

## In Vivo Synergism of Roxithromycin (RU 965) and Interferon against *Toxoplasma gondii*

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**We investigated the activity of roxithromycin (RU 965) and gamma interferon alone and in combination in a murine model of toxoplasmic encephalitis. Roxithromycin at a dosage of 35 or 50 mg per mouse per day decreased mortality. Gamma interferon alone significantly prolonged time to death. When combined, the two agents were remarkably synergistic.**

Patients with acquired immune deficiency syndrome appear particularly prone to the development of adverse reactions associated with sulfonamides (2, 3). Because these agents are part of the standard regimens used to treat *Pneumocystis* pneumonia and toxoplasmic encephalitis, there is a need for alternative therapies for both of these opportunistic infections.

Roxithromycin (RU 965) is a macrolide antibiotic with an in vitro antimicrobial spectrum similar to that of erythromycin. However, roxithromycin has a significantly longer half-life and produces higher levels in serum (7). In addition, its activity against certain pathogens (e.g., *Legionella* and *Chlamydia* spp.) is greater than that of other macrolides (9; C. M. Khurana and P. A. Deddish, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 204, 1985). Its activity against *Toxoplasma gondii* is being investigated because of the known activity of other macrolides, especially spiramycin, against this parasite. Chan and Luft (J. Chan and B. J. Luft, 25th ICAAC, abstr. no. 1114, 1985) have reported that roxithromycin provides protection in mice infected intraperitoneally with *T. gondii*, and more recently, in collaboration with Luft and his colleagues, we noted the efficacy of roxithromycin in a mouse model of toxoplasmic encephalitis developed in our laboratory (B. J. Luft, J. Hofflin, J. Chan, and J. S. Remington, 26th ICAAC, abstr. no. 1105, 1986; J. M. Hofflin, F. K. Conley, and J. S. Remington, J. Infect. Dis., in press).

Another agent which recently was shown to be effective in murine toxoplasmosis is gamma interferon (4). In preliminary studies in the encephalitis model, neither roxithromycin nor gamma interferon was completely protective (unpublished observations). We therefore considered it of interest to further investigate the activity of these two agents alone and in combination in the murine model of toxoplasmic encephalitis.

Nine or ten female Swiss Webster mice, 7 to 8 weeks old and 18 to 20 g in weight (Simonsen Laboratories, Gilroy, Calif.), were used in each treatment group. Tachyzoites of the relatively virulent C56 strain of *T. gondii* were harvested from the peritoneal fluid of mice infected 6 days earlier. The infected peritoneal fluid was filtered through a polycarbonate membrane (3- $\mu$ m pore size; Nuclepore Corp., Pleasanton, Calif.) to obtain organisms free of host cells and debris. After filtration, the purified tachyzoites were washed and sus-

ended to the desired concentration in Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) containing 100 U of penicillin per ml and 100  $\mu$ g of streptomycin per ml. Unless stated otherwise, the concentration of parasites was  $3.3 \times 10^6$ /ml. Viability of the organisms was greater than 95% as determined by trypan blue exclusion.

Injections were delivered into the right frontal lobe of the brain with the use of a stereotactic unit as described previously (Hofflin et al., in press). After anesthesia with methoxyfluorane and placement in the stereotactic unit, the skull was penetrated with a 0.5-in. (ca. 1.3-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- $\mu$ l inoculum ( $10^4$  organisms) of the tachyzoite suspension was injected with a 50- $\mu$ l glass syringe. The 30-gauge needle was checked for patency after each injection, and mice were euthanized if needle blockage had occurred.

Roxithromycin (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.) was thoroughly mixed into powdered mouse feed (Simonsen Laboratories) with a food mixer for a minimum of 6 h. The amount of antibiotic added to the feed was determined based on the observation that mice of this weight consume approximately 4 g of feed per day (1). The mice were given ad libitum access to the feed in containers covered with grids which minimize spillage so that an estimate of ingested feed could be made.

Recombinant murine gamma interferon (Genentech, South San Francisco, Calif.) was diluted in phosphate-buffered saline with 10% fetal bovine serum (PBS), and 0.2 ml was injected intravenously via the tail vein. Control mice received PBS alone. Injections were administered every other day for three doses beginning 6 h after intracerebral infection.

The brains of mice that survived 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- $\mu$ l sample of the resulting suspension was placed on a microscope slide under a coverslip (20 by 50 mm), the entire slide was scanned, and *Toxoplasma* cysts were counted. Because earlier studies from our laboratory (10) showed insignificant differences in the numbers of cysts in multiple samples from an individual suspension, only single preparations were counted. All statistical analyses were performed by the chi-square method.

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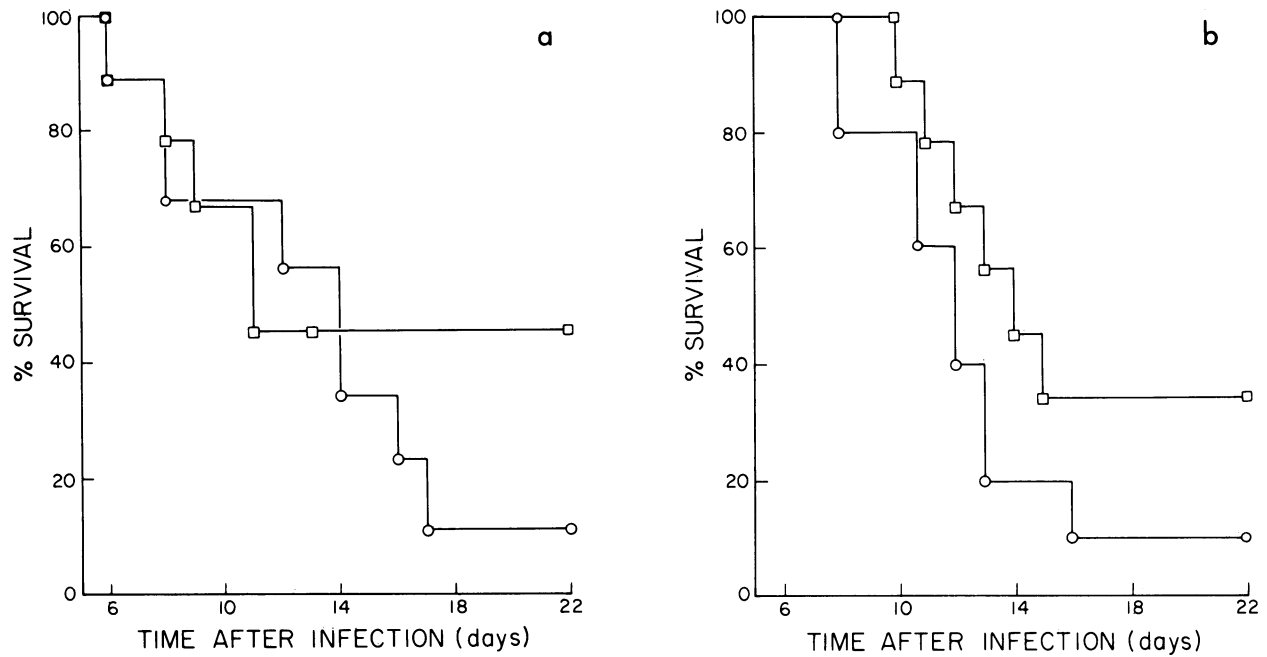


FIG. 1. Percent survival of treated and untreated mice. (a) Mice treated with 50 mg of roxithromycin per day (□); controls (○). (b) Mice treated with 35 mg of roxithromycin per day (□); controls (○).

Roxithromycin (10 mg per mouse per day) that was administered 2 days before infection had no effect on the 100% mortality rate or the median time to death of 10.5 days. When the experiment was repeated with 50 mg of roxithromycin per mouse per day (Fig. 1a), survival increased from 11% in control mice to 44% in the treated

group. Furthermore, the median number of cysts in 50- $\mu$ l samples of brain suspensions of the four treated mice that survived was 41 cysts (range, 21 to 80), compared with 86 cysts in the control mouse that survived. Because it was observed that the treated mice had consumed less feed, perhaps because of the bitter taste of the antibiotic (W.

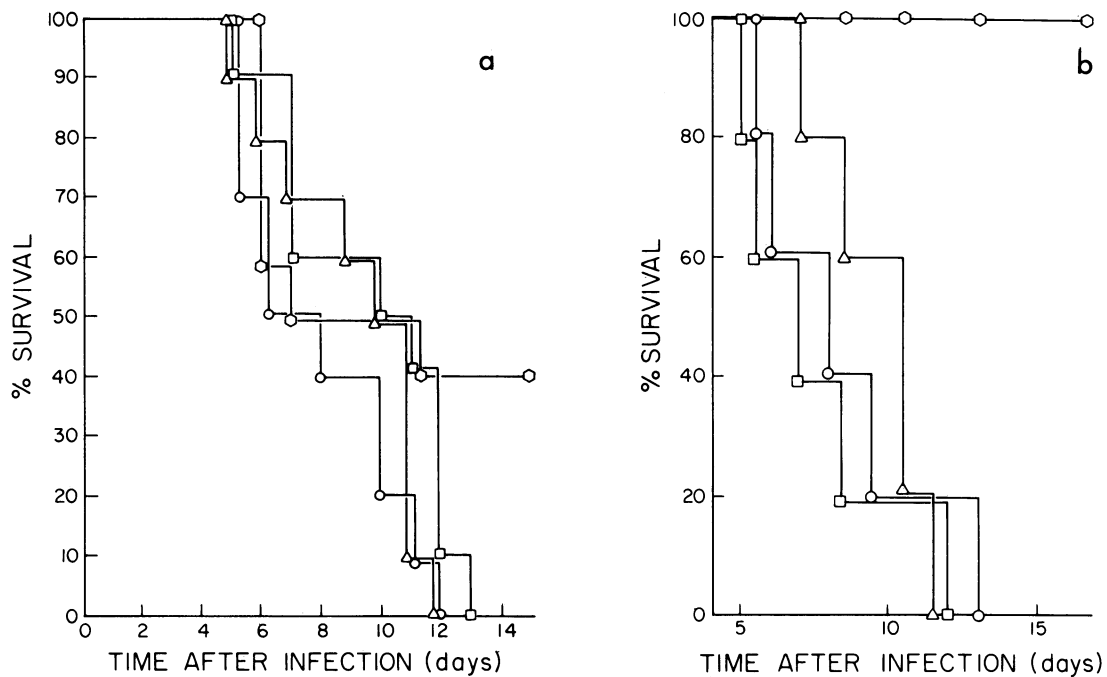


FIG. 2. Percent survival of treated and untreated mice. (a) Mice treated with 35 mg of roxithromycin per day (□) or with gamma interferon ( $\Delta$ ) or with both (○); controls (○). (b) Mice treated with 25 mg of roxithromycin per day (□) or with gamma interferon ( $\Delta$ ) or with both (○); controls (○).

Novick, Hoechst-Roussel, personal communication), we repeated the experiment with a lower dosage of roxithromycin (35 mg per mouse per day [Fig. 1b]), which was administered 1 day before infection. In this experiment, roxithromycin again had a protective effect, and the median number of cysts in treated mice that survived was 142 (range, 44 to 205), compared with 484 in the untreated mouse that survived. However, palatability was still poor, as demonstrated by an average weight loss of 1.8 g in uninfected mice given roxithromycin (35 mg per mouse per day), compared with an average weight gain of 0.8 g in controls over a 3-week period.

In a preliminary experiment, gamma interferon ( $10^6$  U) significantly ( $P = 0.006$ ) decreased mortality on day 8 and prolonged time to death (median, 10 versus 8 days) but did not alter mortality (100%) at 1 month. When gamma interferon ( $5 \times 10^4$  U) and roxithromycin (35 mg per mouse per day) were both administered 2 h before infection with *T. gondii*, there was a remarkable synergistic effect (Fig. 2a). Survival was increased from 0 to 40% ( $P = 0.02$ ). When the experiment was repeated with a lower dosage of roxithromycin, the results were even more impressive ( $P = 0.002$ ; Fig. 2b).

We extended the earlier finding of a protective effect of roxithromycin in *T. gondii*-infected mice to include protection in a model of murine toxoplasmic encephalitis. This series of experiments did not address the issue of whether the death of experimental mice resulted from infection of the brain or other vital organs; however, the reduced numbers of *Toxoplasma* cysts in the brains of roxithromycin-treated mice suggest that the antibiotic did exert an effect in the brain parenchyma. There are no data about the penetration of the blood-brain barrier by roxithromycin (W. Novick, personal communication), but it is anticipated that it will be similar to that by erythromycin which, in the presence of inflamed meninges, achieves levels in the cerebrospinal fluid of 5 to 10% of levels in serum (11). We previously reported that the histopathological changes in the brains of mice after intracerebral inoculation with *T. gondii* resemble those reported in patients with acquired immune deficiency syndrome and toxoplasmic encephalitis (Hofflin et al., in press). These changes, which include inflammation and marked tissue destruction, suggest the possibility that damage to the blood-brain barrier allows roxithromycin to reach effective levels in the involved areas of the brains of such patients.

The ability of gamma interferon to prolong survival in murine toxoplasmosis (4) was confirmed in our studies. The mechanism of protection may be complex. An in vitro inhibitory effect of interferon on *Toxoplasma* replication has been described by several workers (6, 8, 12). In addition to this inhibitory activity, interferon can activate macrophages to inhibit or kill *Toxoplasma* spp. (4, 5). Whether one or both of these activities of interferon contributed to the striking synergistic effect observed with roxithromycin remains to be defined. We consider it likely that the synergy which we observed will occur when gamma interferon is combined with other antibiotics which have activity against *T. gondii* in vivo.

Treatment of toxoplasmic encephalitis in patients with acquired immune deficiency syndrome is unsatisfactory (H. W. Haverkos, J. S. Remington, and J. C. Chan, Am. J. Med., in press). The relapse rate and early and overall

mortality are unacceptably high, and an urgent need exists for new therapeutic agents. In this series of experiments, a remarkable in vivo synergistic effect between a new antibiotic, roxithromycin, and a biological was observed. If further animal studies confirm these results, appropriate trials in patients with acquired immune deficiency syndrome should be undertaken. Also, similar studies with other antibiotics and gamma interferon should be undertaken.

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