Activity of Clarithromycin against *Mycobacterium avium* Complex Infection in Beige Mice

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The activity of clarithromycin alone and in combination with other antimycobacterial agents was evaluated in the beige (C57BL/6J bg/bg’) mouse model of disseminated *Mycobacterium avium* complex (MAC) infection. A dose-response experiment was performed with clarithromycin at 50, 100, 200, or 300 mg/kg of body weight administered daily by gavage to mice infected with approximately 10⁷ viable MAC. A dose-related reduction in spleen and liver cell counts was noted with treatment at 50, 100, and 200 mg/kg. The difference in cell counts between treatment at 200 and 300 mg/kg was not significant. Clarithromycin at 200 mg/kg of body weight was found to have activity against three additional MAC isolates (MICs for the isolates ranged from 1 to 4 μg/ml by broth dilution). Clarithromycin at 200 mg/kg in combination with amikacin, ethambutol, temafloxacin, or rifampin did not result in increased activity beyond that seen with clarithromycin alone. Clarithromycin in combination with clofazimine or rifabutin resulted in an increase in activity beyond that seen with clarithromycin alone. The combination of clarithromycin with clofazimine or rifabutin should be considered for evaluation in the treatment of human MAC infections.

Clarithromycin (CLA) is a new macrolide compound with enhanced in vitro activity against the *Mycobacterium avium* complex (MAC) (4, 7, 10, 18). The activities of clarithromycin in MAC-infected macrophage cultures (19, 27) and murine models of disseminated MAC infection (4, 12) have been demonstrated previously. After a single oral dose of 50 mg/kg of body weight in C57BL/6J mice, CLA achieves a peak level in serum of 2.6 μg/ml (4). After administration of 400 or 1,200 mg of CLA orally in humans, peak levels in serum are 2.1 μg/ml (15) and 4.4 μg/ml (22), respectively. Preliminary results of CLA-containing regimens in the treatment of disseminated MAC infection in persons with AIDS are encouraging (2, 3, 21). The purpose of the present study was to evaluate the activity of CLA alone and in combination with various antimycobacterial agents against several MAC isolates in the beige mouse model of disseminated MAC infection.

**MATERIALS AND METHODS**

**Drugs.** CLA and temafloxacin (TEM) were provided by Abbott Laboratories, Abbott Park, Ill. Clofazimine (CFZ) was provided by CIBA-GEIGY Pharmaceuticals, Summit, NJ. Rifabutin (RBT) was provided by Adria Laboratories, Dublin, Ohio. Amikacin (AMK), ethambutol (EMB), and rifampin (RIF) were obtained from Sigma Chemical Co., St. Louis, Mo.

CLA and TEM were dissolved in absolute ethanol, with subsequent dilution in distilled water prior to administration. CFZ, RIF, and RBT were dissolved in dimethyl sulfoxide, with subsequent dilution in distilled water. The final concentration of ethanol or dimethyl sulfoxide in drug preparations was 0.5%. AMK and EMB were dissolved in distilled water. Drugs were freshly prepared each morning prior to administration.

**MAC isolates.** *M. avium* ATCC 49601 and MAC isolates G and F were clinical isolates obtained from patients with AIDS at the State University of New York Health Science Center in Syracuse. *M. avium* ATCC 49601, serotype 1, has been used previously in beige mouse studies in our laboratory and was designated isolate A (16, 17). MAC isolate 1408-3 was kindly provided by Leonid B. Heifets, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.

The MICs of CLA were determined in modified Mueller-Hinton broth (pH 7.4) (23) supplemented with 10% Middlebrook oleic-acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, Mich.). The MICs of the remaining antimicrobial agents were determined in modified Middlebrook 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) (1) supplemented with 10% Middlebrook OADC enrichment. The MICs for *M. avium* ATCC 49601 (in micrograms per milliliter) are as follows: CLA, 4; TEM, 8; CFZ, 1; RBT, 0.06; AMK, 8; EMB, 4; and RIF, 1. The MICs of CLA for isolates G and F are 2 and 1 μg/ml, respectively. The MICs for MAC isolate 1408-3 (in micrograms per milliliter) are as follows: CLA, 1; CFZ, 0.25; EMB, 4; and RIF, 1.

A cell suspension with a predominantly (>95%) transparent colony morphology was used for infection. Isolates were passaged through beige mice every 3 months to maintain virulence.

**Media.** The organisms were grown in modified Middlebrook 7H10 broth with 10% Middlebrook OADC enrichment and 0.05% Tween 80 (25) on a rotary shaker at 37°C for 3 days. The culture suspension was diluted in 7H10 broth to yield 10 Klett units per ml (Klett-Summner colorimeter; Klett Manufacturing, Brooklyn, N.Y.) or approximately 5 x 10⁷ CFU/ml. The size of the inoculum was determined by titration and counting from duplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% Middlebrook OADC enrichment. The plates were incubated at 37°C for 3 weeks prior to counting.

**Infection studies.** Six-week-old beige (C57BL/6J bg/bg’) mice, bred at our facility, were infected intravenously through a caudal vein. Mice of the same sex were used in
The viable cell counts were converted to logarithms, which were then evaluated with one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the Tukey honestly significant difference tests (14) to make pairwise comparisons among means. The results of the statistical evaluation for the organs are summarized below.

RESULTS

CLA dose-response study. CLA at 50, 100, 200, or 300 mg/kg of body weight was given daily to female mice which had been infected with $1.4 \times 10^7$ viable *M. avium* ATCC 49601 (Fig. 1). CLA at all doses reduced organ cell counts compared with those in mice given no treatment ($P < 0.01$). Treatment at 100 mg/kg reduced cell counts in spleens and livers compared with the counts in the group treated with 50 mg/kg ($P < 0.01$). An increase in cell counts in the lungs of the group treated with 100 mg/kg compared with that in the lungs of the group treated with 50 mg/kg was noted ($P < 0.05$). Treatment with 200 mg/kg reduced the cell counts in spleens and livers compared with the counts in the spleens and livers of the group treated with 100 mg/kg ($P < 0.01$). A reduction in cell counts in lungs in the group treated with 200 mg/kg compared with that in the lungs of the group treated with 100 mg/kg was significant ($P < 0.01$). The difference in cell counts in the lungs of the group treated with 200 mg/kg compared with that in the lungs of the group treated with 50 mg/kg was not significant ($P > 0.05$). The difference in cell counts between mice treated with 200 and 300 mg/kg was not significant for any organ ($P > 0.05$).

Extended duration of therapy. CLA at 200 mg/kg was given daily to female mice which had been infected with $1.1 \times 10^7$ viable *M. avium* ATCC 49601. Mice received treatment for 10 or 20 days. Untreated control groups were sacrificed at the completion of each treatment period. The increase in cell counts between the control groups that received treatment for 10 and 20 days was significant for livers and lungs ($P < 0.01$) but not spleens ($P > 0.05$) (Fig. 2). Treatment with CLA reduced cell counts in organs at each time point compared with the counts in mice given no treatment ($P < 0.01$). Treatment for 20 days further reduced cell counts in each organ compared with the counts in mice treated for 10 days ($P < 0.01$).
Activity of CLA against two additional isolates. In separate experiments, CLA at 200 mg/kg was given daily to mice which had been infected with $2.6 \times 10^7$ viable *M. avium* ATCC 49601 (males), $2.6 \times 10^7$ viable MAC isolate G (females), or $1.4 \times 10^7$ viable MAC isolate F (males). Treatment with CLA for 10 days reduced cell counts of each isolate in spleens compared with those in the spleens of mice given no treatment ($P < 0.01$) (Fig. 3).

Combination of CLA with AMK, EMB, RIF, or CFZ. CLA (200 mg/kg) was given daily alone or in combination with AMK (100 mg/kg), EMB (125 mg/kg), RIF (20 mg/kg), or CFZ (20 mg/kg) to male mice which had been infected with $3.3 \times 10^7$ viable *M. avium* ATCC 49601. A group of early control mice was sacrificed 7 days after infection. The increase in cell counts between early and late control groups was significant for spleens and lungs ($P < 0.01$) (Fig. 4). Treatment with CLA alone and all combinations reduced cell counts in spleens and lungs compared with those in the spleen and lungs of the early and late control groups ($P < 0.01$).

The difference in cell counts between mice treated with CLA alone and those treated with CLA-AMK or CLA-EMB was not significant for organisms in spleens or lungs ($P > 0.05$). CLA alone was more active than the combination of CLA-RIF ($P < 0.05$ for organisms in spleens; $P < 0.01$ for organisms in lungs). The combination of CLA-CFZ was more active than CLA alone against organisms in spleens and lungs ($P < 0.01$).

Combination of CLA with TEM or RBT. CLA (200 mg/kg) was given daily alone or in combination with TEM (100 mg/kg) or RBT (20 mg/kg) to female mice which had been infected with $1.9 \times 10^7$ viable *M. avium* ATCC 49601. A separate group received daily TEM alone at 100 mg/kg. A group of early control mice was sacrificed 7 days after infection. The difference in cell counts between early and late control groups was significant only for lungs ($P < 0.01$) (Fig. 5).

Treatment with CLA alone reduced cell counts in spleens and lungs compared with the counts in the spleens and lungs of mice in the early and late control groups ($P < 0.01$). Treatment with TEM alone did not reduce cell counts in spleens compared with the counts in the spleens of mice in the early or late control groups ($P > 0.05$). Treatment with TEM alone reduced cell counts in lungs compared with the counts in the lungs of late controls ($P < 0.01$) but not in those of early controls.

CLA alone was more active than the combination of CLA-TEM ($P < 0.01$ for organisms in spleens; $P < 0.05$ for organisms in lungs). The combination of CLA-RBT was more active than CLA alone against organisms in spleens and lungs ($P < 0.01$).

Combination therapy against MAC isolate 1408-3. CLA (200 mg/kg) was given daily alone or in combination to female mice which had been infected with $6.2 \times 10^7$ viable MAC isolate 1408-3. Combination groups received either one or two additional agents, as follows: RIF (20 mg/kg), EMB (125 mg/kg), CFZ (20 mg/kg), RIF and EMB, CFZ and EMB, or RIF and CFZ. A group of early control mice was sacrificed 7 days after infection. The difference in cell counts between early and late control groups was not significant for organisms in spleens ($P > 0.05$) (Fig. 6).

The reductions in cell counts seen with CLA alone and all combinations were significant compared with the reductions in the early and late control groups ($P < 0.01$). CLA alone was as active as the combinations of CLA-RIF, CLA-EMB, and CLA-RIF-EMB ($P > 0.05$). The reductions in cell counts seen with any of the CFZ-containing regimens (CLA-
CFZ, CLA-RIF-CFZ or CLA-CFZ-EMB) were significant compared with the reductions after treatment with CLA alone ($P < 0.01$). The differences in cell counts among the three CFZ-containing regimens were not significant ($P > 0.05$).

**DISCUSSION**

CLA at 200 mg/kg had activity against four MAC isolates tested in vivo. The reduction in log CFUs in spleens between the late control and the CLA-treated groups in a 10-day treatment experiment ranged from 0.8 (MAC isolate G, for which the MIC was 1 $\mu$g/ml) to 2.1 (M. avium ATCC 49601, for which the MIC was 4 $\mu$g/ml). There was little correlation between in vitro MICs determined by the broth dilution method and the activity of CLA in the mouse model.

In order to examine the interexperimenal variation in CLA activity against a single MAC isolate (M. avium ATCC 49601), we reviewed seven separate studies (four included in the current report; the others are not given here). The reduction in log CFUs in spleens between the late control and CLA (200 mg/kg)-treated groups occurred in a relatively narrow range (from 1.6 to 2.1; mean, 1.9). Initial inocula (range, $1.1 \times 10^7$ to $3.3 \times 10^7$ viable organisms) did not appear to correlate with activity in vivo. Similarly, mouse gender did not appear to influence CLA activity against this particular isolate. Drug preparation and administration were performed by the same investigator in a similar manner for each experiment.

Previous studies of promising new agents in the beige mouse have often been limited by the use of a single MAC isolate (6, 16). Unlike wild-type strains of *M. tuberculosis*, MAC isolates have considerable variation in vivo susceptibility (9, 11, 26). Parallel testing of additional isolates in vitro and in vivo may help to determine whether in vitro data can be used to predict activity in the mouse model.

The rationale for combination chemotherapy in the treatment of mycobacterial infections is based on minimizing the emergence of drug resistance and, possibly, increasing the activity of agents which have only modest activity when used alone. Although the current studies do not address the question of emergence of resistant organisms, the combination studies demonstrated various interactions in vivo.

The addition of TEM or RIF to CLA resulted in a decrease in activity compared with the activity of CLA alone. TEM as a single agent at 100 mg/kg had poor activity against *M. avium* ATCC 49601. The activity of TEM alone against a second isolate, MAC 1408-3, for which the MIC was more favorable (1 $\mu$g/ml), was minimal in the beige mouse (data not shown). In the in vitro activity of TEM has been variable. Khardori et al. (13) noted an MIC for 90% of strains tested ($n = 22$) of 2.0 $\mu$g/ml using a broth microdilution method, while Van Caekenbergh et al. (24) reported an MIC for 90% of strains tested ($n = 22$) of 32 $\mu$g/ml (range, 1 to 64 $\mu$g/ml) using an agar dilution method. Perrone et al. (20) reported activity of TEM at a concentration of 4 $\mu$g/ml against one of two MAC isolates in human monocyte-derived macrophage cultures. Because the peak concentration of TEM in serum after a single subcutaneous dose of 100 mg/kg in CF-1 mice is 25.2 $\mu$g/ml (8), it is not clear why TEM alone did not have favorable activity against MAC in the beige mouse. The combination of TEM-CLA may have an antagonistic interaction against this particular MAC isolate. Different routes of administration were used for TEM and CLA in combination; therefore, decreased absorption of CLA was unlikely.

The activities of RBT and CFZ as single agents against MAC ATCC 49601 infection in mice have been reported previously (16). The addition of CFZ or RBT to CLA in the current study resulted in an increase in activity compared with that of any of the agents alone. Although the addition of RIF or EMB to the combination of CLA-CFZ did not increase activity beyond that seen with the two-drug combination, the combination of CFZ with a newer rifamycin has been shown to have additive activity in MAC-infected beige mice (5, 16). Therefore, a study of CLA-CFZ-RBT would be of interest to determine whether an even more active regimen may be identified in the beige mouse model. Comparison of these agents singly and in combination would also be warranted to determine whether the emergence of resistant organisms is suppressed. Clinical trials of these promising combinations may lead to identification of an effective oral regimen for the treatment of human *M. avium* complex infections.

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**REFERENCES**

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