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Roxithromycin Alone and in Combination with Either Ethambutol or Levofloxacin for Disseminated *Mycobacterium avium* Infections in Beige Mice

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Roxithromycin alone reduced the level of bacteremia caused by *Mycobacterium avium* complex liver and splenic infection (in CFU per gram) of beige mice and mortality compared with untreated controls ($P < 0.05$). Roxithromycin plus ethambutol resulted in a significant reduction in the number of bacteria in splenic tissue compared with those in control splenic tissues of mice and mice treated with roxithromycin alone and ethambutol alone. Roxithromycin plus levofloxacin was not better than roxithromycin alone. Roxithromycin has in vivo activity against *M. avium* complex strains, and pilot studies with humans may be considered.

New macrolides are among the most active antibiotics against the *Mycobacterium avium* complex (MAC). Clarithromycin and azithromycin (an azalide) have anti-MAC activities in vitro (7, 10, 12, 20), have in vivo activities in beige mice (2, 7, 12, 16), and have been shown to be effective for the treatment of disseminated MAC disease in AIDS patients (6, 23). Another macrolide, roxithromycin, is active in vitro against a number of MAC strains (18, 22) and has shown anti-MAC activity in a macrophage test system (19). Struillou and colleagues (21) compared clarithromycin and roxithromycin treatment of disseminated MAC infection in C57 black mice and found that both agents decreased the numbers of viable MAC organisms in the lungs and spleens.

We have examined the activity of roxithromycin alone and in combination with either ethambutol or levofloxacin in C57 beige mice. Our challenge organism was MAC 101, the most consistently virulent strain in mice isolated from an AIDS patient and proposed by Gangadharam as the standard for in vivo studies (9). The challenge organism and animals were prepared as described in previous publications (5, 8, 12). Roxithromycin (200 mg/kg of body weight per day) was suspended in a saturated sucrose suspension for administration. Levofloxacin (200 mg/kg/day) was dissolved in a pH 5.4 phosphate buffer, and ethambutol (100 mg/kg/day) was dissolved in distilled water. Drugs were administered daily by gavage, and drug treatment was continued for 28 days. Control mice received water in place of antibiotics.

At the end of therapy, mice were bled for quantitation of the numbers of CFU in blood and were harvested. Livers and spleens were obtained, weighed, and homogenized in Middlebrook 7H9 broth containing 20% glycerol. Tissue homogenates were serially diluted and were plated onto Middlebrook 7H10 agar plates. The plates were incubated at 37°C for 7 days for quantitation of viable bacteria.

Treatment with roxithromycin at 200 mg/kg daily for 28 days resulted in the death of 1 of 21 mice (4.8%), whereas 8 of 22 mice (36%) treated with ethambutol, 8 of 21 mice (38%) treated with levofloxacin, and 12 of 21 (57%) untreated control mice died ($P < 0.05$ for roxithromycin versus controls). Figure 1 shows the effects of roxithromycin, ethambutol, and levofloxacin on bacteremia. While treatment with roxithromycin was associated with 0.69 log reduction in the level of MAC bacteremia, ethambutol had static activity ($\Delta = 0.00$), and treatment with levofloxacin resulted in an increase in the number of CFU in the blood of 0.18 log, whereas there was an increase of 0.67 log in untreated controls ($P < 0.001$ for roxithromycin, $P = 0.14$ for ethambutol, and $P = 0.30$ for levofloxacin compared with the untreated controls).

Roxithromycin, ethambutol, or levofloxacin caused a significant decrease in bacterial load in hepatic tissue compared with that in the hepatic tissue of untreated controls ($P < 0.001$ for roxithromycin, $P < 0.001$ for ethambutol, and $P = 0.03$ for levofloxacin compared with the untreated controls). As shown in Fig. 2, treatment with all three antimicrobial agents was associated with reduction in the number of viable organisms in the spleens ($P < 0.001$ for roxithromycin, $P < 0.01$ for ethambutol, and $P < 0.06$ for levofloxacin compared with the untreated controls). Combinations of roxithromycin plus ethambutol and roxithromycin plus levofloxacin reduced the level of bacteremia in comparison with that in the untreated controls ($P < 0.002$ for both comparisons), but neither combination was more effective than roxithromycin alone ($P = 0.45$ for roxithromycin plus ethambutol and $P = 0.32$ for roxithromycin plus levofloxacin compared with roxithromycin alone). Roxithromycin combined with ethambutol exerted an additive effect in splenic tissue but not in the liver when compared with the effect of roxithromycin alone ($P = 0.016$ for ethambutol and roxithromycin in the spleen in comparison with roxithromycin alone; $P = 0.39$ for the combination in the liver compared with roxithromycin alone). Levofloxacin did not add to the effects of roxithromycin alone in tissue ($P = 0.09$).

Treatment with roxithromycin resulted in significant reduction in the number of viable MAC organisms in the blood, liver, and spleen compared with the numbers in the blood, liver, and spleen of the untreated controls, while therapy of MAC infection in beige mice with either ethambutol or levofloxacin was associated with a significant decrease in the bacterial load in the spleen and liver but not in blood (compared with the bacterial load in untreated controls).

Roxithromycin possesses in vitro activity against MAC, and the activity is further enhanced by combination with ethambutol and ofloxacin (18, 19). The present study showed that while

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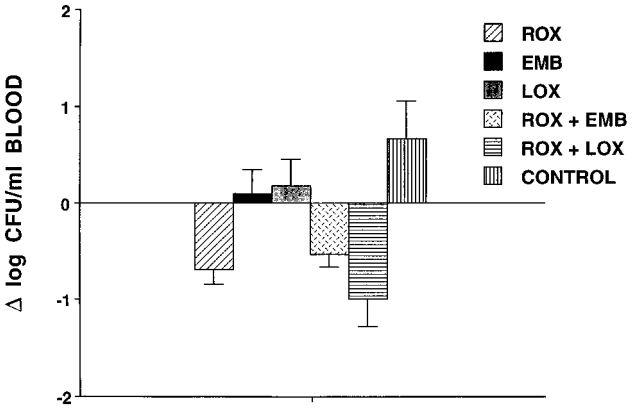

FIG. 1. Effect of roxithromycin (ROX), ethambutol (EMB), or levofloxacin (LOX) alone or in combination against MAC bacteremia in beige mice. Mice were infected with MAC organisms, and treatment was initiated 7 days after infection. The mice were treated for 28 days.

the combination of roxithromycin and levofloxacin did not result in a significant increase in anti-MAC activity compared with the activity of roxithromycin alone, the combination of roxithromycin with ethambutol resulted in significantly greater anti-MAC activity in the spleen (but not in the liver) than that of roxithromycin alone.

Macrolides such as clarithromycin and azithromycin are effective antimicrobial agents for the treatment of human MAC infection (6, 23). Roxithromycin has different pharmacokinetics than clarithromycin and azithromycin (1). Roxithromycin achieves higher levels in serum but does not achieve the same high intracellular concentrations reported for both azithromycin and clarithromycin (1). Furthermore, the MICs of roxithromycin tend to be higher than those of clarithromycin for most MAC strains (2, 3, 11, 13). Struillou and colleagues (21) compared the activity of clarithromycin with the activity of roxithromycin in C57 black mice and found that the drugs have similar anti-MAC activities, as determined by the bacterial CFU in the spleen, liver, and lung. Our results confirm a modest in vivo effect of roxithromycin in MAC-infected beige mice, a model that identified agents of therapeutic promise for further clinical trials with humans.

As single agents, ethambutol and levofloxacin also have

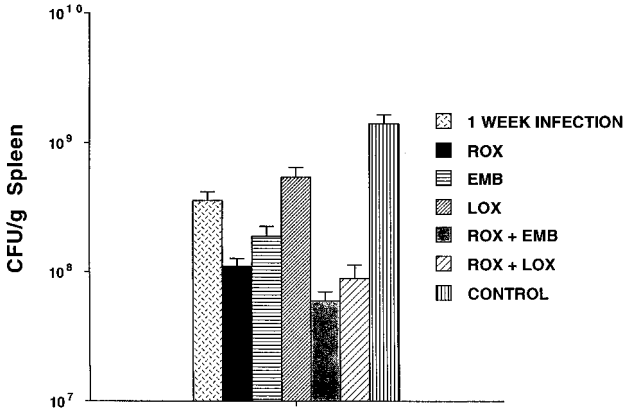

FIG. 2. Effect of roxithromycin (ROX), ethambutol (EMB), or levofloxacin (LOX) alone or in combination against MAC organisms in the spleen. Mice were infected with MAC organisms, and treatment was initiated 7 days after infection. The mice were treated for 28 days.

modest in vivo activities against MAC organisms (3, 15, 17). In the present study, only ethambutol significantly enhanced the activity of roxithromycin, but that was limited to the spleen and was not observed in the liver or blood. However, one concern related to the use of macrolides for the therapy of MAC infection in AIDS patients is the emergence of resistant strains during the course of therapy. In patients treated with clarithromycin as a single agent, MAC resistance to the antibiotic usually develops after approximately 4 months of therapy. This selection of resistant organisms is probably related to the large bacterial load at the onset of treatment. Ji and colleagues (14) as well as our laboratory (4) have shown that the frequency of resistance to clarithromycin is in the range of 10^{-3} to 10^{-4} after 8 weeks of therapy in mice. Since there is no reason to believe that the emergence of resistance to roxithromycin would differ from what has been shown for clarithromycin, future studies should attempt to determine whether the combination of roxithromycin with antibiotics such as ethambutol or levofloxacin would prevent or delay the emergence of resistant subpopulations of MAC during therapy.

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