Activity of Roxithromycin (RU 28965), a Macrolide, against
Toxoplasma gondii Infection in Mice

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Roxithromycin (RU 28965), an ether oxime derivative of erythromycin, protected mice against a lethal infection with the virulent RH strain of Toxoplasma gondii. Therapy begun 24 h before, 2 h after, and 24 h after infection with $2 \times 10^5$ tachyzoites protected 90, 80, and 50% of the mice, compared with 0% of untreated controls ($P < 0.05$ to 0.001). Toxoplasma was isolated in <20% of surviving roxithromycin-treated mice.

Roxithromycin (RU 28965), a new macrolide antibiotic, is an ether oxime derivative of erythromycin (5). The drug has antimicrobial activity similar to that of erythromycin (1, 5). The recommended treatment for toxoplasmosis is the combination of pyrimethamine and sulfadiazine (P-S) (or triple sulfonamides). However, this therapeutic regimen is not without side effects, including bone marrow suppression and skin rash (6-8; L. A. Price and P. K. Bondy, Letter, Lancet i:727, 1973), which sometimes necessitate discontinuation of therapy. Furthermore, pyrimethamine is considered potentially teratogenic (10), and therefore its usefulness in the first trimester of pregnancy is limited. In Europe, spiramycin has been used for the treatment of toxoplasmosis during pregnancy (2, 12). In a prospective study of the offspring of 542 women who acquired toxoplasmosis during pregnancy, treatment with spiramycin reduced the frequency of toxoplasma in the placenta and the number of stillbirths (2).

Because of the apparent efficacy of this macrolide against toxoplasmosis, we studied the effect of another macrolide, roxithromycin, against acute toxoplasmosis in mice.

Female Swiss Webster mice (15 to 16 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used in all experiments. Roxithromycin (a gift from W. Novick, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) was supplied in powder form. It was mixed in with normal powder mouse chow (Rodent Lab Chow 5001; Ralston Purina Co.). The daily dose of roxithromycin was delivered in various amounts per 4 g of food, because mice eat approximately 4 g of the diet per day (4). Control mice received plain food. Treatment was started 24 h before (prophylaxis), 2 h after (early), or 24 h after (delayed) inoculation with tachyzoites of the virulent RH strain of Toxoplasma gondii. The mice ate the plain diet and the food containing roxithromycin at a similar rate. Similarly, pyrimethamine (Burroughs Wellcome Co., Research Triangle Park, N.C.) and sulfadiazine (Eli Lilly & Co., Indianapolis, Ind.) were administered 24 h after inoculation at daily doses of 0.044 and 5 mg, respectively (3). Therapy was continued for 3 weeks for mice inoculated with $2 \times 10^5$ organisms. Mice inoculated with $2 \times 10^5$ or $2 \times 10^4$ organisms were treated for 4 weeks. Levels of roxithromycin in serum were obtained from uninfected mice that were fed 10 mg of the drug per day. Samples were collected by axillary bleeding at 0, 3, 6, 9, 24, 48, and 96 h after the start of therapy. Levels in serum were measured by A. Barry of the Clinical Microbiology Institute, Inc., Tualatin, Ore., by a standard bioassay with Micrococcus luteus ATCC 9341 ("Sarcina lutea") as the indicator organism (personal communication). The peak level in serum achieved by 10 mg of roxithromycin orally per day was 2.0 $\mu$g/ml.

Tachyzoites of the virulent RH strain of T. gondii were harvested and prepared as described previously (9) and suspended at $10^5$, $10^4$, and $10^3$ organisms per ml in phosphate-buffered saline. The organisms were injected intraperitoneally in a volume of 0.2 ml, and the mice were monitored for time to death and percent mortality. There were 10 mice per group. All survivors remained clinically healthy 4 weeks after the discontinuation of roxithromycin. They were then sacrificed, and homogenates of their liver, spleen, and brain were injected intraperitoneally into normal mice to assess residual infection. Residual infection was demonstrated by the ability of the subinoculum to kill mice injected with the organs of survivors. In preliminary experiments, fewer than 10 organisms of the RH strain injected in homogenates of normal murine liver, spleen, or brain killed 100% of the mice (11).

A dose-response experiment with the early-treatment regimen demonstrated significant protection against $2 \times 10^3$ organisms at doses of 7.5 mg/day (60% mortality, $P < 0.05$) and 10 mg/day (100% mortality, $P < 0.001$), when compared with the control regimen (100% mortality) (Table 1). Mice treated with 3 mg of roxithromycin per day had 100% mortality. Mice treated with 5 mg of the drug per day had no significant decrease in mortality compared with the controls ($P > 0.05$); however, there was an increase in time to death. Prophylactic treatment with 10 mg of roxithromycin per day protected 100, 90, and 100% of the mice inoculated with $2 \times 10^5$, $2 \times 10^4$, and $2 \times 10^3$ organisms, respectively; 100% of the controls died ($P < 0.001$). Residual infection was detected in 30% (the brain of one animal; the liver of another; and the brain, liver, and spleen of a third animal), 0%, and 20% (the liver of one animal and the spleen of another animal) of survivors inoculated with $2 \times 10^5$, $2 \times 10^4$, and $2 \times 10^3$ organisms, respectively. Early treatment with the same dose protected 80% of the mice inoculated with either $2 \times 10^5$ or $2 \times 10^4$ organisms; 100% of the controls died ($P < 0.001$). Residual infection was detected only in the group inoculated with $2 \times 10^3$ organisms (37%; the spleens of three animals).

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To evaluate the efficacy of roxithromycin against toxoplasmosis under more stringent conditions, we used the delayed-treatment regimen comparing roxithromycin (10 mg/day) with P-S (0.044 and 5 mg/day). The dosage of P-S used was previously found to be effective in the treatment of murine toxoplasmosis (3). P-S (10% mortality) was more effective in preventing death than was roxithromycin (50% mortality), although the difference was not statistically significant ($P > 0.05$; Table 1).

Our results indicate that the new macrolide, roxithromycin, at a dose of 10 mg orally per day, is effective in the treatment of acute infection with T. gondii RH in mice. However, this dose of roxithromycin was less effective in preventing death than was the standard treatment, P-S. Therefore, roxithromycin may offer a safer alternative therapy against toxoplasma infection. Further studies in this regard are warranted.

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**LITERATURE CITED**


