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Clarithromycin, Minocycline, and Rifabutin Treatments before and after Infection of C57BL/6 Mice with Mycobacterium avium

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C57BL/6 mice were pretreated with rifabutin or clarithromycin alone or combined with minocycline 3 days before intravenous challenge (day 0) with Mycobacterium avium. Treatment was continued until sacrifice at days 1, 8, 15, and 21. Rifabutin or clarithromycin decreased the level of infection in both the lungs and the spleen. Rifabutin was as effective as clarithromycin in the lungs but was more effective in the spleen. The clarithromycin-minocycline combination was as effective as clarithromycin alone.

There is no antibiotic combination able to eradicate Mycobacterium avium infection in patients with AIDS (6). Therefore, prophylactic regimens should be studied. In a recent trial, rifabutin reduced the frequency of M. avium infection in patients with AIDS with CD4 counts of <200/ mm³ (2, 10). Clarithromycin is one of the most active antibiotics against in vitro and in vivo M. avium infection (7, 15) and is already used for the treatment of M. avium infection in patients with AIDS (4, 5). Minocycline may enhance the activity of clarithromycin against M. avium within human macrophages (3). The aim of the present study was to compare the activity of clarithromycin alone, or combined with minocycline, with that of rifabutin in the normal C57BL/6 mouse model of M. avium infection (14). Drug treatment was started before bacterial challenge, and drugs were subsequently administered after the challenge to mimic prophylactic treatment which might be given to patients with AIDS. In our model, mice were challenged intravenously with a high inoculum of M. avium (12). In humans, the infection is progressive with a respiratory or gastrointestinal portal. Thus, drugs able to decrease the infection after this strong challenge in mice may be able to prevent infection in patients.

Randomly selected normal C57BL/6 female mice (7 weeks old) were used for all studies. Mice were quarantined for 1 week before being used for experiments.

The MO-1 strain of M. avium used in our previous studies (3, 14), and which was isolated from a patient with AIDS, was used after a single subculture on mycobacterium 7H11 agar (Difco Laboratories, Detroit, Mich.) supplemented with Middlebrook OADC enrichment (Difco). One flat transparent colony of the strain was picked and cultivated at 37°C in Middlebrook 7H9 broth (Difco) supplemented with Middlebrook OADC enrichment (Difco) in Falcon tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif.). After 26 days of culture, the bacterial suspension was adjusted to a density of 1 mg/ml with a turbidimeter (Institut Pasteur Production). Counts of CFU on 7H11 agar correlated this density to a bacterial concentration of 5 × 10⁸ CFU/ml. Aliquots of the bacterial suspension were frozen at −80°C. Before challenge, M. avium was diluted in sterile water to a final concentration of 2.5 × 10⁸ CFU/ml.

The following antimicrobial agents were used: rifabutin (Farmitalia-Carlo Erba, Milan, Italy), clarithromycin (Abbott Laboratories, North Chicago, Ill.), and minocycline (Lederle Laboratories, Oullins, France). Working solutions were prepared in accordance with the manufacturers’ instructions. All compounds were diluted in sterile water and administered subcutaneously (0.1 ml per day), except rifabutin, which was administered orally by gavage (0.2 ml per day), since the manufacturer did not recommend its use by the subcutaneous route. The daily dose of rifabutin was 40 mg/kg of body weight, that of clarithromycin was 50 mg/kg, and that of minocycline was 25 mg/kg. Control mice were injected with saline.

The MICs of rifabutin, clarithromycin, and minocycline were determined by the agar macrodilution method (12). Mice were pretreated 3 days before challenge. On the fourth day of treatment (day 0 of infection), mice were challenged with 0.1 ml of the M. avium suspension (inoculum of 2.5 × 10⁷ CFU) injected in the retro-orbital plexus. Mice were treated every day for 3 weeks. Animals were sacrificed at days 1, 8, 15, and 21 after infection. The spleen and the right lung were removed aseptically, weighed, homogenized, diluted, and cultured on 7H11 agar (Difco). To assess the efficacy of the treatment, the numbers of CFU per gram of tissue in the lungs and spleens of six mice per time point were measured.

CFU counts at days 15 and 21 were analyzed by the Student t test. Results were expressed as log₅ CFU per gram (means ± standard errors of the means).

The MICs of the antibiotics for strain MO-1 of M. avium were 64 μg of minocycline per ml, 2 μg of rifabutin per ml, and 2 μg of clarithromycin per ml at pH 7.4.
Results are shown in Fig. 1. At day 15, compared with control experiments, only clarithromycin significantly decreased the CFU counts in the spleen \( (P = 0.029) \) and only rifabutin significantly decreased the CFU counts in the lungs \( (P = 0.0018) \); the comparison of the antibiotics showed that rifabutin was as effective as clarithromycin in the spleen \( (P = 0.15) \) and in the lungs \( (P = 0.16) \). At day 21, compared with control treatments, rifabutin and clarithromycin significantly decreased the level of infection in the spleen \( (P < 0.0001) \) and in the lungs \( (P < 0.0001) \); clarithromycin had an activity in the lungs similar to that of rifabutin but was more active in the spleen \( (P = 0.012) \). The combination of clarithromycin and minocycline was as effective as clarithromycin alone.

The purpose of this study was to compare antibiotic regimens started before \( M. avium \) infection in C57BL/6 mice. Fernandes et al. have shown that clarithromycin was effective against \( M. avium \) infection in the beige mouse model when used at a daily dose ranging from 10 to 100 mg/kg (7). The activity of rifabutin administered orally has been evaluated in different mouse models of \( M. avium \) infection at daily doses ranging from 5 to 40 mg/kg (8, 9, 13). In our study, the daily doses of clarithromycin (50 mg/kg) were given according to the experience of Ji et al. in their model of \( Mycobacterium leprae \) infection of mice, and the daily dose of rifabutin was given according to the experience of Orme and to that of Furney et al. in their models of \( M. avium \) infection of mice (8, 11, 13). In these studies, the peak levels of antibiotics, given orally, in the sera of mice at the doses we used were 1.5 to 4.3 \( \mu \)g/ml for clarithromycin and 0.25 \( \mu \)g/ml for rifabutin. The peak levels of clarithromycin in the sera of mice are at least twice as high when the antibiotic is given by subcutaneous injection (7). Compared with controls, clarithromycin was the most active antibiotic in the lungs and spleen. Clarithromycin had an activity in the lungs similar to that of rifabutin but was more active in the spleen. This difference seems relevant since the infection level is 100 times higher in the spleen than in the lungs. The combination of clarithromycin and minocycline may be more effective than clarithromycin alone against \( M. avium \) infection within human macrophages (3). The activity of minocycline has not been previously evaluated in an in vivo model of \( M. avium \) infection. In our study, the daily dose of minocycline (25 mg/kg) was, as for clarithromycin, given according to the experience of Ji et al. (11). In our C57BL/6 mouse model, the combination of clarithromycin and minocycline was as effective as clarithromycin alone. Further studies should evaluate the ability of minocycline to prevent the emergence of clarithromycin-resistant mutants.

We conclude that clarithromycin should be further evaluated for the prophylaxis of \( M. avium \) infection. As it has been shown that the combination of clarithromycin and minocycline was efficient in a murine model of toxoplasmosis (1), this combination could be of interest for the prevention of both opportunistic infections.

REFERENCES


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Volume 37, no. 8, p. 1690, line 4 of abstract: “more effective” should read “less effective.”