ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1992, p. 2454–2457
0066-4804/92/112454-04$02.00/0

Vol. 36, No. 11

Activity of Clarithromycin Alone or in Combination with Other Drugs for Treatment of Murine Toxoplasmosis

FAUSTO G. ARAUJO,[1] PHILLIPPE PROKOCIMER,[2] TERI LIN,[1] AND JACK S. REMINGTON[1,3]*

Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301[1]; Abbott Laboratories, Abbott Park, Illinois 60064[2]; and Stanford University School of Medicine, Stanford, California 94305[3]*

Received 14 May 1992/Accepted 31 August 1992

The activity of the macrolide antibiotic clarithromycin was examined alone or in combination with other drugs for the treatment of acute or chronic infections with *Toxoplasma gondii* in mice. A dose of 300 mg of clarithromycin per kg per day administered alone for 10 days, beginning 24 hours after infection, protected 10 to 30% of mice infected with lethal inocula of tachyzoites or tissue cysts of different strains of *T. gondii*, including some strains isolated from patients with both AIDS and toxoplasmosis. Although clarithromycin was protective, a wide variation in its activity against different strains was observed. Survival of infected mice was increased significantly by treatment with clarithromycin in combination with pyrimethamine or with sulfadiazine. Treatment of chronically infected mice with clarithromycin at 300 mg/kg/day administered alone for 8 weeks resulted in significant reduction in the numbers of *T. gondii* cysts in their brains. The combination of clarithromycin and minocycline resulted in an activity against *T. gondii* cysts that was significantly greater than the activity of clarithromycin or minocycline administered alone. These results indicate a role for clarithromycin in the treatment of human toxoplasmosis, particularly when this antibiotic is used in combination with other drugs with activity against *T. gondii*.

At present the treatment of choice for toxoplasmosis remains the synergistic combination of pyrimethamine and a sulfonamide (14). However, untoward side effects that may require discontinuation of the therapy are relatively frequent when this combination is used to treat toxoplasmosis in immunocompromised individuals, particularly patients with AIDS (13). Therefore, a considerable research effort is in progress to identify new therapeutic agents and new therapeutic combinations for treatment of toxoplasmosis, particularly toxoplasmic encephalitis. A number of azalide/macrolide antibiotics have been demonstrated to have in vitro and in vivo activities against *Toxoplasma gondii* (1, 2, 5–7, 10). Of these, clarithromycin, a 6-*O*-methyl derivative of erythromycin, has been shown to have in vitro and in vivo activity against *T. gondii* (8, 9). We have examined the activities of clarithromycin alone and in combination with other drugs in treating acute and chronic infections with different strains of *T. gondii*, including strains isolated from patients with AIDS.

MATERIALS AND METHODS

Drugs. Clarithromycin (lot 134-974-AX; Abbott Laboratories, Abbott Park, Ill.) was dissolved in phosphate-buffered saline (PBS; pH 6.8), sonicated before each administration, and given orally through a feeding needle. Minocycline HCl (lot 798; Lederle Laboratories, Division of American Cyanamid Co.) was dissolved in distilled water, the desired concentrations were prepared in polyethylene glycol 500 as previously described (6), and the drug was administered orally. Pyrimethamine (lot 3F0991; Burroughs-Wellcome Co.) was dissolved in 0.25% carboxymethyl cellulose containing 0.05% Tween 20 and administered orally. Sulfadiazine (Sigma Chemical Co., St. Louis, Mo.) was dissolved and administered in drinking water.

* Corresponding author.

Mice. Swiss Webster or CBA/Ca female mice, weighing 20 g at the beginning of each experiment, were used.

***T. gondii*.** Tachyzoites of *T. gondii* RH or tissue cysts of *T. gondii* ME49 or C56 were used. In addition, tachyzoites of *T. gondii* MO, SOU, and CAST, isolated from patients with both AIDS and toxoplasmic encephalitis (6), were used. Tachyzoites were obtained from the peritoneal cavities of previously infected mice (2); cysts were collected from brains of chronically infected mice (12).

Statistical analysis. Statistical analysis was performed by using the Mann-Whitney U test.

RESULTS

Determination of the activity of clarithromycin against acute murine toxoplasmosis. Swiss Webster mice were infected intraperitoneally (i.p.) with 2.5×10^3 RH tachyzoites or orally with 10 cysts of the C56 strain. Treatment with clarithromycin or with the other drugs alone or in combination was initiated at different intervals after infection. Mice were observed for survival, and brains of survivors were examined for *T. gondii* cysts as previously described (3).

Although there was variation in the activity of clarithromycin against acute murine toxoplasmosis, repeated experiments consistently demonstrated protection, as represented by survival of 10 to 30% of mice infected with a lethal inoculum of RH tachyzoites (Fig. 1) or C56 cysts (Fig. 2) and treated with a daily dose of 300 mg of clarithromycin per kg for 10 days beginning 24 h after infection. A similar dose and schedule of treatment was also active against strains of *T. gondii* isolated from patients with AIDS, but a wide variation in activity of the antibiotic against different strains of the parasite was observed. Thus, five experiments performed on different days revealed survival of 10 to 30% of mice infected with RH (Fig. 1) or MO (Fig. 3A) tachyzoites and 20% of mice infected with SOU tachyzoites (Fig. 3B). However, in three experiments 100% of mice infected with CAST tachyzoites died (Fig. 3C). An increase in the dose of clarithro-

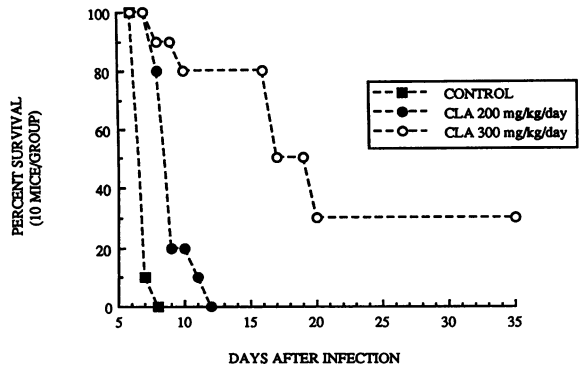

FIG. 1. Survival of mice infected i.p. with 2.5×10^3 tachyzoites of *T. gondii* RH and treated orally with clarithromycin (CLA) at 200 or 300 mg/kg/day for 10 days beginning 24 h after infection (P = 0.018 for the dose of 300 mg; Mann-Whitney U test).

mycin to 400 or 500 mg/kg/day did not result in improved survival of mice infected with any of the strains of *T. gondii*.

Activities of clarithromycin in combination with other drugs. Synergistic activity was observed when clarithromycin was combined with other drugs that are active against *T. gondii*. In two experiments, when mice infected with RH tachyzoites were treated with clarithromycin at 300 mg/kg/day combined with pyrimethamine at 15 mg/kg/day, at least 70% survived, whereas no more than 10% of control animals treated with just one of the drugs survived (Fig. 4). Similarly, combination of a noncurative dose of clarithromycin with a noncurative dose of sulfadiazine resulted in 60% survival (Fig. 5).

Effect of treatment on cysts in brains of chronically infected mice. To determine activity against *T. gondii* cysts, we infected CBA/Ca mice orally with 10 cysts of *T. gondii* ME49 as previously described (3). The infection was allowed to develop in these mice for 6 weeks without treatment. Then oral treatment with clarithromycin alone at 300 mg/kg/day was initiated and continued for 8 weeks. At 2-week intervals after initiation of treatment, groups of three mice were killed and their brains were collected and divided in half. One-half of each brain was mixed with PBS and ground with a mortar and pestle, and the number of cysts in five samples of 20 μl each was determined. The other portion of each brain was fixed and used for histopathologic testing (3).

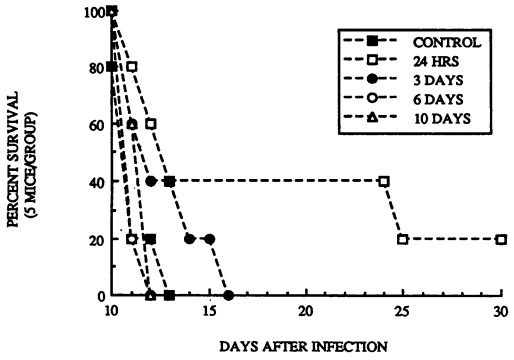

FIG. 2. Survival of mice infected orally with cysts of *T. gondii* C56 and treated orally with clarithromycin at 300 mg/kg/day beginning at the indicated times after infection (P = 0.02 for the 24-h period).

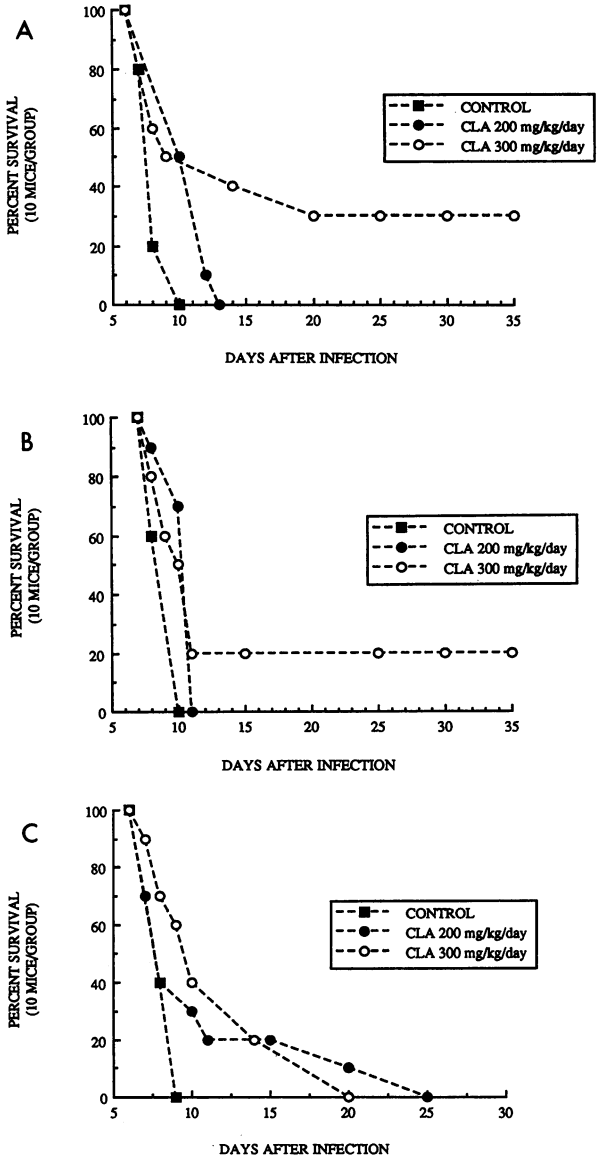

FIG. 3. Activity of clarithromycin (CLA) in mice infected i.p. with 2.5×10^3 tachyzoites of *T. gondii* MO (A), SOU (B), and CAST (C). All three strains were isolated from patients with both AIDS and toxoplasmic encephalitis (P = 0.019 for the dose of 300 mg in panel A, and P = 0.1 for the dose of 300 mg in panel B).

Treatment with clarithromycin at 300 mg/kg/day for 8 weeks resulted in a significant reduction in the number of cysts in the brains of the infected mice (Fig. 6). Because of these results and our previous demonstration of the synergistic effect of the combination of clarithromycin and the tetracycline analog minocycline in the treatment of acute murine toxoplasmosis (4), we considered it of interest to determine whether a larger reduction in the numbers of *T. gondii* cysts in the brains of mice would be observed in mice treated with the clarithromycin-minocycline combination. For this experiment, we infected groups of CBA/Ca mice i.p. with 20 cysts of strain ME49 and, 6 weeks later, treated them orally with clarithromycin at 200 mg/kg/day plus minocycline at 50 mg/kg/day or with an equal concentration of clarithromycin or minocycline administered alone. Treatment was

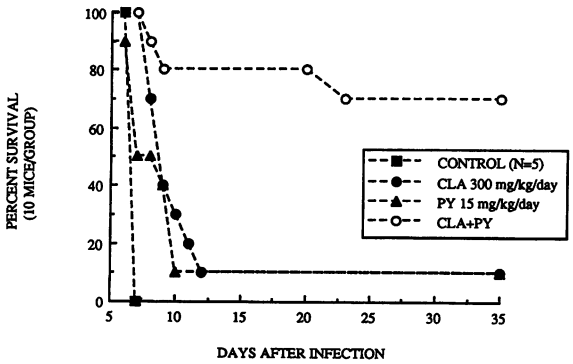

FIG. 4. Synergistic effect of the combination of clarithromycin (CLA) and pyrimethamine (PY) in treatment of acute murine toxoplasmosis. Mice were infected i.p. with 2.5×10^3 RH tachyzoites. Oral treatment was initiated 24 h after infection and was continued for 10 days ($P = 0.011$ when clarithromycin alone was compared with the combination).

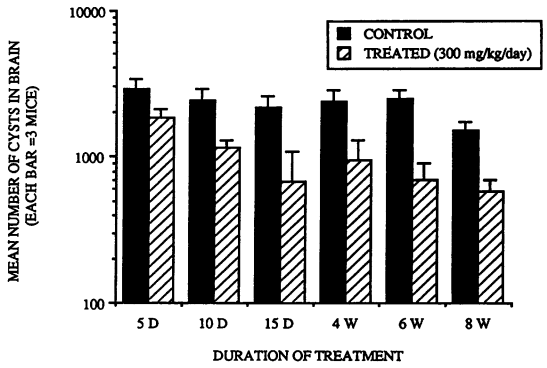

FIG. 6. Activity of clarithromycin alone against cysts of *T. gondii* in brains of chronically infected mice. CBA/Ca mice were infected orally with 10 cysts of strain ME49. Oral treatment with clarithromycin at 300 mg/kg/day was initiated 6 weeks after infection and continued for 8 weeks. Bars indicate the mean (and standard error) of the number of cysts in the brains of three treated and three control mice at the indicated days or weeks after initiation of treatment. Differences between treated and control mice from day 10 through 8 weeks were statistically significant ($P < 0.001$, Mann-Whitney U test). Abbreviations: D, days; W, weeks.

continued daily for 4 weeks. At 2 and 4 weeks after initiation of treatment, mice were killed and their brains were processed as described above for cyst counts and histopathologic testing. The number of *T. gondii* cysts observed in brains of mice treated with the combination was significantly decreased from that in the controls (Fig. 7). In addition, histopathologic testing revealed less brain inflammation in the mice treated with the combination than in the mice treated with each drug alone.

DISCUSSION

The above results demonstrate that clarithromycin is active in the treatment of toxoplasmosis in mice infected either i.p. or orally with lethal inocula of different strains of *T. gondii*. However, a wide variation in the response to the treatment was noted in mice infected with different strains of the parasite. This observation may have implications for therapy of toxoplasmosis in humans, particularly those with AIDS. However, it may not necessarily predict a relative lack of efficacy for certain strains in humans, since the pharmacokinetics of clarithromycin in mice and humans may differ considerably. In fact, results of a trial in patients with both AIDS and toxoplasmic encephalitis revealed that treatment with the combination of clarithromycin and pyrimethamine resulted in partial resolution of brain lesions on computed tomography and in striking clinical responses (11).

A synergistic effect was observed when clarithromycin was used in combination with pyrimethamine, sulfadiazine, or minocycline. The observed synergism of the combination of clarithromycin and minocycline is of particular interest since it indicates that the combination of clarithromycin and drugs other than pyrimethamine or sulfadiazine might be effective for treatment of human toxoplasmosis in patients with AIDS. This observation is important because drugs that can replace pyrimethamine or sulfadiazine for treatment of toxoplasmosis in patients with AIDS are urgently needed.

Clarithromycin alone or in combination with minocycline

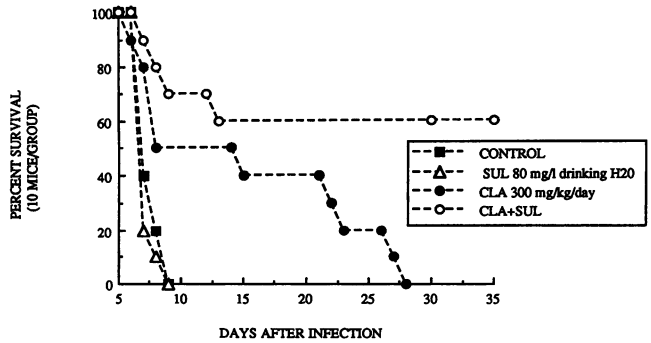

FIG. 5. Synergistic effect of the combination of clarithromycin (CLA) plus sulfadiazine (SUL) in treatment of acute murine toxoplasmosis. Infection and treatment were as for Fig. 4 ($P = 0.012$ when clarithromycin alone was compared with the combination).

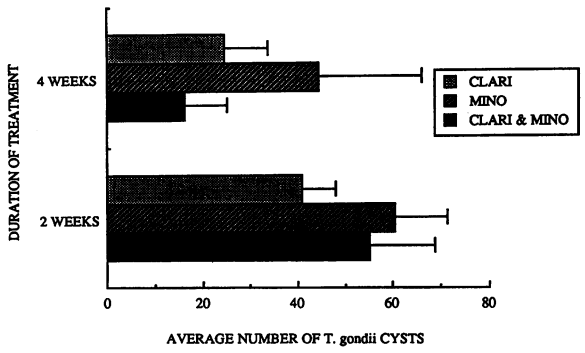

FIG. 7. Activity of the combination of clarithromycin (CLARI) and minocycline (MINO) against *T. gondii* cysts in brains of chronically infected mice. Mice were infected as for Fig. 6. Treatment with 200 mg of clarithromycin per kg in combination with 50 mg of minocycline per kg was administered orally beginning 6 weeks after infection and continued, daily, for 6 weeks. Groups of mice were killed at 2 and 4 weeks of treatment. Each bar represents the mean (and standard error) of the number of cysts in five mice. *P* values of the differences between clarithromycin alone or in combination with minocycline were 0.004 at 4 weeks and 0.03 at 2 weeks. For the combination of clarithromycin and minocycline and for minocycline alone the values were 0.0002 at 4 weeks and 0.3 at 2 weeks (Mann-Whitney U test).

was also found to be active against cysts of *T. gondii* in vivo. This activity was evidenced by a reduction in the number of cysts in the brains of treated mice. The reduction was noticed as early as 5 days after initiation of treatment and was statistically significant after 2 and 4 weeks of treatment. However, there were few differences between the inflammatory responses in the brains of treated and untreated mice.

In the mouse model used in the present studies, the pathogenesis of the encephalitis differs from what is believed to occur in patients with AIDS. Thus, in the model used, the toxoplasmic encephalitis results from a primary infection and the animals are treated while they still have considerable inflammation in their brains. In patients with AIDS, toxoplasmic encephalitis is believed to originate from reactivation of a latent *T. gondii* infection. Despite this, the remarkable activity of clarithromycin alone or in combination with other drugs against the acute infection in mice and against the cysts in the chronic infection supports its use in carefully designed studies of treatment of toxoplasmosis.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI04714 and AI30230 and a grant from Abbott Laboratories.

REFERENCES

1. **Araujo, F. G., D. R. Guptill, and J. S. Remington.** 1988. Azithromycin, a macrolide antibiotic with potent activity against *Toxoplasma gondii*. Antimicrob. Agents Chemother. **32**:755–757.
2. **Araujo, F. G., J. Huskinson, and J. S. Remington.** 1991. Remarkable in vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against tachyzoites and cysts of *Toxoplasma gondii*. Antimicrob. Agents Chemother. **35**:293–299.
3. **Araujo, F. G., J. Huskinson-Mark, W. E. Gutteridge, and J. S. Remington.** 1992. In vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against the cyst form of *Toxoplasma gondii*. Antimicrob. Agents Chemother. **36**:326–330.
4. **Araujo, F. G., P. Prokocimer, and J. S. Remington.** 1992. Clarithromycin-minocycline is synergistic in a murine model of toxoplasmosis. J. Infect. Dis. **165**:788.
5. **Araujo, F. G., and J. S. Remington.** 1991. Synergistic activity of azithromycin and gamma interferon in murine toxoplasmosis. Antimicrob. Agents Chemother. **35**:1672–1673.
6. **Araujo, F. G., R. M. Shepard, and J. S. Remington.** 1991. In vivo activity of the macrolide antibiotics azithromycin, roxithromycin and spiramycin against *Toxoplasma gondii*. Eur. J. Clin. Microbiol. Infect. Dis. **10**:519–524.
7. **Chang, H. R., and J.-C. Pechère.** 1988. In vitro effects of four macrolides (roxithromycin, spiramycin, azithromycin [CP-62,993], and A-56268) on *Toxoplasma gondii*. Antimicrob. Agents Chemother. **32**:524–529.
8. **Chang, H. R., F. C. Rudareanu, and J. C. Pechere.** 1988. Activity of A-56268 (TE-031), a new macrolide, against *Toxoplasma gondii* in mice. J. Antimicrob. Chemother. **22**:359–361.
9. **Derouin, F., and C. Chastang.** 1988. Activity in vitro against *Toxoplasma gondii* of azithromycin and clarithromycin alone and with pyrimethamine. J. Antimicrob. Chemother. **25**:708–711.
10. **Derouin, F., J. Nalpas, and C. Chastang.** 1988. Mesure in vitro de l'effet inhibiteur de macrolides lincosamides et synergestines sur la croissance de *Toxoplasma gondii*. Pathol. Biol. **36**:1204–1210.
11. **Fernandez-Martin, J., C. Leport, P. Morlat, M. C. Meyohas, J. P. Chauvin, and J. L. Vilde.** 1991. Pyrimethamine-clarithromycin combination for therapy of acute *Toxoplasma* encephalitis in patients with AIDS. Antimicrob. Agents Chemother. **35**:2049–2052.
12. **Huskinson-Mark, J., F. G. Araujo, and J. S. Remington.** 1991. Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*. J. Infect. Dis. **164**:170–177.
13. **Israelski, D. M., B. R. Dannemann, and J. S. Remington.** 1990. Toxoplasmosis in patients with AIDS, p. 241–264. *In* M. A. Sande and P. A. Volberding (ed.), The medical management of AIDS. The W. B. Saunders Co., Philadelphia.
14. **McCabe, R. E., and J. S. Remington.** 1990. *Toxoplasma gondii*, p. 2090–2103. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennet (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, Inc., New York.