Mefloquine, Moxifloxacin, and Ethambutol Are a Triple-Drug Alternative to Macrolide-Containing Regimens for Treatment of Mycobacterium avium Disease

Luiz E. Bermudez,1,a Peter Kolonoski,1 Mary Petrofsky,1 Martin Wu,1,a Clark B. Inderlied,1 and Lowell S. Young1

1Kuzell Institute for Arthritis and Infectious Diseases, California Pacific Medical Center Research Institute, San Francisco, and 1University of Southern California, Children’s Hospital of Los Angeles, Los Angeles, California

Macrolides are the core of effective drug regimens for the treatment of Mycobacterium avium complex (MAC) disease. Mefloquine (MFQ), moxifloxacin (MXF), and ethambutol (EMB), in combination, were evaluated against both clarithromycin-resistant (CLR-R) and CLR-susceptible (CLR-S) MAC; MFQ (40 mg/kg), MXF (100 mg/kg), or EMB (100 mg/kg/day) was given to mice for 4 weeks. MFQ was bactericidal, whereas MXF and EMB were bacteriostatic against both MAC 101 CLR-S and CLR-R. The combination of MFQ and EMB reduced (P < .05, for comparison with controls), and the combination of MFQ and MXF significantly reduced, the load of CLR-R in both the liver and the spleen. Treatment with all 3 drugs was associated with ~1-log reduction of CLR-R after 1 week, 2.1-log reduction of CLR-R after 4 weeks, and 2.17-log reduction in MAC/mL blood. Treatment of MAC 101 CLR-S strain had comparable results.

In patients with advanced stages of AIDS, organisms of the Mycobacterium avium complex (MAC) are a common cause of bacteremia and disseminated disease, [1, 2]. MAC may cause disease in children (lymphoadenopathy), the elderly, and people with chronic lung disease [3, 4], as well as in a small group of people who have no identified risk factor. At present, only a limited number of compounds—such as macrolides (azithromycin and clarithromycin [CLR]), amikacin, and ethambutol (EMB)—have demonstrated therapeutic activity against MAC in humans. Both the emergence of macrolide resistance and drug interactions between rifamycins and protease inhibitors emphasize the need for additional drugs with anti-MAC activity.

Mefloquine (MFQ) (a 4-quinoline methanol) is an anti-malarial agent widely used for prophylaxis of chloroquine-resistant Plasmodium falciparum malaria. MFQ has been shown to concentrate in tissues 80-fold, compared with serum concentrations, and has a long half-life estimated at 80 h. We have demonstrated elsewhere that MFQ has anti-M. avium activity in an experimental mouse model [5]. In addition MFQ is bactericidal against MAC in vivo [5]. Moxifloxacin (MXF) (a fluoroquinolone) exhibits activity against a wide range of Gram-positive and Gram-negative bacteria. In a recent study, we have shown that MXF is active against MAC in the beige-mouse model [6]. Here we extend our previous observations, to evaluate the efficacy of MFQ and MXF in combination with EMB, an antimicrobial with known anti-MAC activity in humans. This triple-drug regimen was evaluated against both CLR-resistant (CLR-R) and CLR-susceptible (CLR-S) strains of MAC.

Materials and methods. MAC 101 is a well-characterized human isolate that is susceptible to macrolides and has been used in many other in vivo studies [5]. MAC 101 CLR-R was obtained from C57BL/6 mice undergoing long-term CLR therapy [7]. MAC organisms were cultured on Middlebrook 7H11 agar (Difco) supplemented with oleic acid, albumin, dextrose, and catalase, for 10 days at 37°C, as described elsewhere [5, 6]. Transparent colony morphotypes were harvested and were suspended in Hanks’ balanced salt solution, to a concentration of 3 × 10^8 cfu/mL, for murine challenge. The number of bacteria in the suspension was confirmed by colony plating onto 7H11 agar. The minimal inhibitory concentration for CLR-S strain is 2 µg/mL, and that for CLR-R strain is >128 µg/mL.

Antimicrobial preparations for therapy were made by suspending the agent with 2.5% gum arabic (Sigma), in 0.2% Tween 80 (Sigma). MFQ was purchased from the pharmacy at the Children’s Hospital of Los Angeles. EMB was purchased from Sigma. MXF was provided by Bayer AG.

C57BL/6J-bgj/bj female mice, 8–10 weeks old, were obtained from Jackson Laboratories, for challenge studies. After being quarantined for 2 weeks, mice were infected, through the caudal vein, with 3 × 10^8 cfu of either MAC 101 CLR-S or MAC 101 CLR-R. After 7 days, all mice were bled for quantitative blood culture with MFQ at 40 mg/kg, EMB at 100 mg/kg, and/or
MXF at 100 mg/kg, daily treatment was then initiated with each drug separately, with each of the 3 different combinations of 2 drugs, and with all 3 drugs, since these doses had been shown to be effective for treatment of the infection in other studies [5, 6]. Each treatment group contained ≥15 mice. In addition, to establish a baseline level of infection in tissues before treatment, an experimental group of 10 mice was collected after 7 days of infection. Mice were treated for 4 weeks, were bled for quantitative culture 48 h after having received the last dose, and were killed. Livers and spleens of all mice were aseptically dissected, were weighed, and were homogenized in 5 mL of 7H9 Middlebrook broth containing 20% glycerol. Tissue suspensions were serially diluted and were plated onto 7H11 agar plates, to quantify viable organisms. Plates were incubated for 10 days at 37°C [5, 6].

The statistical significance of the differences between tissue bacterial loads was assessed by analysis of variance. Differences between experimental groups were considered to be significant at \( P < .05 \).

**Results.** The efficacy of treatment with MFQ, MXF, and EMB, alone and combined in all possible combinations, against macrolide-susceptible MAC is shown in figure 1. Alone, MFQ was bactericidal, whereas MXF alone and EMB alone inhibited bacterial growth in the liver and the spleen. All 3 drugs were bacteriostatic in the blood. Combinations of (1) MFQ and MXF and (2) MFQ and EMB were bactericidal and were significantly more active than was each drug alone (\( P < .05 \) for comparisons with each of MFQ, MXF, and EMB alone) in the blood, the liver, and the spleen. Although the combination of MXF and EMB was significantly more efficacious than was each drug alone (\( P < .05 \)) than the combination of MFQ and EMB, in both the liver and the spleen. When MAC-infected mice were treated with MFQ, MXF, and EMB in combination, the reduction in bacterial load was significantly greater than it was with either any drug alone or any combination of 2 drugs. In fact, the reduction obtained (1.8 log in the spleen and 1.6 log in the liver, compared with levels in untreated controls, at 4 weeks) is comparable to the reduction obtained when mice

**Figure 1.** Effect of treatment with mefloquine (MFQ), moxifloxacin (MXF), and ethambutol (EMB), alone or in combination, on the number of viable *Mycobacterium avium*-complex bacteria in spleen (A), liver (B), and blood (C). Mice were treated orally for 4 weeks. At least 15 mice/experimental group were used. Data are mean ± SE.
are treated with macrolides (authors’ personal observations during many years of personal experience).

The anti-MAC activity of MFQ, MXF, and EMB in combination was also evaluated against macrolide-resistant MAC. MAC 101 CLR-R, described elsewhere [7], was used to challenge mice. As shown in table 1, the activity of MXF, MFQ, and EMB, either alone or in combination, against macrolide-resistant MAC was comparable to its activity against macrolide-susceptible MAC. In both the liver and the spleen, the combination of all 3 drugs led to an ~2-log decrease in the number of bacteria, compared with that in the controls at 4 weeks, and to both an ~1.3-log reduction in the liver and an ~1.1-log reduction in the spleen (both reductions signify bactericidal effect), compared with the controls before treatment.

Discussion. The treatment of MAC infection can present a major problem for medical practitioners. In patients with AIDS, as well as in individuals without AIDS, infections caused by MAC can be life threatening and persistent and can recur after apparently successful initial treatment [8]. Although the introduction of the modern macrolides—such as CLR, azithromycin, and roxithromycin—has had a significant and positive effect on the ability to treat MAC infections in both patients with AIDS and patients without AIDS, once resistance to 1 of the macrolides develops, the resistance is cross-reactive, and, as a consequence, the therapy for the infection becomes suboptimal at best. Therefore, the development of a nonmacrolide regimen that offers comparable efficacy is a necessity.

Recent studies have established that MFQ and MXF are active against MAC in mice, by use of a test system that has identified the most active anti-MAC antibiotics currently used in the treatment of human infection [5, 6, 9, 10]. EMB is the most active anti-tuberculosis antimicrobial with anti-MAC activity in humans [11]. MFQ is bactericidal against MAC in vivo, and its effect is significantly enhanced by combination with EMB [5]. Likewise, the quinolone MXF has anti-MAC activity that is augmented in the presence of EMB [6]. In the present report, we have shown that the combinations of (1) MFQ and MXF and (2) MFQ and EMB have significant anti-MAC activity. In addition, when mice were treated with the combination of MFQ, MXF, and EMB, we were able to achieve bacterial clearance that is comparable to that achieved by macrolide-containing regimens [9, 10, 12]. In fact, the regimen using all 3 drugs was also shown to have activity in vivo against MAC 101 CLR-R, an isogenic strain of MAC 101 with a characterized mutation in the 16S rRNA [7].

MFQ used for prophylaxis of malaria is administered once a week (because of the long half-life of MFQ). In our studies, MFQ was given daily, in a dose adapted for mouse metabolism, and it should be borne in mind that there are no published accounts of more-frequent dosing with MFQ. Our previous study had demonstrated that MFQ administered 3 times a week, as well as daily, has significant anti-MAC activity.

The efficacy of the combinations containing EMB, compared with the efficacy of either MFQ or MXF alone, might be explained by the effect that EMB has on the mycobacterial cell wall—that is, increasing the latter’s the uptake of other drugs. However, the increased activity of the regimen containing MXF and MFQ, compared with the activity of each drug alone, is noteworthy. Since the target of MFQ is known to be gyrase, one can speculate, to explain the significant increase in anti-MAC activity when the 2 drugs are combined, that the target of MFQ may be involved in cell-wall synthesis. We have described an anti-MAC regimen that, in mice, has activity comparable to that of regimens containing modern macrolides, and evaluation of this regimen in humans is certainly warranted.

Acknowledgments

We are indebted to Chris Lambros for reviewing the manuscript and to Karen Allen for typing it.

References


