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Clarithromycin, Dapsone, and a Combination of Both Used To Treat or Prevent Disseminated *Mycobacterium avium* Infection in Beige Mice

LUIZ E. BERMUDEZ,[1]* CLARK B. INDERLIED,[2] PETER KOLONOSKI,[1] MARY PETROFSKY,[1] AND LOWELL S. YOUNG[1]

Kuzell Institute for Arthritis and Infectious Diseases, California Pacific Medical Center Research Institute, San Francisco, California 94115,[1] and Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, University of Southern California, Los Angeles, California 90027[2]

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Bacteremic infection caused by organisms of the *Mycobacterium avium* complex (MAC) is common in patients with AIDS. We evaluated both clarithromycin and dapsone alone and in combination for the treatment and prevention of disseminated MAC disease in beige mice. In the therapeutic model, C57BL/6 beige mice were infected intravenously with strain 101 of MAC (serovar 1). After 1 week postinfection, mice were given clarithromycin (200 mg/kg of body weight per day) and dapsone (15 mg/kg of body weight per day) alone or in combination by gavage. Treatment with clarithromycin resulted in a significant reduction in bacteremia and the numbers of CFU of MAC in the liver and spleen. Treatment with dapsone had no effect on the mycobacterial counts in blood, liver, or spleen, and the combination of dapsone with clarithromycin was no better than clarithromycin as a single agent. Clarithromycin and dapsone were used to prevent systemic disease in beige mice infected orally with MAC 101. Clarithromycin prophylaxis was associated with a significant reduction in the numbers of bacteria in the liver, spleen, and appendix compared with those in controls. Prophylaxis with dapsone resulted in a mild reduction in the numbers of MAC in the spleen but not in the other tissues. Clarithromycin both treats and prevents MAC disease in beige mice. Dapsone has no therapeutic effect, but it does have a slight prophylactic effect, and in combination with clarithromycin it does not abrogate the effect of clarithromycin.

Organisms of the *Mycobacterium avium* complex (MAC) are the most common cause of bacterial infection in patients with AIDS. Autopsy studies in AIDS patients have shown a large number of acid-fast bacilli in the liver, spleen, bone marrow, and intestines (17, 20, 28). Current observations suggest that most of the cases of MAC infection in AIDS patients are acquired by the gastrointestinal tract, and colonization of the gut appears to precede the onset of bacteremia (5, 8).

MAC is resistant to many of the standard antituberculosis antimicrobial agents, but some strains may be susceptible in vitro and in vivo to agents such as ethambutol, amikacin, rifabutin, macrolides, and azalides (clarithromycin, roxithromycin, and azithromycin), clofazimine, and certain fluoroquinolones (9, 10, 12–14, 22, 23). Clarithromycin, a new macrolide that is structurally related to erythromycin, is active in vitro (19, 25–27), inhibiting 90% of MAC strains (MICs, 1 to 8 μg/ml). Clarithromycin concentrates within macrophages and tissues (2), and it has also been shown to be active in experimental animals (12, 24) and AIDS patients with MAC infection, as shown in clinical trials (7, 9–11). The development of MAC resistance to clarithromycin has been observed in patients taking the antibiotic (18). For instance, in a recent compassionate-use study, resistance following clarithromycin therapy developed in 33% of patients (9).

Dapsone is a synthetic antifolate that has activity against *Mycobacterium leprae* (15) and, when it is used alone or in combination with pyrimethamine, prevents *Pneumocystis carinii* pneumonia (6, 21); dapsone has been reported to have in vitro activity against MAC (16). We now report on the use of clarithromycin alone or in combination with dapsone to treat and prevent disseminated MAC infection in beige mice. The aims of the study were (i) to determine if clarithromycin in combination with dapsone is more active (additive or synergistic) than clarithromycin alone and (ii) to assess the prophylactic utilities of clarithromycin and dapsone alone and in combination.

MATERIALS AND METHODS

Antibiotics. Clarithromycin was provided by Abbott Laboratories, Abbott Park, Ill., and dapsone was provided by Jacobus Pharmaceutics Co., Inc., Princeton, N.J. Clarithromycin was dissolved in absolute ethanol, with subsequent dilutions in saturated sucrose syrup prior to administration to mice. Dapsone was dissolved in a minimal amount (4 to 5 drops) of ethanol and was then diluted into 50 ml of saturated sucrose syrup to form a suspension. The final concentration was 15 mg/ml.

Mycobacteria. MAC 101 (serovar 1) was isolated from a patient with AIDS (4). MAC was subcultured onto Middlebrook agar 7H10 medium (Difco Laboratories, Detroit, Mich.) for 10 days at 37°C. Transparent colonies were harvested in Hank's buffered salt solution and washed twice, and the suspension was adjusted to 3×10^8 bacteria per ml (for the therapeutic model) or 1×10^9 bacteria per ml (for the prophylactic model) by comparison with a McFarland turbidity standard. Samples obtained from the bacterial suspensions were plated to confirm the inoculum size. One hundred milliliters of the original suspension was used to infect mice. Before infecting the animals, the final suspension of mycobac-

* Corresponding author. Mailing address: Kuzell Institute for Arthritis and Infectious Diseases, 2200 Webster Street, Suite 305, San Francisco, CA 94115.

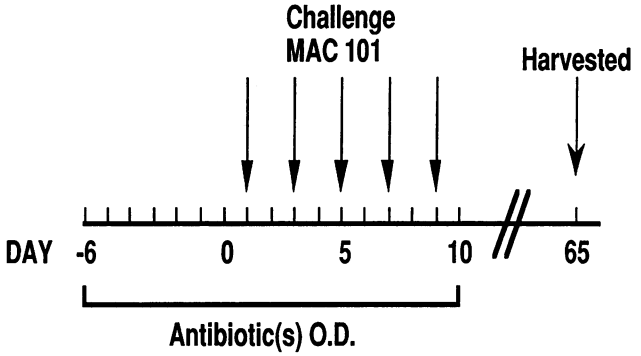

FIG. 1. Representation of the prophylaxis model. O.D., oral dose.

teria was vortex agitated for 2 min to prevent clumping. MAC 101 is the most virulent strain that we have tested in experimental animals and it causes reproducible levels of infection and mortality in beige mice (4).

Mice. Experiments were carried out with 6- to 7-week-old female C57BL/6 bg^+/bg^+ mice (Jackson Laboratories, Bar Harbor, Maine).

Therapeutic model. The therapeutic efficacies of clarithromycin and dapsone alone and in combination were examined by the beige mouse therapeutic model as described previously (4). Briefly, mice were infected through the caudal vein with 3×10^7 bacteria, and after 7 days treatment was initiated with clarithromycin (200 mg/kg of body weight per day) or dapsone (15 mg/kg of body weight per day) alone or in combination. Drugs were administered by gavage for 28 days. A control group of mice was infected, but received saline instead of antibiotics. An additional group of mice was harvested 7 days after infection in order to establish the level of infection just before the initiation of therapy. At the termination of treatment, the livers and spleens of control and treated mice were aseptically removed, and the organs were weighed and then homogenized in 5 ml of Middlebrook 7H9 broth with a tissue homogenizer. The tissue suspensions were serially diluted in 7H9 broth and plated onto 7H10 agar (Difco) plates supplemented with oleic acid, albumin, glucose, and catalase (Difco) for the quantitation of viable bacteria. The level of mycobacteremia was determined by collecting 0.05 ml of blood at day 7 and day 28. The number of CFU per milliliter of blood was determined by inoculating the blood into 4 ml of BACTEC 12B radiometric medium (Johnson Laboratories, Sparks, Md.) by the T_{100} method of data analysis described previously (22).

Prophylaxis model. Beige mice were slightly anesthetized by halothane inhalation and were given 1×10^8 CFU of mycobacteria delivered by gavage on alternate days over 9 days (total of 5×10^8 organisms on days +1, +3, +5, +7, and +9). Clarithromycin and dapsone, alone or in combination, were administered daily by gavage on days −6 to +10. Mice were observed for 64 days and were then sacrificed (Fig. 1). The livers, spleens, and appendixes were obtained, homogenized, serially diluted, and subsequently plated onto 7H11 agar to determine the number of viable organisms as described above. Blood samples were obtained at the end of the experiment and were submitted to the same process described above for the therapeutic model. In this model, approximately 50% of the mice develop bacteremia after 4 weeks, and 100% of the mice develop disseminated disease after the same period (1).

Statistical analysis. The statistical significance of the differences between the number of viable organisms recovered from the spleen, liver, appendix, and blood were evaluated by one- or two-variable analyses of variance. Differences were considered statistically significant if P values were less than 0.05.

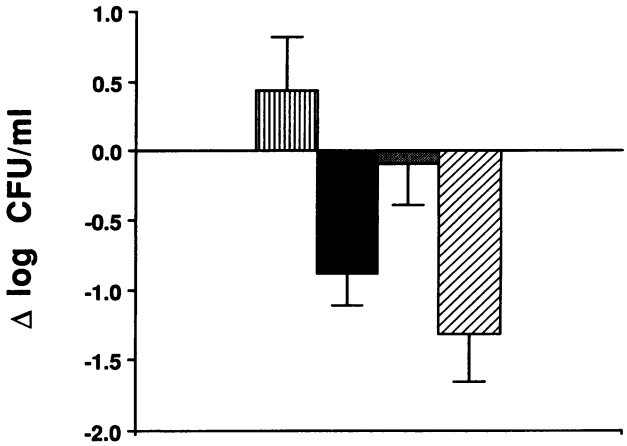

FIG. 2. Therapeutic effects of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) on the numbers of CFU per milliliter in the blood of beige mice infected with MAC 101. Bars indicate control mice, clarithromycin-treated mice, dapsone-treated mice, and clarithromycin- and dapsone-treated mice from left to right, respectively.

RESULTS

Evaluation of clarithromycin and dapsone as single therapeutic agents. Treatment of MAC infection in beige mice with clarithromycin (200 mg/kg/day) was associated with a significant reduction in the numbers of bacteria in the blood, spleen (98.1%; $P = 0.02$), and liver (95.8%; $P = 0.04$) (Fig. 2, 3, and 4), respectively). The mortality in mice receiving clarithromycin alone was 11%, whereas it was 33% in the control group ($P = 0.02$).

Treatment with dapsone alone was not associated with a significant reduction in the numbers of MAC organisms in blood or solid organs. Likewise, dapsone had no significant effect on the mortality (21%, versus 33% of control; $P > 0.05$).

Evaluation of combination of clarithromycin and dapsone as therapeutic agents. Dapsone administered in combination with clarithromycin did not improve the microbiologic response in the liver (97.1% reduction compared with the implantation inoculum) or the spleen (97.9%) when compared with the responses in the livers and spleens of mice receiving

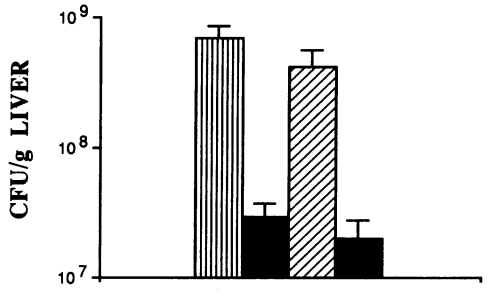

FIG. 3. Therapeutic effects of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) on the spleens of beige mice infected with MAC 101. There were $(9.1 \pm 0.3) \times 10^7$ bacteria at day 7, before the initiation of therapy. See legend to Fig. 2 for descriptions of bars.

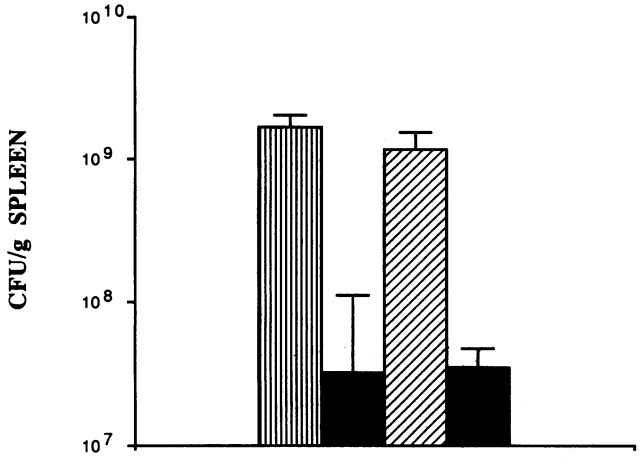

FIG. 4. Therapeutic effects of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) on the livers of beige mice infected with MAC 101. There were (7.8 ± 0.4) × 10^7 bacteria at day 7, before the initiation of therapy. See legend to Fig. 2 for descriptions of bars.

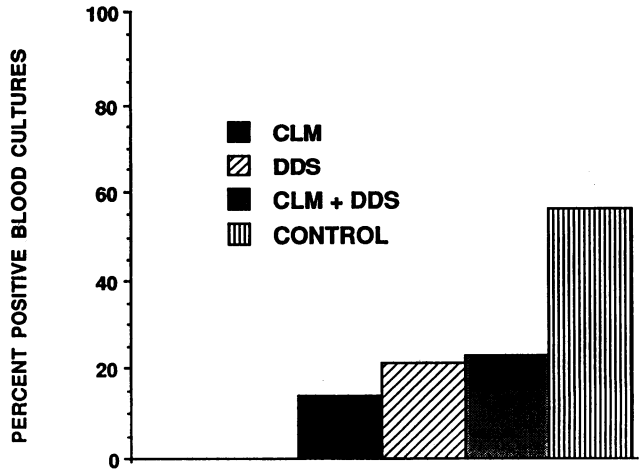

FIG. 5. Prophylactic effects of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) on the incidence of bacteremia in beige mice infected with 5 × 10^8 CFU of MAC 101. Animals received antibiotics from day −6 to day +10.

clarithromycin alone ($P > 0.05$). The combination of clarithromycin-dapsone resulted in a slightly greater decrease in the numbers of bacteria in the blood than was observed with the use of clarithromycin alone (1.32 and −0.89 Δlog CFU/ml, respectively; $P = 0.06$) (Fig. 2, 3, and 4).

Evaluation of clarithromycin and dapsone as single prophylactic agents. Effective MAC prophylaxis is desirable for the reduction of morbidity in AIDS patients. We evaluated the use of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) administered from days −6 to day +10 by gavage. Clarithromycin alone was an effective prophylactic agent, reducing the incidence of bacteremia in treated mice compared with that in the untreated control animals (2 of 14 treated mice [14%] compared with 5 of 9 control mice [56%]; $P = 0.04$). There was a significant reduction in the numbers of organisms isolated from the livers, spleens, and appendixes of clarithromycin-treated mice compared with the numbers isolated from control mice (99.8% for liver, 94.8% for spleen, and 91.2% for appendix; $P = 0.04$ for the three organs compared with the controls). (Fig. 5 and 6).

The prophylactic use of dapsone caused a consistent reduction in the colony counts in the liver (92%; $P = 0.06$), spleen (90%; $P = 0.04$), and appendix (76.4%; $P > 0.05$) compared with the colony counts in the organs of the control group. Only in splenic tissues was this difference significant. Three of 14 (21%) mice that received dapsone developed bacteremia, whereas 5 of 9 (56%) control mice developed bacteremia ($P > 0.05$).

Evaluation of combination of clarithromycin and dapsone for prophylaxis. Clarithromycin in combination with dapsone led to a decrease in the number of mycobacteria in the liver (99.2%), spleen (97.1%), and appendix (99.4%) ($P < 0.005$ for liver, spleen, and appendix compared with untreated control mice) (Fig. 5 and 6). Three of 13 (23%; $P < 0.005$ compared with control mice) of the mice that received the combination developed bacteremia, whereas 5 of 9 (56%) of the control mice developed bacteremia. Overall the use of clarithromycin in combination with dapsone did not result in an improvement in prophylactic activity when compared with the activity of either clarithromycin alone or dapsone alone.

DISCUSSION

In the current study, clarithromycin used as therapy was associated with a significant reduction in the numbers of mycobacteria in the blood, liver, and spleen compared with the numbers in untreated control mice. In addition, clarithromycin therapy resulted in a significant reduction in mortality. These observations are similar to the observations of others (12, 19, 24, 25), and they are consistent with the results of trials in humans receiving clarithromycin monotherapy (7, 9–11). Nonetheless, more recent clinical trials in AIDS patients have demonstrated that the therapeutic use of clarithromycin results in the emergence of MAC resistance in 33 to 50% of the patients (9, 10), indicating that clarithromycin should always be given in combination with other antimicrobial agents with anti-MAC activity. Dapsone alone, however, had no therapeutic effect, and when it was combined with clarithromycin it did not add to the effect of the macrolide. Dapsone was evaluated because a large percentage of AIDS patients receive dapsone for the prophylaxis and treatment of *P. carinii* and the potential interaction with clarithromycin had not been examined (6, 18). Furthermore, previous studies have suggested that dapsone has anti-MAC activity (16).

The prophylactic model in mice uses the same mouse strain (beige mouse) as the therapeutic model. However, bacteria are administered by gavage during a period of 10 days. Bacteremia is predictably observed in approximately 40 to 50% of the animals, and the period necessary to detect bacteremia is approximately 4 weeks. Disseminated infection is observed in 100% of the animals after the same period of time (1). The gastrointestinal route of infection appears to be the mode of acquisition in most AIDS patients (8).

When used as a prophylactic agent, clarithromycin caused a significant reduction in the number of MAC organisms in murine tissues. In the case of this oral model of MAC infection, the prophylactic effect of an antibiotic can be due to one or more of the following: (i) high levels of antibiotic in serum; (ii) high concentrations of antibiotic in tissue; or (iii) high concentrations of antibiotic in the intestinal lumen that are either above the MIC of the antibiotic and can kill the microorganism before invasion or below the MIC but can

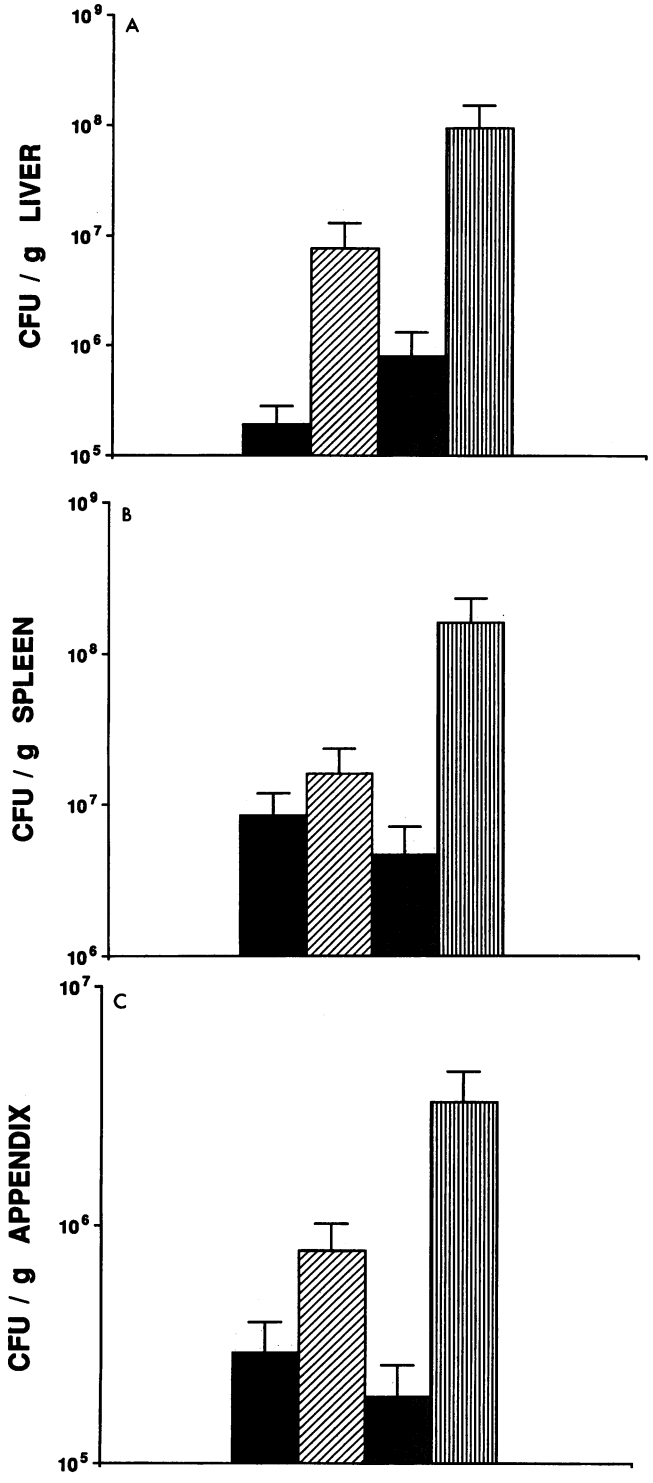

FIG. 6. (A) Prophylactic effects of clarithromycin (200 mg/kg/day) and dapsone (15 mg/kg/day) on the appendixes of beige mice. (B) Prophylactic effects of clarithromycin and dapsone on the spleens of beige mice. (C) Prophylactic effects of clarithromycin and dapsone on the livers of beige mice. Bars indicate clarithromycin-treated mice, dapsone-treated mice, clarithromycin- and dapsone-treated mice, and control mice from left to right, respectively.

influence bacterial behavior, i.e., inhibit binding of the bacterium to intestinal cells. Clarithromycin probably was effective as a prophylactic agent because it is able to achieve high concentrations in tissues (12). Since clarithromycin was given only once a day we believe that the concentration in serum cannot explain the prophylactic effect. Alternatively, it is also possible that the high concentration of clarithromycin in the intestinal lumen was responsible for the prophylactic effect by killing MAC, because previous results showed that both clarithromycin and azithromycin have no effect on the ability of MAC to bind to intestinal mucosal cells, making this hypothesis less likely (3).

Dapsone had a modest prophylactic activity in splenic tissue only, but overall dapsone was not efficient. The lack of effect of dapsone can be due to the excess of thymidine and thymine available in rodent tissues, which would obviate the effects of inhibitors of the folate pathway. Dapsone is widely used as prophylaxis for *P. carinii* in AIDS patients (6, 21), and it is not unusual that an AIDS patient either with MAC disease or under a prophylactic regiment for MAC infection be given dapsone as part of therapy for opportunistic infections. Our data showed that the combination of dapsone and clarithromycin is no better than clarithromycin alone, ruling out the possibility of an antagonistic interaction.

Clinical trials with clarithromycin as a prophylactic agent against MAC are in progress. The lessons taught by the previous clinical trials indicate that clarithromycin monotherapy is associated with the development of resistance after 2 to 7 months. Although no current evidence exists, further studies are needed to determine whether a regimen with clarithromycin used as a prophylactic agent for MAC infection would be associated with the emergence of resistance.

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