Synergistic Activity of Clarithromycin and Minocycline in an Animal Model of Acute Experimental Toxoplasmosis

FRANCIS DEROUIN,1,4 BRUNO CAROFF,2 FRANÇOISE CHAU,2 PHILIPPE PROKOCIMER,3 AND JEAN JACQUES PO CIDALO2

Laboratoire de Parasitologie-Mycologie, Hôpital Saint-Louis, 1, Avenue Claude Vellefaux, 75475 Paris Cedex 10,1 and Institut National de la Santé et de la Recherche Médicale U 13, Hôpital Claude Bernard, Paris,2 France, and Abbott Laboratories, Abbott, Illinois 60064-35002

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The efficacy of clarithromycin in a murine model of acute toxoplasmosis was studied. Clarithromycin was administered alone and concurrently with minocycline, and efficacy was assessed by survival rates and sequential determination of parasite burden in blood, brains, and lungs. Limited protection resulted from administration of each drug alone, whereas a remarkable synergistic effect followed concurrent administration. Survival of mice treated with 200 mg of clarithromycin plus 20 mg of minocycline per kg of body weight daily was 95%; that of mice treated with 50 mg of clarithromycin plus 50 mg of minocycline per kg daily was 93%. The parasite burden in the blood and organ tissues of these mice was markedly reduced compared with that in mice treated with a single agent. In mice treated with 200 mg of clarithromycin plus 50 mg of minocycline per kg per day, survival was 100% during the 30-day experiment; no parasites were found in blood and tissues.

The combination of pyrimethamine and sulfadiazine is the standard therapy for toxoplasmic encephalitis. However, the incidence of side effects is high; skin reaction due to the sulfonamide and hematologic toxicity due to pyrimethamine have been reported with high frequency (8). Alternative regimens involving the replacement of one of the two components by another drug are currently being investigated. The combination of pyrimethamine plus clindamycin and the combination of pyrimethamine plus clarithromycin proved efficient for treatment of patients with toxoplasmic encephalitis (5, 7); other drug combinations using a macrolide have also been effective in an experimental model of acute toxoplasmosis (6). We designed a study to identify a combination of drugs that might make the use of folate inhibitors unnecessary. Since clarithromycin and minocycline are individually active against Toxoplasma gondii (3, 4), we evaluated the efficacy of their combination in an animal model of acute toxoplasmosis by sequential determination of parasite burden in blood and tissues.

(This study was presented in part at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, Calif., 10 to 14 October 1992.) Experiments were conducted with Swiss albino mice acutely infected with the highly virulent RH strain of T. gondii; infection was induced by intraperitoneal injection of 10⁶ tachyzoites. In initial experiments, groups of 10 animals each were treated with the following regimens: no treatment (controls), minocycline alone at 20 and 50 mg/kg of body weight daily, clarithromycin alone at 50 and 200 mg/kg daily, and all possible combinations of minocycline at 20 and 50 mg/kg daily and clarithromycin at 50 and 200 mg/kg daily. Minocycline (Lederle Laboratories) and clarithromycin (Abbott Laboratories) were prepared daily as liquid suspensions in distilled water and then briefly sonicated and administered orally to mice via tube feeding. Treatments were administered for 10 days beginning on day 1 after infection.

The treatment regimens associated with at least 50% survival by day 30 were then reevaluated by using larger groups of mice. Both survival rate and the kinetics of blood and tissue infection were evaluated. In these larger-sample experiments, mice were randomly allocated to separate groups: 30 mice served as controls (no treatment), and each treatment group consisted of 40 mice. The regimens consisted of minocycline and clarithromycin alone at each of their dose levels and the combination regimens at the following doses (per kilogram of body weight per day): 20 mg of minocycline plus 200 mg of clarithromycin, 50 mg of minocycline plus 50 mg of clarithromycin, and 50 mg of minocycline plus 200 mg of clarithromycin. The combination of 20 mg of minocycline plus 50 mg of clarithromycin was not tested, since the initial experiment indicated that only 30% of the mice on this regimen survived for 30 days.

Mice were studied for 30 days after infection. Monitoring of infection involved the estimation of survival rates by using the Kaplan Meier product limit method and sequential examination of parasite burden in blood, brains, and lungs on days 4, 7, 10, 14, 22, and 30 after infection; on day 30, liver and spleen tissues were also examined. At each time point, five mice from each group were sacrificed and blood and organ homogenates were cultured as described previously (12). Briefly, serial fourfold dilutions of each blood or organ suspension were prepared in the culture medium, and then 40 µl of each dilution was inoculated into duplicate wells of tissue culture plates. Previous experiments using the same experimental procedure had shown that antimicrobial agents in ground tissue had no effect on parasitic growth in the cultures (12). After 72 h of incubation at 37°C, cultures were fixed and examined for T. gondii with an indirect immunofluorescence assay. The presence of parasitic foci was recorded in each well; the final titer was the last dilution which gave at least one parasitic focus. The parasite burden was calculated as the reciprocal titer of parasites in tissue culture per milliliter × 1,000 or per gram × 1,000. Results were expressed as the log of the number of parasites per gram of tissue or per milliliter of blood. The mean value for

* Corresponding author.
the parasite burden for five mice (±1 standard error) was calculated for each time point.

Examination of survival rates (Table 1) and culture results indicated that clarithromycin treatment at 50 mg/kg daily was ineffective in prolonging survival or reducing the parasite burden. On day 4, the parasite burdens for control mice and clarithromycin-treated mice were 5.37 ± 0.2 and 4.96 ± 0.75 log units in lungs, 1.87 ± 0.62 and 1.81 ± 0.74 log units in brains and 0.6 ± 0.83 and 0.81 ± 0.74 log units in blood, respectively. Clarithromycin treatment at 200 mg/kg daily increased the mean survival time of mice, but protection was only partial, as 100% of the mice died within 18 days (Table 1). When parasite burden was examined, parasitic infection in lungs and blood was found to decrease from day 7 while that in brains increased between days 4 and 10 (Fig. 1A).

Minocycline treatment at 20 mg/kg daily also had limited efficacy, as 100% of the mice died within 10 days after infection, with high parasite burden in lungs (Fig. 1B). With a dose of 50 mg/kg daily, 27% of the mice were alive on day 30 (Table 1). When tissue cultures were studied, no parasites were detectable in blood throughout the follow-up period. In tissues, parasites were detectable only at a small number in brains on day 7 while parasite burdens in the lungs increased until day 7, became negative from day 10 to day 15, and then increased at day 22 (Fig. 1C).

The combination of clarithromycin and minocycline was associated with marked increases in survival (Table 1). On day 30, the survival rate was 93% for mice treated with 50 mg of clarithromycin plus 50 mg of minocycline per kg per day; it was 95% for those treated with 200 mg of clarithromycin plus 20 mg of minocycline per kg per day. The kinetics of parasite infection in blood and tissues were comparable. With both regimens, parasitemia remained undetectable. Parasite burdens decreased rapidly while mice were under treatment and, thereafter, remained at low levels in lungs and in brains (Fig. 1D and E). On day 30, no parasites were observed in livers; however, small numbers of parasites were observed in the spleens of two of the five mice treated with 200 mg of clarithromycin plus 20 mg of minocycline per kg per day (parasite burden = 0.6 ± 0.9 log units); this was also true for two of the five mice treated with 50 mg of clarithromycin plus 50 mg of minocycline per kg per day (parasite burden = 0.8 ± 1.2 log units). For mice treated with 200 mg of clarithromycin plus 50 mg of minocycline per kg per day, survival was 100% on day 30. Throughout the follow-up, parasites remained undetectable in blood and tissues, including spleen and liver tissues, on day 30 (Fig. 1F).

Our results confirm that minocycline alone is partially effective against murine toxoplasmosis. Examination of parasite burdens in blood and tissues of mice treated with 50 mg/kg daily indicated that, although drug efficacy was limited in lung tissue, a marked and prolonged reduction of parasite burden occurred in brain tissue. Previously, Chang et al. (3) observed a significant reduction in the number of brain cysts in chronically infected mice treated with minocycline at 50 mg/kg/day for 21 days. Taken together, these results suggest that minocycline is effective in the treatment of brain toxoplasmosis, probably because of its excellent penetration into brain tissue, which is related to its high lipophilicity (2, 11). Clarithromycin alone was ineffective when administered at 50 mg/kg daily, but a marked reduction of parasite burden in lung tissue was noted for mice treated with 200 mg/kg daily. However, the drug did not protect the animals from death; it only delayed it. When clarithromycin and minocycline were combined, a synergistic effect was observed. Our results are in agreement with those of Araujo et al. (1), who used a strain of lower virulence and also found 100% survival for mice treated with 200 mg of clarithromycin plus 50 mg of minocycline per kg daily; the survival rate was only 50% for mice treated with minocycline alone and 20% for those treated with clarithromycin alone. In our study, we evaluated this in vivo synergism between the two drugs by examining the parasite burden in blood and tissues of treated mice; parasites were never observed in blood or tissues when the combination of 50 mg of minocycline plus 200 mg of clarithromycin per kg daily was administered. With lower doses of either drug, the synergistic effect remained but the parasitic infection was detectable at a low level in some tissues. Since the mode of action of clarithromycin and minocycline on T. gondii is unknown, the explanation for this synergism is unclear. Our results lead us to hypothesize that the in vivo synergism reflects the complementary effect of the two drugs at the different sites of infection.

Finally, these results offer a reliable experimental basis for assessing the combination of clarithromycin and minocycline in the treatment of toxoplasmosis in humans, particularly those intolerant of sulfadiazine and/or pyrimethamine. Our findings also offer a rationale for combining the two drugs for the prophylaxis of toxoplasmosis; prolonged drug efficacy, as evidenced by maintenance of low parasite burden after discontinuation of therapy, was observed in our experiments. Also, minocycline has demonstrated possible efficacy on brain cysts, as reported elsewhere (3). Because of the antiparasitic spectrum of the two drugs (9, 10, 13), it may be worthwhile to explore the possibilities of preventing multiple opportunistic infections.

### Table 1. Survival of mice after infection with T. gondii

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>% of mice surviving on day:</th>
<th>Mean survival (days)</th>
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</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Minocycline (20)</td>
<td>100</td>
<td>88</td>
</tr>
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<td>100</td>
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<td>Clarithromycin (50)</td>
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<td>Clarithromycin (200)</td>
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<td>Minocycline (50) + clarithromycin (50)</td>
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<tr>
<td>Minocycline (50) + clarithromycin (200)</td>
<td>100</td>
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*Survival was estimated on the date of examination of parasite burden in blood and tissues (Fig. 1).
*In milligrams per kilogram of body weight per day. n = 40 except for the control group (n = 30).*
FIG. 1. Kinetics of parasite burdens in the blood (○), lungs (■), and brains (●) of mice infected on day 0 with 10⁴ tachyzoites of the RH strain. Each point represents the mean ± standard error of the mean for five mice. Shaded areas represent the period of administration of the antimicrobial agent(s) (i.e., days 1 to 10 after induction of infection). Treatments represented in the graphs are as follows (with doses in milligrams per kilogram per day): (A) clarithromycin, 200; (B) minocycline, 20; (C) minocycline, 50; (D) clarithromycin, 50, plus minocycline, 50; (E) clarithromycin, 200, plus minocycline, 20; (F) clarithromycin, 200, plus minocycline, 50.
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REFERENCES