ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1974, p. 647-651

Vol. 5, No. 6
Printed in U.S.A.

Effect of Clindamycin on Acute and Chronic Toxoplasmosis in Mice

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Received for publication 4 February 1974

The effect of clindamycin on survival of mice during acute infection with the RH and C56 strains of *Toxoplasma* and the ability of this drug to prevent congenital transmission during the acute stage of the infection in the mother and to eradicate the parasite from tissues of mice chronically infected with the C56 strain were evaluated. The drug effectively prevented death due to the acute infecton and, in the experimental model employed, eradicated the organism at least from the liver, spleen, and brain of approximately 30 to 50% of the acutely infected animals which survived. Clindamycin also effectively prevented congenital transmission during the acute infection in the mother. During short-term treatment (7 days), persistent parasitemia in the chronic infection was effectively diminished or eradicated. More prolonged treatment resulted in a significant clearing of the organisms from the spleens and livers, but not from the brains, of chronically infected mice.

The present drug regimen used in the treatment of toxoplasmosis is the combination of pyrimethamine with sulfadiazine (or triple sulfanomides). The potential toxicity to the bone marrow associated with pyrimethamine treatment of toxoplasmosis in both immunologically normal and immunologically deficient individuals (12) has caused investigators to search for alternative modes of therapy. In addition, since pyrimethamine is an antimetabolite, it must be considered potentially teratogenic (11), and we, as well as another (14), have advised against use of this drug early in pregnancy. In the United States, no other drug or drug combination has been considered acceptable for treatment of toxoplasmosis. In Europe, spiramycin (7) has been employed extensively, but there are few control studies (2) in animals or in man to allow one to determine objectively the efficacy of this antibiotic.

What is needed is an effective, nontoxic drug or drug combination that can be employed safely in normal infants, in older children and adults, and in patients on immunosuppressive therapy during pregnancy. We have been evaluating certain of the newer antimicrobial agents as they have appeared—most recently, rifampin (19) and trimethoprim (4)—both of which have proven disappointing in experiments carried out in vivo.

The recent demonstration of the efficacy of clindamycin in the treatment of acute toxoplasmosis in a mouse model by McMaster and his colleagues (10) was the impetus for the present study. Since *Toxoplasma* can be transmitted congenitally (13) and persists in the tissues of a large percentage of the normal population of the United States and in the rest of the world (5), we were interested in determining the effect of this antibiotic in preventing congenital transmission and its effect on the persistence of the parasite during the chronic (latent) infection.

MATERIALS AND METHODS

Mice. Swiss-Webster mice purchased from Simonsen Laboratories, Inc., Gilroy, Calif., and weighing 22 to 24 g, were employed in all experiments.

Preparation and administration of clindamycin. Clindamycin HCl hydrate, supplied as a powder (lot no. 962BEK2) by the Upjohn Co., Kalamazoo, Mich., was mixed with normal mouse chow in powder form by using a water-cooled mechanical mixer. Control mice were given the powdered diet without clindamycin. The amount of drug in the diet was given in milligram amounts per 4 g of food since mice consumed approximately 4 g of the diet each day (3). The drug in the doses employed in these experiments had no effect on survival of normal mice when administered for a period of 4 months. Differences in mortality were subjected to chi-square analysis.

Sulfadiazine. Sodium sulfadiazine, supplied by Lederle Laboratories, Pearl River, N.Y., was administered in the drinking water in a concentration of 40 mg%. This provided an intake of approximately 1.6 mg of sulfadiazine per mouse per day.

Toxoplasma inoculum. Chronic infection with

Toxoplasma gondii was accomplished as described previously (20). For acute infection, trophozoites of the C56 or RH strain were harvested from the peritoneal fluids of infected mice as described previously (8), washed with saline, and counted in a hemacytometer. The organisms were inoculated intraperitoneally (i.p.) in a volume of 0.2 ml of saline. Control mice were inoculated with the same volume of saline alone.

Congenital transmission. For the congenital transmission experiments, three females were kept with a single male for 3 days. Infection of the pregnant females with *Toxoplasma* was performed 11 days after removal of the male. Females were placed in separate cages just prior to delivery. Organs of newborn mice or mice obtained by caesarian section were processed and inoculated into normal mice as described previously (18). Recipient mice were followed and tested for seroconversion and presence of organisms (18).

Serology. The Sabin-Feldman dye test was performed as described by Frenkel and Jacobs (6). A titer of 1:16 (final dilution) was considered positive in this study.

RESULTS

Effect of clindamycin on survival of mice with acute toxoplasmosis. In a pilot experiment, four groups of 10 mice each were infected i.p. with 10^5 trophozoites of the highly virulent RH strain of *Toxoplasma*. Immediately after infection, three groups were given a diet containing 1, 3, or 6 mg of clindamycin per 4 g of food, respectively, for 14 days. Mice in the fourth group were fed the same food without clindamycin. Mortality was 90, 100, and 0%, respectively, in the three treated groups and 100% in the untreated group. The efficacy of these doses of clindamycin were then tested against varying inoculum sizes of *Toxoplasma* (Table 1). The lowest dose of clindamycin (1 mg) provided excellent protection against inocula of *Toxoplasma* RH trophozoites ranging from 200 to 20,000 organisms, but no protection was demonstrable when the inoculum contained 200,000 organisms. Interestingly, the results with the 6-mg dose were approximately the same as with the 1-mg dose. The reason for the difference observed with the 3-mg dose is unclear. Reproducibility of results was tested in three separate experiments using groups of 10 mice infected i.p. with 5×10^4 *Toxoplasma* RH and fed 6 mg of clindamycin per 4 g of food for 15 days. The mortality, recorded at 30 days, in the three experiments was 0, 20, and 20%, respectively. All untreated control mice died.

To determine the effect of three different doses of clindamycin (1, 3, and 6 mg/4 g of food) on survival of mice infected with 10^5 trophozoites of the less virulent C56 strain, three groups of 10 mice each were infected i.p. and given the diet containing clindamycin immediately thereafter for 10 days. A fourth group was given diet without the antibiotic. Mortality was recorded daily for 30 days. The cumulative results at 30 days are shown in Table 2. Whereas 100% of untreated mice died, 40% of those receiving the 1-mg dose of clindamycin and 10% of those receiving 3-mg of clindamycin died. All mice treated with 6 mg of clindamycin survived. All surviving mice were sacrificed on day 40, and their brains, spleens, and livers were subinoculated into normal mice to determine whether clindamycin had prevented persistent infection. Two normal mice were injected with brain and two were injected with a combination of liver and spleen of each survivor (Table 3). Toxoplasma was demonstrated in 50, 44, and 55% of the recipient mice subinoculated with brain from survivors which had been treated with 1, 3, and 6 mg of clindamycin, respectively. The figures for mice subinoculated with combined liver and spleen were 75, 44, and

TABLE 1. *Effect of the dose of clindamycin on mortality of mice infected i.p. with different numbers of trophozoites of the RH strain of Toxoplasma gondii*[a]

Clindamycin dose (mg/4 g of food)	Mortality (no. dead at 30 days/no. inoculated)			
	2×10^2[b]	2×10^3	2×10^4	2×10^5
1	0/6	1/6	2/6	6/6
3	0/6	1/6	3/6	3/6
6	0/6	0/6	2/6	6/6
None	6/6	6/6	6/6	6/6

[a] Six mice in each group. Treatment was started immediately after infection and continued for 10 days.

[b] Number of *Toxoplasma* injected.

TABLE 2. *Effect of the dose of clindamycin on mortality of mice infected i.p. with 10^5 trophozoites of the C56 strain of Toxoplasma gondii*[a]

Clindamycin dose (mg/4 g of food)	Cumulative mortality[b] on day 30
1	4/10[c] (40)
3	1/10 (10)
6	0/10 (0)
None	10/10 (100)

[a] Treatment with clindamycin was for 10 days starting immediately after infection.

[b] Number dead/number inoculated: figures in parenthesis = percent mortality.

[c] Two more mice died in this group, one on day 20 and one on day 24.

60%. Thus, clindamycin administered in a dose of 1, 3, or 6 mg/4 g of food for 10 days reduced mortality and prevented latent infection in some of the animals.

Effect of clindamycin on eradication of Toxoplasma from tissues of chronically infected mice. To determine whether *Toxoplasma* can be eradicated from the central nervous system and peripheral tissues of chronically infected hosts, groups of mice chronically infected with the C56 strain of *Toxoplasma* were treated for 6 to 8 weeks with either clindamycin (6 mg/4 g of food) or sulfadiazine (40 mg/100 ml of drinking water). The mice had been infected approximately 15 weeks prior to their use in the experiments. At 6 to 8 weeks, treatment was discontinued; 48 h thereafter all mice were sacrificed, and the entire brain, spleen, and liver of each were each ground separately in 4 ml of saline using a mechanical tissue grinder (H. A. Thomas Co., Philadelphia). Tenfold dilutions of the tissue suspension were made in saline, and 0.5 ml of each dilution was injected i.p. into three normal mice. In the case of the undiluted suspension, 0.2 ml was inoculated i.p. Mice surviving were bled 6 weeks after inoculation, and their sera were tested by the Sabin-Feldman dye test. Mice dying during the experiment were examined for the presence of *Toxoplasma* in their peritoneal fluid. The results of two separate experiments are shown in Tables 4 and 5. In the first experiment (Table 4), a significant difference in survival of mice subinoculated with liver and spleen, but not with brain, was noted between the clindamycin-treated group and controls. When mice which survived the subinoculation were tested by the Sabin-Feldman dye test, all those which had been inoculated with tissues of the nine mice treated with clindamycin were negative, whereas those inoculated with tissues of eight of the untreated mice were positive. In an attempt to confirm and extend the results of this initial experiment, another study was performed with the addition of a sulfadiazine-treated group (Table 5). There were 15 animals in each group and the groups were treated for 8 weeks. The results revealed that both clindamycin and sulfadiazine decreased significantly the number of parasites in liver and spleen, but no significant difference was detectable in the degree of latent infections between the treated and untreated groups in the case of brain.

Effect of clindamycin on congenital transmission of Toxoplasma during the acute infection in the mother. To determine whether treatment with clindamycin can prevent transmission of *Toxoplasma* from an acutely infected mother to her offspring in utero, pregnant mice were infected i.p. with 10^6 trophozoites of the RH strain of *Toxoplasma* and given clindamycin (6 mg/4 g of food) in their diet immediately thereafter. Controls were infected pregnant mice which received no clindamycin in their diet. Subinoculation results revealed that of the 11 infected controls, 9 gave birth to infected offspring or were delivered of infected offspring by caesarian section. They had a total of 44 infected offspring. Of the 7 infected mice treated with clindamycin, none gave birth to or were delivered of infected offspring (the size of the litters varied from 4 to 14 offspring). The number of days of clindamycin treatment prior to delivery in both infected and treated and untreated groups was approximately 8 to 14 days. There was no appreciable difference in duration of infection between the treated group and the untreated control group. The number and health of the offspring delivered by uninfected pregnant mice which had been treated with clindamycin alone was similar to that observed in untreated, uninfected normal pregnant mice in our laboratory.

TABLE 3. *Presence of Toxoplasma in tissues of acutely infected (C56 strain) mice after 10 days of treatment with clindamycin*

Clindamycin dose (mg/4 g of food)	Presence[a] of *Toxoplasma* in mice subinoculated with:	
	Brain	Liver, spleen
1	4/8 (50)	6/8 (75)
3	8/18 (44)	8/18 (44)
6	11/20 (55)	12/20 (60)

[a] Number with *Toxoplasma*/number subinoculated with tissues: figures in parenthesis = percentage.

Effect of clindamycin on persistent parasitemia in chronically infected mice. Mice chronically infected with the C56 strain 5 months previously were treated with a diet containing 6 mg of clindamycin per 4 g of food. On days 7, 14, and 21 of treatment, the clindamycin was discontinued in the diet; 48 h later mice were bled, and the total volume of blood (approximately 1.5 ml) was subinoculated i.p. into normal mice. Parasitemia was considered proved only if *Toxoplasma* organisms (proliferative form in the peritoneal fluid or cysts in the brain) were demonstrated in the recipient mice. Two experiments were performed and, in each, 12 experimental and 12 control mice were bled for each time period. In the second experiment, mice were treated for only 7 days. The com-

TABLE 4. *Mortality in mice subinoculated with dilutions of brain, liver, and spleen of mice chronically infected with the C56 strain of Toxoplasma gondii and treated with clindamycin for 6 weeks*

Group	Dilution of subinoculated tissue suspension[a]	Mortality[b] in mice subinoculated with:			
		Brain	*P* value	Liver, spleen	*P* value
Treated with clindamycin (9 mice)	Undiluted	27/27 (100)	>0.05	3/27 (11)	<0.001
	1:10	27/27 (100)	>0.05	0/27 (0)	<0.001
	1:100	27/27 (100)	>0.05	0/27 (0)	<0.007
	1:1000	26/27 (96)	>0.05	1/27 (3)	<0.05
	1:10,000	20/27 (74)	>0.05	1/27 (3)	<0.05
Untreated (8 mice)	Undiluted	24/24 (100)		14/24 (58)	
	1:10	24/24 (100)		12/24 (50)	
	1:100	22/24 (92)		6/24 (25)	
	1:1000	21/24 (87)		1/24 (4)	
	1:10,000	14/24 (58)		3/24 (12)	

[a] Three normal mice were inoculated intraperitoneally with each dilution (see text).
[b] Number dead/number injected: figures in parenthesis = percentage.

TABLE 5. *Mortality and dye test results in mice subinoculated with tissues of mice chronically infected with the C56 strain of Toxoplasma gondii which had been treated with clindamycin or sulfadiazine for 8 weeks*

Group[a]	Dilution of subinoculated tissue suspension	Subinoculated with brain		Subinoculated with liver & spleen		
		Mortality[b]	DT in survivors[c]	Mortality[b]	*P* value	DT in survivors[c]
Clindamycin	Undiluted	30/30 (100)		3/30 (10)	<0.001	0/27 (0)
	1:10	30/30 (100)		1/30 (3)	<0.001	3/29 (10)
	1:100	28/30 (93)	0/2 (0)	0/30 (0)	<0.001	0/30 (0)
	1:1000	26/30 (86)	0/4 (0)	0/30 (0)	0.04	0/30 (0)
	1:10,000	10/30 (33)	0/20 (0)	0/30 (0)	<0.05	3/30 (10)
Sulfadiazine	Undiluted	30/30 (100)		9/30 (30)	<0.001	0/21 (0)
	1:10	30/30 (100)		5/30 (16)	<0.001	1/25 (4)
	1:100	26/30 (86)	2/4 (50)	5/30 (16)	>0.05	1/25 (4)
	1:1000	22/30 (73)	2/8 (25)	2/30 (6)	>0.05	1/28 (3)
	1:10,000	11/30 (36)	1/19 (5)	3/30 (10)	>0.05	1/27 (3)
No drug	Undiluted	30/30 (100)		20/27 (74)		1/7 (14)
	1:10	30/30 (100)		15/27 (55)		5/12 (41)
	1:100	29/30 (96)	0/1 (0)	8/27 (29)		3/19 (15)
	1:1000	16/30 (53)	4/14 (28)	4/27 (14)		0/23 (0)
	1:10,000	8/30 (26)	3/22 (13)	3/27 (11)		2/24 (8)

[a] There were 15 mice in each group.
[b] Number dead/number subinoculated: figures in parenthesis = percentage. Two normal mice were inoculated intraperitoneally with each dilution (see text). Mice dying in first 3 days after inoculation from causes other than toxoplasmosis account for the numbers in the denominators which are less than 30.
[c] Number of survivors with a positive DT/number tested: figures in parenthesis = percentage.

bined results are shown in Table 6. Whereas parasitemia was demonstrable in 50% of untreated mice, none of the treated mice had demonstrable parasitemia after 7 days of treatment. Similar results were found after 14 and 21 days of therapy.

DISCUSSION

The results described above demonstrate the efficacy of clindamycin in the treatment of acute and chronic toxoplasmosis in a mouse model and in prevention of congenital transmission during the acute stage of infection in the mother. Clindamycin was effective not only in protecting mice from the lethal effects of the acute infection, but also in eradicating the organisms from host tissues (spleen, liver, and blood) during the chronic (latent) infection as well. The latter experiments were performed to determine whether the degree of latent infection might be lessened in the central nervous system and peripheral tissues during the chronic infec-

TABLE 6. *Effect of clindamycin on persistent parasitemia in chronically infected mice*

Group	Infected mice[a]		
	7[b]	14	21
Clindamycin	0/24 (0)	1/12 (8)	1/12 (8)
No drug	12/24 (50)	5/12 (41)	7/12 (58)

[a] Number of mice with parasitemia/number of mice tested: figures in parenthesis = percent mortality.
[b] Days of treatment.

tion. The results revealed that a significant decrease in the number of organisms occurred only in the peripheral tissues. It is not surprising, however, that clindamycin did not effectively eradicate organisms from the brain, since lincomycin penetrates into the nervous system and cerebrospinal fluid poorly (9). Of interest is the fact that sulfadiazine also effectively reduced the number of parasites in peripheral tissues of mice with the latent infection. Its lack of effect on the infection in the brain may have been due to too low a dose, since this drug does cross the cerebrospinal fluid and blood-brain barrier (21). Our results suggest that each of these antimicrobial agents is able to effectively enter the tissue cyst and act on the encysted parasites. Whether this is the mechanism whereby the drugs acted, however, remains to be clarified.

The doses of clindamycin most effective in the present study appear higher than those ordinarily used in man (approximately 250 mg per kg per 24 h in the mice compared with approximately 15 to 30 mg per kg per 24 h used in man) when calculated on a milligram per kilogram basis. However, when the doses are equated on a comparable surface area basis (a much more reliable measure), the dose for the mice was 750 mg/m^2, which is comparable to the dose of 550 to 1,100 mg/m^2 usually used in man. Whether clindamycin is metabolized in mice at the same rate as in man is unknown.

The results reported here suggest that clindamycin may be of value in treatment of latent infection with *Toxoplasma* and, in agreement with the results of McMaster et al. (10), in the treatment of acute toxoplasmosis. Before any recommendations can be made, however, results of carefully controlled studies in man must be forthcoming.

ACKNOWLEDGMENTS

This study was supported by a grant-in-aid from the Upjohn Company and from Conselho Nacional de Pesquisas, Brazil.

We acknowledge the excellent technical assistance of William Dougherty.

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