 10^6 inoculum size, the survival rates

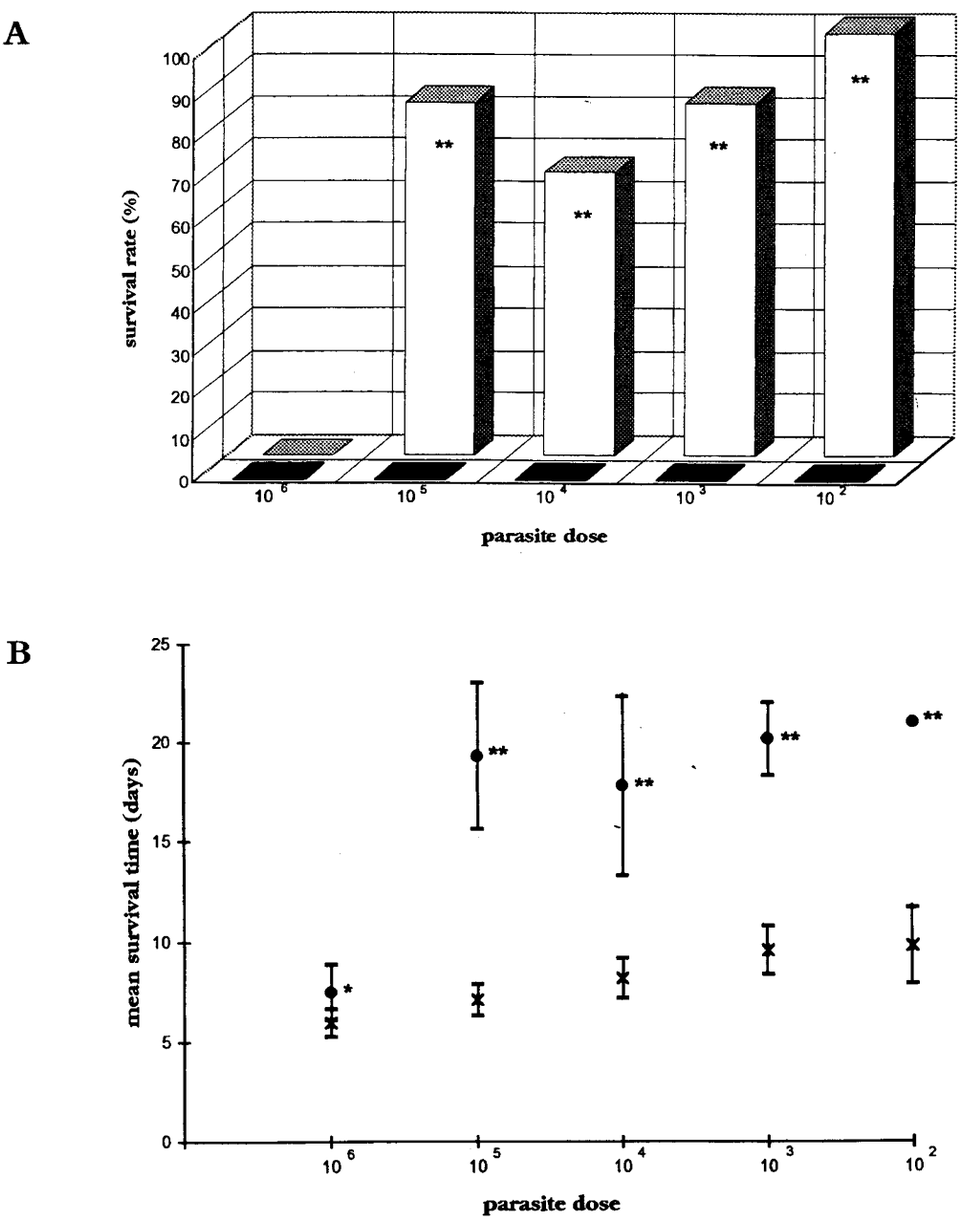

Figure 1 Effect of clindamycin treatment as compared to no treatment in mice intraperitoneally infected with a 10^6–10^2 dose range of RH *T. gondii* tachyzoites. (A) Survival rates. □=clindamycin-treated mice; ■=untreated mice. (B) Length of survival. Results expressed as mean survival time ± SD. ●=clindamycin-treated mice; ×=untreated mice. *$p<0.02$, **$p<0.0001$.

of clindamycin-treated animals were significantly higher than those of untreated controls. In addition, as shown in Figure 1B, mean survival times of clindamycin-treated animals infected with any parasite dose were significantly higher as compared to their respective controls.

Since the above experiment clearly showed 10^6 and 10^2 to be the inoculum sizes which elicited extreme responses to treatment, further experiments were restricted to the use of these.

To assess whether the therapeutic efficacy of clindamycin depends on the time of drug introduction, treatment was initiated 24 h prior to infection, at the time of infection, and 48 h postinfection. Clindamycin treatment in animals infected with 10^6 parasites as compared to both sulfamethoxasole-treated animals and untreated controls showed no protective effects in terms of survival (Figure 2). On the other hand, sulfamethoxazole treatment initiated prior to and at the time of infection resulted in survival rates of 44.4% ($p<0.0001$) and 55.6% ($p<0.0001$), respectively. Survival times of mice that succumbed to infection were significantly higher ($p<0.0001$) than those of animals treated with clindamycin. Treatment by either drug initiated 48 h postinfection, however, had no effect as judged by either parameter, as compared to the negative control group ($p>0.05$).

Figure 3 presents the results of the efficacy of treatment of mice infected with 10^2 parasites. Clindamycin treatment initiated at any time point significantly prevented mortality when compared to untreated controls. The mouse survival rates in the groups treated 24 h prior to and at the time of infection amounted to 100% ($p<0.0001$) and 83–100% (depending on particular experiment) ($p<0.0001$), respectively, while in the group treated 48 h postinfection survival was 58.3% ($p<0.0001$). The mean survival times were also significantly higher than in untreated animals ($p<0.0001$). On the other hand, the effects of clindamycin and sulfamethoxazole were comparable if treatment was introduced prior to or at the time of infection ($p>0.05$), while sulfamethoxazole showed better results when initiated 48 h postinfection ($p<0.02$).

The absence of lethal events in the survivors from all experiments during the 5-week observation period following the discontinuation of treatment suggested clearance of tachyzoites from the host organisms. The clearance of tachyzoites was further supported by the absence of mortality in animals intraperitoneally subinoculated with survivor mice brain tissue. On the other hand, 100% survival of mice perorally subinoculated with the same material suggested the absence of encysted toxoplasma. No toxoplasma cysts were found by examination of native brain tissue

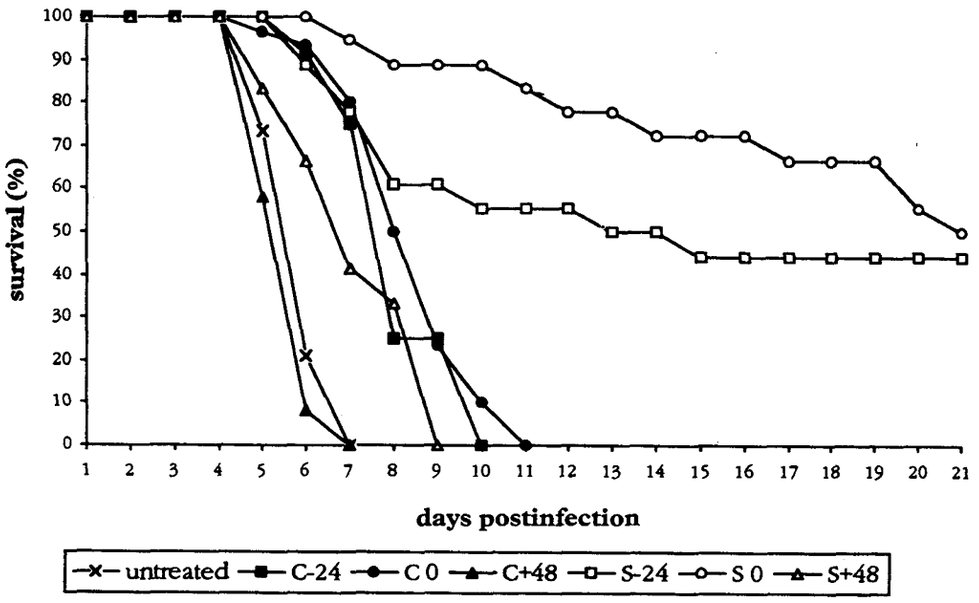

Figure 2 Survival in clindamycin-treated mice infected with 10^6 RH *T. gondii* tachyzoites according to the time of initiation of treatment versus untreated and sulfamethoxazole-treated mice. C=clindamycin; S=sulfamethoxazole; −24=treatment initiated 24 h prior to infection; 0=treatment initiated at the time of infection; +48=treatment initiated 48 h postinfection.

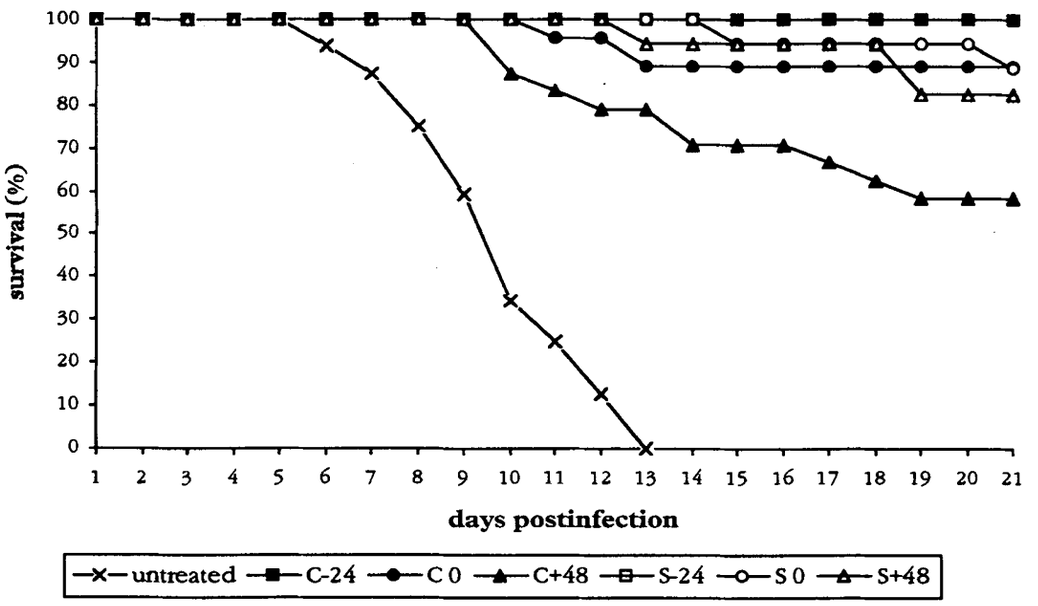

Figure 3 Survival in clindamycin-treated mice infected with 10^2 RH *T. gondii* tachyzoites according to the time of initiation of treatment versus untreated and sulfamethoxazole-treated mice. C=clindamycin; S=sulfamethoxazole; −24=treatment initiated 24 h prior to infection; 0=treatment initiated at the time of infection; +48=treatment initiated 48 h postinfection.

preparations. Furthermore, neither tachyzoites nor cysts were seen in histologic sections of the brains and lungs in any of the animals analyzed, and no signs of encephalitis or pneumonia/pneumonitis were observed at any time point. In contrast, in sulfamethoxazole-treated animals the overall mortality rate following discontinuation of the drug was 46% during the 5 weeks of observation (data not shown), while histologic sections of brain and lung tissue showed evidence of both encephalitis and pneumonia.

DISCUSSION

The in vivo antitoxoplasma activity of clindamycin has been evaluated in a number of animal models. Some studies showed protective effects of clindamycin in both systemic toxoplasmosis [13,23] and TE [14], while others failed to do so [15,16]. These conflicting results may be attributed to differences in parasite strains and inoculation routes, drug dosage, and mode and duration of treatment.

The murine model we developed for the evaluation of drug efficacy showed the protective effect of clindamycin administered for 3 weeks at a dose of 400 mg/kg to be a function of the inoculum size, ranging from 100% survival of animals infected with 10^2 RH strain parasites to no survivals in animals infected with 10^6 parasites. The efficacy of clindamycin was also initiation time dependent; thus, if clindamycin was introduced before or at the time of infection, animals infected with 10^2 parasites were 100% protected, versus 58% if it was introduced 48 h postinfection. The therapeutic potential of clindamycin was further demonstrated by increased mean survival times of animals infected with any inoculum size, irrespective of the time of initiation of treatment, except in animals infected with 10^6 parasites if initiated 48 h postinfection. The lower efficacy of clindamycin if introduced 48 h postinfection may be a consequence of its delayed mode of action. Fichera et al. [18] recently showed that clindamycin acts by markedly inhibiting the replication of toxoplasma within the parasitophorous vacuole of the second generation. Given the RH parasite replication rate of one cycle per 6–8 h, when treatment is initiated 48 h postinfection the initial parasite burden has already risen up to 250-fold. Therefore, a later initiation time acts ultimately as an initially higher parasite burden. However, our results show that at an appropriate parasite dose/drug dose ratio, clindamycin can provide complete protection.

Whether clindamycin can eliminate *T. gondii*, as suggested by the absence of biological and histologic evidence for the presence of either tachyzoites or cysts in the clindamycin-treated survivors in our model,

remains to be elucidated by investigations at the molecular level.

The toxoplasmacidal capacity of clindamycin in acute toxoplasma infection supports the use of clindamycin in clinical situations involving the proliferative stage of the parasite. A limitation, however, to direct extrapolation of the data presented may be the dose of clindamycin used in our experimental model, which surpasses the dose usually administered in human therapy by 5–10-fold. However, preliminary experiments involving doses reduced to 50 mg/kg and 25 mg/kg per day have shown promising results in terms of efficacy, and further work is currently underway in our laboratory.

Acknowledgments

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