Clindamycin in a Murine Model of Toxoplasmic Encephalitis

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We investigated the efficacy of clindamycin in a murine model of toxoplasmic encephalitis using direct intracerebral inoculation. Clindamycin reduced mortality from 40% in normal mice and 100% in cortisone-treated mice to 0% in both groups. Although we were unable to document appreciable levels of clindamycin in the brains of infected mice, the histological features of cerebral infection were markedly altered. The formation of large numbers of cysts and the intense inflammatory response seen in the brains of normal mice and the unchecked infection and tissue necrosis in the brains of cortisone-treated mice were absent in the brains of clindamycin-treated mice. Enumeration of cysts in the brains of mice 10 weeks after infection revealed a significantly lower number in the clindamycin-treated mice. Spread of infection to other organs was also decreased during clindamycin administration. These observations suggest that clindamycin may have a role in the therapy of toxoplasmic encephalitis.

The remarkable number of cases of toxoplasmic encephalitis associated with the acquired immunodeficiency syndrome epidemic has served as an impetus for the search for treatment regimens which are not associated with the untoward side effects of the conventional regimen of pyrimethamine plus a sulfonamide. Because of its activity in a mouse model of toxoplasmosis (1, 11), clindamycin, which does not achieve adequate levels in cerebrospinal fluid even in the presence of bacterial meningitis (6), has been used in several cases of toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome, usually in combination with pyrimethamine (12, 15). Based on the available anecdotal data, no conclusion can be made regarding its efficacy in this disease.

Recently, we developed a mouse model of toxoplasmic encephalitis by direct injection of the organism into the brain (J. M. Hofflin, F. K. Conley, and J. S. Remington, *J. Infect. Dis.*, in press). The histopathological changes in the brains of such mice resemble those reported in patients with acquired immunodeficiency syndrome and toxoplasmic encephalitis (9). These changes, which include inflammation and marked tissue destruction, suggest that damage to the blood-brain barrier may allow clindamycin to reach effective levels in the involved areas of the brains of such patients. For this reason, and because of the great need for alternative drugs for treatment of this entity, we examined the efficacy of clindamycin in our mouse model of toxoplasmic encephalitis.

MATERIALS AND METHODS

Mice. Swiss Webster female mice (Simonsen Laboratories, Gilroy, Calif.), 7 to 10 weeks old and 17 to 25 g in weight, were used in all experiments.

Toxoplasma organisms. Tachyzoites of the C56 strain were harvested from the peritoneal fluid of mice infected 6 days earlier (8). These mice had been treated with cyclophosphamide 2 days before infection to prevent antibody coating of the organisms (7). The infected peritoneal fluid was filtered through a polycarbonate membrane (pore size, 3 μ m; Nuclepore Corp., Pleasanton, Calif.) to obtain organisms

free of host cells and debris. After filtration, the purified tachyzoites were washed once and suspended at a concentration of 3.3×10^6 organisms per ml in Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) containing 100 U of penicillin and 100 μ g of streptomycin per ml (HBSS). Viability of the organisms was greater than 95%, as determined by trypan blue exclusion.

Chronic infection. Chronic infection was established as described previously (13). Beginning 4 days after mice were injected intraperitoneally with 10^4 tachyzoites, sulfadiazine (40 mg%) was added to their drinking water for 2 weeks. During this time, immunity develops such that when sulfadiazine is discontinued, the mice remain healthy. Chronic infection is considered established by 6 weeks after inoculation.

Intracerebral infection. Injections were delivered into the right frontal lobe of the brain with a stereotactic unit as previously described (3). After anesthesia with methoxyflurane and placement in the stereotactic unit, the skull was penetrated with a 0.5-in. (1.27-cm) short-beveled 23-gauge needle. A 30-gauge needle was passed through the 23-gauge needle into the brain to a measured depth of 3 mm, and a 3- μ l inoculum (10^4 organisms) of the tachyzoite suspension was injected with a 50- μ l glass syringe. Animals injected intracerebrally with 3 μ l of HBSS were used as controls. The 30-gauge needle was checked for patency after each injection, and mice were euthanized if needle blockage had occurred.

Immunosuppression. Beginning 2 days before intracerebral infection, mice received 25 mg of cortisone acetate (Merck, Sharpe & Dohme, West Point, Pa.) per kg (body weight) or normal saline administered daily by subcutaneous injection.

Treatment with clindamycin. Beginning 1 day after intracerebral infection, mice received 150 mg of clindamycin phosphate (The Upjohn Co., Kalamazoo, Mich.) per kg or normal saline intraperitoneally twice daily for 14 days. In earlier studies from this laboratory, a similar dosage was administered orally and provided complete protection (100% survival) from intraperitoneal infection with the highly virulent strain *Toxoplasma* sp. RH (1).

Clindamycin assays. Microbiological assay for clindamycin was performed by Theodore Spiro at Upjohn.

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FIG. 1. Untreated intracerebral *Toxoplasma* infection, day 7. Intense mononuclear inflammation at injection site.

Histological evaluation. Each experimental group was divided into two cages: one with 10 mice for observation of time to death and the other for sacrifice by carbon dioxide asphyxiation of 3 mice each on days 4, 7, 14, and 21 after infection. At autopsy, the brains were removed and fixed in 10% buffered Formalin. Four to six paraffin-embedded 5- μ m-thick coronal sections of brain in the region of the injection were stained with hematoxylin and eosin.

Isolation studies. At 4, 7, and 14 days of infection, the livers, spleens, hearts, and lungs of three intracerebrally infected mice in each group were harvested. The livers and spleens were combined in 4 ml of normal saline and processed with a tissue grinder; the hearts were processed separately in 2 ml of normal saline, as were the lungs. Samples (0.2 ml) of 10-, 100-, and 1,000-fold dilutions of the resulting suspensions were inoculated intraperitoneally into three mice. *Toxoplasma* infection of these recipient mice was determined by examination of peritoneal fluid of any that died or by serologic testing of those that survived 6 weeks. Positive serologic results were confirmed by the examination of brain suspension for the presence of cysts, as described below.

Enumeration of cysts. The brains of mice surviving 10 weeks after infection were each triturated in a mortar and mixed with 1 ml of normal saline. The suspension was drawn up and expressed through a 22-gauge needle four times. A 50- μ l sample of the resulting suspension was placed on a microscope slide under a cover slip (20 by 50 mm), the entire slide was scanned, and *Toxoplasma* cysts were counted. Because earlier studies from our laboratory (14) showed insignificant differences in the numbers of cysts in multiple samples from an individual suspension, only single preparations were counted. Student's *t* test was performed to compare the numbers of cysts.

RESULTS

Mortality. Intracerebral inoculation with HBSS alone did not cause mortality or overt neurological damage. Intracerebral infection alone resulted in a mortality of 40%; when clindamycin was administered, the mortality was 0%. Intracerebral infection in cortisone-treated mice resulted in a mortality rate of 100%; when clindamycin was administered,

the mortality was 0% ($P = 0.0001$ by chi-square analysis). No mortality occurred in mice which had been inoculated intracerebrally with HBSS and treated with cortisone during the 1-month period of observation.

Histology. Details of the histology in normal and cortisone-treated mice are reported elsewhere (Hofflin et al., in press). Briefly, in untreated mice at 4 days after infection, hemosiderin was present in phagocytic cells, and there were numerous intracellular vacuoles which contained *Toxoplasma* cells. There was no associated inflammation at this time. On day 7, the histologic features included meningeal and perivascular infiltration of mononuclear inflammatory cells in the region of the injection site, which was intensely inflamed and edematous, often with foci of necrotic debris (Fig. 1). Extracellular tachyzoites, as well as intracellular vacuoles filled with *Toxoplasma* organisms, were present. By day 14, necrosis was more prominent, and extracellular tachyzoites were only rarely observed. Instead, a remarkable number of small cysts were present in the region of the injection site, and scattered isolated cysts were also observed in the contralateral cerebral hemisphere.

In mice treated with clindamycin, examination on day 7 of infection revealed a much smaller number of vacuoles which contained *Toxoplasma* organisms and markedly less inflammation than in nontreated controls (Fig. 2). Free organisms were not identified, and no tissue destruction was present. The histology on day 14 was essentially unchanged from that observed on day 7.

In mice that were treated with 25 mg of cortisone per kg daily beginning 2 days before intracerebral infection, there was minimal meningeal and perivascular inflammation observed at any time. In marked contrast to what we observed in noncortisonized mice, the inoculation site contained remarkably few inflammatory cells. There was an expanding central region of tissue destruction and a peripheral zone in which tachyzoites were present in both extracellular and intracellular sites (Fig. 3). There was no evidence of cyst formation. Other foci of tachyzoites and tissue damage could be detected in the contralateral hemisphere and caudal to the inoculated region.

In cortisonized mice treated with clindamycin, the histological appearance on days 7 and 14 was similar to that in

FIG. 2. Clindamycin-treated intracerebral *Toxoplasma* infection, day 7. Injection site with minimal inflammation or tissue destruction.

nonimmunosuppressed mice treated with clindamycin (Fig. 4). There was no evidence of the overwhelming proliferation of organisms and tissue destruction that was seen in cortisonized mice that were not treated with clindamycin.

Isolation studies. As can be seen in Table 1, during treatment of nonimmunosuppressed mice with clindamycin, the ability of tissue suspensions to transmit *Toxoplasma gondii* was prevented in all except the most concentrated (1:10 dilution) suspension of lung tissue. Clindamycin was discontinued on day 14 after intracerebral infection. Seven days later, tissue infection was detected in liver or spleen and lung suspensions but not in heart tissue. In cortisone-treated mice, clindamycin markedly reduced the concentrations of the parasite but did not eradicate it from any of the tissues tested; clindamycin appeared to be least effective in the lung.

Cyst enumeration. To determine whether the numbers of cysts in the brains of clindamycin-treated mice were comparable to the numbers in normal mice more than 1 month after discontinuation of the antibiotic, the brains of three mice in each of the surviving groups were harvested 10 weeks after infection and were individually suspended in normal saline. The average number of cysts in the brains of the normal mice was 71 (range, 54 to 86) compared with 9 (range, 6 to 12) ($P = 0.004$) in the brains of the clindamycin-treated mice and 51 (range, 41 to 58) in the brains of the clindamycin- and cortisone-treated mice.

Clindamycin levels. Simultaneous levels in serum and brain tissue were measured in mice after administration of 150 mg of clindamycin per kg intraperitoneally twice daily for 4 days (Table 2). This was done in normal mice and in mice chronically infected with *Toxoplasma* organisms to determine whether the diffuse perivascular and meningeal inflammation known to accompany the chronic infection (2) might influence the entry of clindamycin into the brain parenchyma. Simultaneous with levels in serum comparable to those achieved with usual doses in humans, low but detectable levels of clindamycin were present in brain parenchyma at 1 and 2 h after administration. No differences were detected between the two groups by Student's *t* test.

We also compared levels in control mice with those in mice that had received an intracerebral injection of *Toxo-*

FIG. 4. Clindamycin- and cortisone-treated intracerebral infection, day 7. Injection site with less inflammation than in noncortisonized mice but without tissue destruction seen in non-clindamycin-treated, cortisonized mice (c.f. with Fig. 3).

plasma organisms or of HBSS alone 1 week earlier (Table 3). As described above, at this time significant intracerebral inflammation had developed in response to the infection. In this study, mice were treated with 100 mg of clindamycin per kg every 2 h for four doses. This schedule was chosen to compensate for the different serum half-lives of clindamycin in humans and mice (17). Levels in serum were slightly lower than those obtained with the higher dose, and levels in the brain could be detected by bioassay only at 1 h postadministration. The intracerebral infection and associated inflammation had no discernable effect on clindamycin levels in the brain when infected animals were compared with the control groups.

DISCUSSION

Clindamycin had a significant protective effect in this model of toxoplasmic encephalitis. Protection was demonstrated in terms of mortality, histopathology, spread of infection, and cyst formation. Most remarkable was the reduction in mortality from 100% in cortisone-treated mice to 0% when clindamycin was added to the regimen. In previous experiments, cortisone treatment of intracerebrally infected mice has been uniformly fatal (Hofflin et al., in press). Thus, this reduction in mortality is highly significant and important statistically. Whether the increased survival rate can be attributed primarily to the effect of clindamycin on intracerebral *Toxoplasma* multiplication or to an effect on the spread of the organism to tissues outside of the central nervous system is unclear.

The impact of clindamycin on the proliferation of intracerebral *Toxoplasma* organisms was demonstrated by the dramatic change in the histological features of the infection from those of a progressive destructive process to those of a well-localized, nondestructive one. Histologically, there were remarkably fewer *Toxoplasma* organisms in the brains of the clindamycin-treated mice than in the brains of the untreated controls. This observation was confirmed and quantitated by evaluation of the numbers of *Toxoplasma* cysts in the brains of the mice at 10 weeks after intracerebral infection. In addition, clindamycin markedly decreased the

FIG. 3. Cortisone-treated intracerebral *Toxoplasma* infection, day 7. Border (arrowheads) of necrotic area with remarkable lack of inflammation.

TABLE 1. Presence of *T. gondii* in different dilutions of triturated tissues from mice in four treatment groups

Organ(s) and treatment regimen	No. of mice infected ^a at indicated tissue dilution on:											
	Day 4			Day 7			Day 14			Day 21		
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³
Liver or spleen or both												
Control	3	1	0	1	0	0	2	0	0	3	0	0
Clindamycin	0	0	0	0	0	0	0	0	0	1	0	0
Cortisone	2	1	0	3	0	0	3	3	3	NA ^b		
Clindamycin + cortisone	2	0	0	0	0	0	2	1	0	3	3	3
Heart												
Control	0	0	0	2	0	0	3	2	0	3	0	0
Clindamycin	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone	0	0	0	2	0	0	3	3	0	NA		
Clindamycin + cortisone	3	0	0	0	0	0	0	1	0	3	2	0
Lung												
Control	3	0	0	3	0	0	3	3	2	3	3	3
Clindamycin	1	0	0	2	0	0	0	0	0	3	1	0
Cortisone	3	2	0	2	1	0	3	3	3	NA		
Clindamycin + cortisone	3	0	0	3	1	0	2	2	0	2	3	3

^a Three mice were inoculated with each dilution (see Materials and Methods).

^b NA, Not applicable, because none of the mice survived to 21 days.

inflammatory infiltrate which occurs in response to *Toxoplasma* infection in nonimmunosuppressed mice. Enhanced survival cannot, however, be attributed solely to a reduction in inflammation, because mice which were treated with cortisone alone did not have a significant inflammatory response, and 100% of them died. However, the brains of the latter did exhibit marked necrosis and unimpeded multiplication of *T. gondii*.

These histological findings were unexpected in light of the known poor penetration of normal and inflamed meninges by clindamycin and the low levels of clindamycin measured in the brains of the mice in this study. We did not detect a significant difference between clindamycin levels in the brains of normal mice and mice with acute or chronic inflammation of the central nervous system resulting from toxoplasmosis. However, our methodology did not allow for measurement of clindamycin levels specifically in the region of the injection site. The infection at this site may have resulted in disruption of the blood-brain barrier, with local accumulation of higher amounts of clindamycin than reflected by our measurement of the concentration of antibiotic in the entire brain. This may be important because reports of therapeutic levels (for anaerobic infections) of clindamycin in human brain abscesses have appeared (5), and we have documented a level of ≥ 2 $\mu\text{g/ml}$ in a brain

abscess in a patient 2 h after administration of 600 mg intravenously (unpublished observations).

The spread of infection to organs outside the central nervous system was enhanced by cortisone and markedly inhibited by clindamycin administration. Treatment with cortisone resulted in an increase in the number of organisms in peripheral organs. This difference from controls became more pronounced with time. In contrast, the effect of clindamycin was observable at day 4 of infection and persisted during the period of administration. Whether the reduction in spread of infection with clindamycin resulted from its effect primarily on organisms in the brain, blood, or peripheral organs was not determined in this study. After discontinuation of clindamycin, an increase in infection of all organs examined was observed in both normal and immunosuppressed mice. This observation suggests renewed proliferation or parasitemia or both. As is true of other anti-*Toxoplasma* agents, clindamycin was suppressive rather than curative.

Although the studies described are the first to examine the effect of clindamycin specifically on intracerebral toxoplasmosis, several published studies demonstrated that clindamycin has an in vivo effect on experimental murine infection with *T. gondii*. McMaster and co-workers (11) in 1973 cured

TABLE 2. Clindamycin levels in brains and sera of normal mice and mice chronically infected intraperitoneally with *T. gondii*

Time after intraperitoneal administration of clindamycin (h)	Clindamycin level ^a			
	Chronic <i>Toxoplasma</i> infection		Normal	
	Brain	Serum	Brain	Serum
1	1.2	15.9	2.4	12.6
2	0.6	2.0	0.6	1.7
4	— ^b	0.3	—	0.1

^a Concentrations are expressed as micrograms per gram of brain and as micrograms per milliliter of serum detectable by microbiological assay. Values are means for three mice.

^b —, Below the lower limit of detection of the assay.

TABLE 3. Clindamycin levels in brains and sera of normal mice and mice infected intracerebrally with *T. gondii*

Time after intraperitoneal administration of clindamycin (h)	Clindamycin level ^a			
	Intracerebral <i>Toxoplasma</i> infection		Normal	
	Brain	Serum	Brain	Serum
0.5	1.3	32.5	0.9	19.1
1	0.2	11.99	0.2	9.4
2	— ^b	2.1	—	1.2
3	—	0.6	—	0.4

^a Concentrations are expressed as micrograms per gram of brain and as micrograms per milliliter of serum detectable by microbiological assay. Values are means for three mice.

^b —, Below the lower limit of detection of the assay.

mice of infection with the virulent strain *Toxoplasma* sp. RH with clindamycin given by gavage or mixed into the food. Previous studies from this laboratory (1) confirmed the ability of clindamycin to decrease mortality in mice acutely infected with *Toxoplasma* organisms and documented a reduction in the prevalence of parasitemia in chronically infected mice. In addition, it was found that both clindamycin and sulfadiazine decreased the parasite load in the livers and spleens but not in the brains of chronically infected mice. In mice acutely infected intraperitoneally with a highly virulent *Toxoplasma* strain, Thiermann and co-workers (16) found the combination of clindamycin and sulfamethoxy-pyridazine to more effectively prevent infection in the brain than did spiramycin or trimethoprim in combination with the same sulfonamide. However, pyrimethamine in combination with sulfamethoxypyridazine was more effective.

In the single report in the literature (10) and in extensive unpublished studies from our own laboratory of in vitro assays of clindamycin activity against *T. gondii*, no efficacy was demonstrated. The reason for the discrepancy between this result and the efficacy found in the in vivo studies may relate to the need to hydrolyze clindamycin phosphate to the active clindamycin compound (4).

Only anecdotal reports of the use of clindamycin for toxoplasmic encephalitis in humans have appeared. Interpretation of these reports is difficult because the drug has usually been used in combination with agents with known efficacy. Nevertheless, given the urgent need for alternative agents for the therapy of toxoplasmic encephalitis, our results suggest that controlled trials of clindamycin in combination with agents of known efficacy should be undertaken.

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