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SRI-286, a Thiosemicarbazole, in Combination with Mefloquine and Moxifloxacin for Treatment of Murine *Mycobacterium avium* Complex Disease

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Treatment of *Mycobacterium avium* disease remains challenging when macrolide resistance develops. We infected C57 beige mice and treated them with mefloquine, SRI-286, and moxifloxacin. SRI-286 (80 mg/kg) was bactericidal in the liver. Mefloquine plus moxifloxacin or mefloquine plus SRI-286 were better than mefloquine alone.

Organisms of the *Mycobacterium avium* complex (MAC) are a common cause of bacteremia and disseminated disease in patients in advanced stages of AIDS (9, 13). Currently, only a limited number of compounds, such as macrolides (azithromycin and clarithromycin), amikacin, and ethambutol, have demonstrated therapeutic activity against *M. avium* in humans. The emergence of macrolide resistance and drug interactions between rifamycins and protease inhibitors emphasizes the need for additional compounds with anti-*M. avium* activity.

Mefloquine (MFQ; a derivative of 4-quinoline-methanol) is an antimicrobial agent widely used for prophylaxis of chloroquine-resistant *Plasmodium falciparum* malaria (7). It has been demonstrated to have anti-*M. avium* activity in an experimental mouse model (MIC of 16 μg/ml) (2, 3). Moxifloxacin (MXF) is a fluoroquinolone with activity against a wide range of gram-positive and gram-negative bacteria, including *Mycobacterium tuberculosis* (11). Recently, we have shown that MXF is active against *M. avium* in the beige mouse model (MIC of 4 μg/ml) (1). Thiacetazone is a thiosemicarbazole antimicrobial agent that has been widely used for therapy of tuberculosis. SRI-286, a thiosemicarbazole analog, also has good activity against *M. avium* both in vitro and in vivo (MIC of 2 μg/ml) (4). In vivo, SRI-286 has been shown to be bacteriostatic against *M. avium* (4). In contrast, thiacetazone, the parent compound, has no effect on *M. avium* in mice (4). Although thiacetazone has a synergistic effect with isoniazid against *M. tuberculosis*, neither of the single compounds is active against *M. avium* (2, 4, 9). We now extend our previous observation to evaluate the effect of SRI-286 in combination with MFQ and MXF, two compounds with activity in vivo against *M. avium* (1, 3).

Mycobacteria. MAC 101 is a well-characterized human isolate that is susceptible to macrolides and has been used in many previous in vivo studies (1, 4). *M. avium* organisms were cultured on Middlebrook 7H11 agar (BBL) supplemented with oleic acid-albumin-dextrose-catalase (Hardy Diagnostics) for 10 days at 37°C. Transparent colony morphotypes were harvested and suspended in Hanks' buffered salt solution to a concentration of 3×10^8 CFU/ml for murine challenge. The inoculum was confirmed by colony plating onto 7H11 agar.

Antibiotics. 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 hospital pharmacy with a prescription. SRI-286 was provided by the Southern Research Institute (Birmingham, Ala.). MXF was provided by Bayer AG.

Experimental design. C57BL/6J-bg^j/bg^j female mice, aged 8 to 10 weeks, were obtained from Jackson Laboratories for challenge studies. Mice were infected through the caudal vein with 3×10^7 CFU of MAC 101. After 7 days, all mice were bled for quantitative blood culture to ensure an adequate baseline level of infection. Treatment was then initiated with MFQ at 40 mg/kg, SRI-286 at 40 or 80 mg/kg, or MXF at 100 mg/kg daily as oral monotherapy; all permutations of two-drug therapy; or all three drugs in combination. Each treatment group contained at least 12 evaluable animals. In addition, an experimental group of 10 mice was harvested after 7 days of infection in order to establish a baseline level of infection in tissues. Animals were then treated daily for an additional 4 weeks and then euthanized after allowing 2 days without therapy to reduce carryover. In the original work (3), it was determined that there was no carryover effect. The livers and spleens of all of the mice were aseptically dissected, weighed, and homogenized in 5 ml of 7H9 broth containing 20% glycerol. Tissue suspensions were serially diluted and plated onto 7H11 agar plates to quantitate viable organisms. Plates were incubated at 37°C for 10 days.

We defined bactericidal activity as a reduction in the number of bacteria in the liver and spleen after 4 weeks of treatment (5 weeks) to a number smaller than the number of bacteria in those organs prior to treatment (control at 1 week). One also needs to consider that once a drug decreases the number of

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TABLE 1. Activities of MFQ, MXF, and SRI-286 alone or in combination against *M. avium*

Regimen (dose [mg/kg])	*n*	Mean no. of CFU/g ± SEM	
		Liver	Spleen
MFQ (40)	19	$(5.2 \pm 1.6) \times 10^{7a,b}$	$(1.2 \pm 0.4) \times 10^{8a,b}$
MXF (100)	13	$(1.5 \pm 0.2) \times 10^{8b}$	$(7.0 \pm 1.0) \times 10^{8b}$
SRI-286 (40)	18	$(1.1 \pm 0.2) \times 10^{8b}$	$(5.1 \pm 0.8) \times 10^{8b}$
SRI-286 (80)	16	$(5.1 \pm 1.1) \times 10^{7b}$	$(2.1 \pm 0.2) \times 10^{8b}$
MFQ (40) + SRI-286 (40)	14	$(2.5 \pm 0.9) \times 10^{7a,b,c}$	$(5.6 \pm 2.1) \times 10^{7a,b,c}$
MFQ (40) + SRI-286 (80)	12	$(8.8 \pm 2.4) \times 10^{6a,b,c}$	$(1.6 \pm 0.4) \times 10^{7a,b,c}$
MFQ (40) + MXF (100)	14	$(9.2 \pm 2.8) \times 10^{6a,b}$	$(1.4 \pm 0.8) \times 10^{7a,b}$
MXF (100) + SRI-286 (40)	12	$(5.6 \pm 1.2) \times 10^{7b}$	$(1.1 \pm 0.2) \times 10^{8b}$
MXF (100) + SRI-286 (80)	13	$(3.8 \pm 0.9) \times 10^{7a,b}$	$(6.0 \pm 1.9) \times 10^{7a,b}$
MFQ (40) + MXF (100) + SRI-286 (40)	15	$(1.2 \pm 0.4) \times 10^{7a,b,c}$	$(2.4 \pm 0.9) \times 10^{7a,b,c}$
MFQ (40) + MXF (100) + SRI-286 (80)	12	$(9.5 \pm 3.4) \times 10^{6a,b,c}$	$(2.9 \pm 1.3) \times 10^{7a,b,c}$
5-week control	14	$(9.8 \pm 1.6) \times 10^{8}$	$(4.7 \pm 0.8) \times 10^{9}$
1-week control	10	$(8.5 \pm 1.0) \times 10^{7}$	$(2.4 \pm 0.3) \times 10^{8}$

[a] $P < 0.05$ compared to 1-week control.
[b] $P < 0.05$ compared to 5-week control.
[c] $P < 0.05$ compared to both drugs alone.

viable bacteria, the host immune system would participate in the control of the infection.

Statistical analysis. The statistical significance of the difference between tissue bacterial loads was assessed by analyses of variance. Differences between experimental groups were considered significant at the $P < 0.05$ level.

Treatment of mice with MFQ alone resulted in bactericidal activity in 5 weeks. These results agree with previously published information demonstrating that MFQ is bactericidal for *M. avium* in both the liver and the spleen (3, 7). MXF at 100 mg/kg had bacteriostatic activity against *M. avium*, which has been observed previously (1). SRI-286 at 40 mg/kg, a dose comparable to the dose of thiacetazone in mice, was bacteriostatic, with activity comparable to that of MXF. When administered at 80 mg/kg/day, it had bactericidal activity in the liver and bacteriostatic activity in the spleen (Table 1). No toxicity was revealed by visual observation of the mice.

Combination of MFQ with SRI-286 significantly improved the anti-*M. avium* activity of MFQ and SRI-286 alone. Combination with SRI-286 at 80 mg/kg was superior to treatment with the combination with SRI-286 at 40 mg/kg and resulted in a 1-log reduction in the liver and spleen compared with the control prior to treatment. Combination of MFQ with MXF was associated with results similar to those of the combination of MFQ and SRI-286 at 80 mg/kg. MXF and SRI-286 at either 40 or 80 mg/kg/day resulted in a significant decrease in the load of bacteria in the liver and spleen, compared with MXF or SRI-286 alone (Table 1). The combination of the three drugs was not more active than the combination of MFQ and MXF or MFQ and SRI-286 (Table 1). Our results confirm previous observations that MFQ is bactericidal alone and when combined with other compounds.

Infections caused by the MAC are difficult to treat, and only regimens containing macrolides or azalides have been shown to be effective (6, 12, 15). More recently, we have shown that in mice the combination of MFQ, MXF, and ethambutol was as effective as the macrolide- and azalide-containing regimens (2). In fact, it was the first time that an alternative to a macrolide-containing regimen was proposed.

Now, we have shown that a regimen consisting of MFQ, MXF, and SRI-286 is also as active as macrolide-containing regimens against *M. avium* (8, 10). Although no side-by-side comparison was performed, we have experience of more than 70 experiments using macrolide therapy. SRI-286 is bacteriostatic against *M. avium*, while thiacetazone has no activity (4). Thiacetazone, however, is bacteriostatic against *M. tuberculosis* but is synergistic with isoniazid (5). It appears that SRI-286, when combined with MFQ, had an increasing effect on *M. avium*.

The triple regimen may be superior to the combination of two compounds because of the smaller chance that antibiotic resistance will develop. It can also be an alternative to treat infections resistant to a macrolide-containing regimen or for patients who cannot tolerate macrolide therapy.

One of the unknown aspects of the MFQ regimen is whether the drug would be well tolerated. When MFQ is used for prophylaxis of *P. falciparum* malaria, it is administered once a week. Almost certainly, for the treatment of *M. avium* infection, more frequent doses will be required. Recently, a report of a case of *M. avium* infection successfully treated with MFQ has been published (14). No side effects were observed (14). The definitive answer to this question awaits future studies with humans.

MFQ may also have another advantage in the treatment of *M. avium* infection. Thus far, despite trying several strategies, no resistant clone has been identified by attempting induction in vitro, by treating mice for 12 weeks, and by chemical mutagenesis (unpublished data), which suggests that the drug target in *M. avium* might be a lethal target or may indicate that killing does not occur by a simple mode of action. Future studies will attempt to address this possibility.

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