ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1988, p. 755–757
0066-4804/88/050755-03$02.00/0

Vol. 32, No. 5

Azithromycin, a Macrolide Antibiotic with Potent Activity against *Toxoplasma gondii*

FAUSTO G. ARAUJO,[1] DOUGLAS R. GUPTILL,[1] AND JACK S. REMINGTON[1,2]*

Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301,[1] and Stanford University Center, School of Medicine, Stanford, California 94305[2]*

Received 13 July 1987/Accepted 3 February 1988

Doses of 200 mg of azithromycin per kg (body weight) administered by the oral route daily for 10 days completely protected mice against death caused by intraperitoneal infection with *Toxoplasma gondii*. The same treatment regimen also protected 80% of mice infected intracerebrally, which suggests that azithromycin attains active concentrations in the inflamed central nervous system.

Toxoplasma gondii has long been recognized as an important opportunistic protozoan for immunocompromised individuals (10). With the increased use of immunosuppressive therapy and the advent of acquired immunodeficiency syndromes, *T. gondii* has become one of the leading causes of encephalitis in immunosuppressed patients (2). The treatment of choice for toxoplasmosis is the synergistic combination of pyrimethamine and a sulfonamide (5). The optimum duration of treatment has not been determined because of the variable course and severity of the infection. Both drugs are potentially toxic; pyrimethamine is an inhibitor of dihydrofolate reductase, and prolonged therapy may result in depression of the bone marrow, a potentially serious side effect particularly in immunocompromised patients (7, 10). In addition, pyrimethamine is teratogenic, and its use during pregnancy is not recommended. In this case, spiramycin, a macrolide antibiotic that is not readily available to physicians in the United States, is recommended and widely used in Europe, particularly in France (6, 9). Alternative drugs, such as trimethoprim-sulfamethoxazole or clindamycin, have been shown to have activity in vitro and in mouse models, but clinical studies to establish their efficacy have not been conducted. There is thus a critical need for an evaluation of new drugs and drug combinations for the treatment of toxoplasmosis, especially drugs that can be safely used during pregnancy and in immunocompromised patients and drugs that are active in infections of the central nervous system.

Azithromycin, a macrolide antibiotic, was provided in powdered form by Pfizer Inc. (Groton, Conn.). The drug was dissolved in a small volume of 95% ethanol, and the desired concentrations for oral administration by gavage to Swiss-Webster adult female mice (18 to 20 g; Simonsen Laboratories, Gilroy, Calif.) were prepared in polyethylene glycol 200 (J. T. Baker Chemical Co., Phillipsburg, N.J.). Control animals received polyethylene glycol 200 only. Water and pelleted chow were available to the animals at all times.

Trophozoites of the RH or C56 strain of *T. gondii* were obtained from acutely infected mice as previously described (1, 8). In experiments in which RH trophozoites were used, mice were inoculated intraperitoneally (i.p.); when C56 trophozoites were used, mice were inoculated intracerebrally as previously described by Hofflin et al. (8). A statistical analysis of the results was performed by the chi-square test (11).

* Corresponding author.

To determine the activity of azithromycin against *T. gondii*, mice were infected i.p. with 10^2 RH trophozoites and 24 h later they were treated with the antibiotic administered by gavage in two daily doses for 10 days. Dosages higher than 100 mg/kg (body weight) per day resulted in a statistically significant ($P \leq 0.001$) survival rate compared with the rate in untreated controls (Table 1). Some mice treated with 100 or 150 mg/kg per day showed signs of acute infection (lethargy and ruffled fur), but those treated with 200 mg/kg per day did not. A slight accumulation of ascitic fluid was noted in some of the latter mice, but no toxoplasmas were found by microscopy of aspirated fluid. Because of these results and the fact that uninfected mice treated with 200 mg of azithromycin per kg per day did not show signs of drug toxicity, this concentration was used for subsequent experiments. In addition, in separate experiments, a single daily dose provided results that were equal to those obtained with two daily doses (data not shown). In studies in which 200 mg of azithromycin per kg per day was administered mixed with powdered chow (3), no protection was noted, possibly because the chow-containing drug was not palatable to the animals.

To determine the number of doses capable of providing protection, groups of 10 mice were infected i.p. with 10^2 RH trophozoites and treated with 200 mg of azithromycin per kg per day for 1, 2, or 3 days beginning 24 h after infection. The results revealed that, like controls, mice treated for 1 day had 100% mortality on day 8 after infection. By this time, mice treated for 2 and 3 days had 50 and 0% mortalities, respectively. When the experiment was terminated, 35 days after infection, 20 and 90% of the animals treated for 2 or 3 days were alive ($P < 0.01$ and $P < 0.001$, respectively).

To determine the activity of azithromycin administered at different times after infection, groups of 10 mice were infected i.p. with either 10^2 or 10^3 RH trophozoites per animal and treated with 200 mg of azithromycin per kg per day starting 24, 48, 72, 96, or 120 h after infection. In mice infected with 10^2 organisms, there was 100% survival when treatment was started 24, 48, or 72 h after infection (Fig. 1). The survival rate was still highly significant ($P < 0.001$) when treatment was started at 96 or 120 h after infection. In mice infected with 10^3 organisms, 100% survival occurred only in the group in which treatment started 24 h after infection; after 48 h, 80% of the mice survived ($P < 0.001$). There was no difference between treated and control mice when treatment was started 72 h after infection or later.

The potent ability of azithromycin to prevent death fol-

TABLE 1. Activity of azithromycin against death resulting from the i.p. inoculation of mice with 10^3 trophozoites of the RH strain of *T. gondii*

Treatment (mg/kg per day)[a]	% Mortality on day:			
	7	9	11	35
None	40	100		
25	20	80	100	
50	0	80	80	100
100	0	0	20	40
150	0	0	0	0
200	0	0	0	0

[a] Azithromycin was administered orally by gavage. The cumulative results of two experiments are given. In each experiment, there were five mice per group.

lowing i.p. infection of a lethal inoculum of *T. gondii* prompted us to examine the activity of the drug after intracerebral infection. Mice were infected with 10^4 trophozoites of the C56 strain of *T. gondii* by the intracerebral route of inoculation (8). Treatment with 200 mg of azithromycin per kg per day, administered orally by gavage, was started 24 h after infection and lasted for 10 days. Treatment resulted in a highly significant survival rate: only 2 of 10 treated mice died by day 14 of infection, whereas 9 mice of the control group were dead by that time ($P < 0.001$) (Fig. 2). All treated survivors after 14 days were still alive and well 35 days after infection, when the experiment was terminated. The single surviving control was noticeably sick on day 30, when it was killed. An examination of its brain revealed numerous cysts (8 cysts per 50 μl of a 2-ml suspension of the entire brain). The brains of three treated mice were examined, and in only one animal a single cyst was observed in 150 μl of a 2-ml suspension of the entire brain.

In vitro susceptibility studies have indicated superior or equivalent activity for azithromycin compared with erythromycin, tetracycline, and clindamycin when it is tested against several species of bacteria (12). Our results indicate that azithromycin is highly active in protecting mice against death due to a lethal infection with *T. gondii*. Compared with published data on roxithromycin, another macrolide with activity against *T. gondii*, the data on azithromycin showed

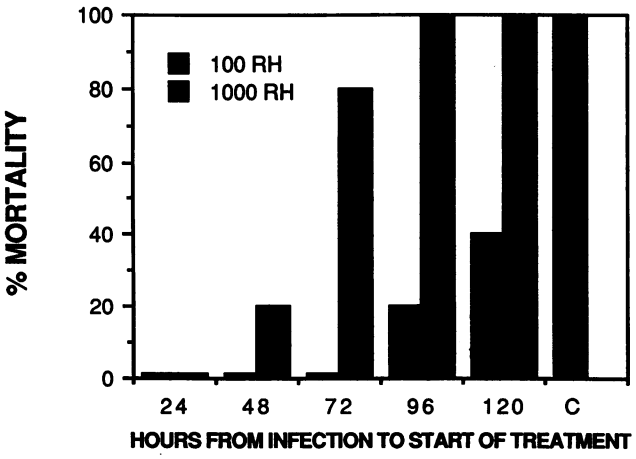

FIG. 1. Cumulative percent mortality in mice in which treatment with 200 mg of azithromycin per kg per day was started at different times after i.p. infection with 10^2 or 10^3 RH trophozoites of *T. gondii*. There were 10 mice per group. Mice were observed for 30 days after the termination of the treatment. C, Controls.

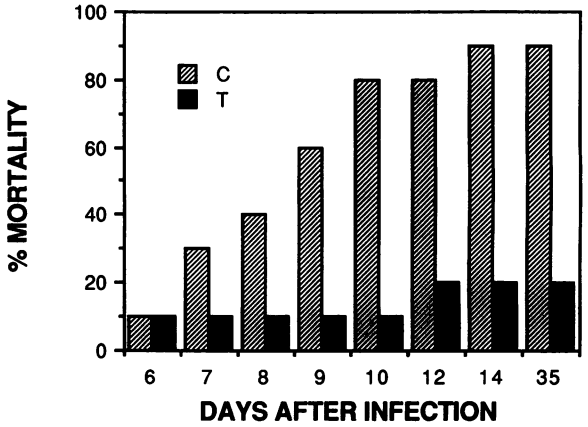

FIG. 2. Percent mortality in mice infected intracerebrally with 10^4 C56 trophozoites of *T. gondii* and treated with azithromycin. C, Controls ($n = 10$); T, treated ($n = 10$). Treatment with 200 mg of azithromycin per kg per day was started 24 h after infection and lasted for 10 days. Mice were observed for 35 days after infection.

significant activity at considerably lower concentrations. Thus, 10 mg of roxithromycin per mouse per day (3) or 540 mg of roxithromycin per kg per day (4) was necessary to protect 80 (3) and 100% (4) of mice infected with 1×10^3 or 5×10^3 organisms of the RH strain of *T. gondii*, respectively, whereas 100% of mice infected with 10^3 organisms of the same strain were protected by 200 mg of azithromycin per kg per day. Preliminary pharmacological studies provided by the manufacturer (*Azithromycin:Investigator's Reference Manual*, Pfizer Inc., 1986) indicate that azithromycin is well absorbed in humans and has a remarkable affinity for tissues. The half-life of the drug in humans appears to vary between 5 and 11 h, depending on the dose. Peak concentrations are attained between 1.5 and 3.3 h after oral administration. The drug is protein bound in serum and appears to be eliminated slowly, possibly because of low serum clearance and extensive distribution in the tissues. Thus, detectable levels of the drug can be found in the urine 7 to 14 days after the administration of a single dose. The relatively long half-life, the affinity for tissues, and the slow elimination of azithromycin indicate potential for a once-a-day dosing regimen. This was confirmed by our experiments, which showed that a relatively low concentration (200 mg/kg) administered daily for only 3 days was highly effective in protecting mice, even when the animals were infected with 10^3 organisms of the virulent RH strain of *T. gondii*. The remarkable protection provided by oral administration of azithromycin against death due to toxoplasmic encephalitis in a murine model is of great interest, because it suggests that the drug attains active concentrations in the inflamed central nervous system. This observation, with the results from infection by the i.p. route, indicates that studies with azithromycin as an alternative therapy for toxoplasmosis in humans are warranted.

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