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 toxoplasma 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 toxoplastic 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 toxoplastic 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), both in preliminary studies in the encephalitis model, neither roxithromycin nor gamma interferon was completely protective (unpublished observations).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 toxoplastic 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-μm pore size; Nuclepore Corp., Pleasanton, Calif.) to obtain organisms free of host cells and debris. After filtration, the purified tachyzoites were washed and sus-

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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-μl 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.

![Graph of percent survival vs. time after infection for treated and untreated mice.](image)
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 infected 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 x 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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