Comparison of the Intracellular Activities of Clarithromycin and Erythromycin against Mycobacterium avium Complex Strains in J774 Cells and in Alveolar Macrophages from Human Immunodeficiency Virus Type 1-Infected Individuals

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The intracellular activities of clarithromycin and erythromycin, alone and in combination with other antimicrobial agents, were tested against Mycobacterium avium complex (MAC) strains isolated from mouse J774 cells and inside alveolar macrophages obtained from human immunodeficiency virus type 1-infected individuals. Clarithromycin alone had greater intracellular activity than erythromycin alone, and drug combinations that included clarithromycin were usually more active than combinations that included erythromycin.

Clarithromycin is a new macrolide antimicrobial agent that has in vitro activity against Mycobacterium avium complex (MAC) strains (3, 8–11) and may have therapeutic value in treating MAC infections in patients with AIDS (2, 3, 9). The susceptibility of MAC strains to clarithromycin has been measured in broth cultures (8, 11), on agar (3, 8, 10), and inside monocyte-derived macrophages obtained from healthy donors (9, 10). Another test system that has been used to measure the intracellular activity of drugs against MAC strains is the mouse macrophage-derived cell line J774 (11, 12, 15). Rastogi and Labrousse (11) demonstrated that clarithromycin is active against MAC strains inside this cell line. The use of alveolar macrophages obtained from human immunodeficiency virus type 1 (HIV-1)-infected patients has also been described as a means of measuring the activities of drugs against MAC strains and thus evaluating their potential therapeutic value in treating MAC infection in HIV-1-infected patients (16). There are no published reports on the activity of clarithromycin in alveolar macrophages from HIV-1-infected individuals.

In contrast to clarithromycin, the older macrolide erythromycin has been shown to have poor activity against most strains of MAC in broth and on agar (3, 4, 6–8, 14). However, erythromycin-resistant clarithromycin is known to concentrate inside phagocytes (5, 13), and the intracellular activity of erythromycin in combination with other antimicrobial agents against MAC strains is not known. Thus, the potential therapeutic value of erythromycin in treating MAC infection in patients with AIDS has yet to be determined.

Because of the resistance of MAC strains to most single antimicrobial agents and the possibility of the development of in vivo resistance to drugs, patients with MAC infection are often treated with a combination of drugs. In the study by Rastogi and Labrousse (11), the intracellular activity of clarithromycin was enhanced by the addition of ethambutol and rifampin, but similar studies using erythromycin have not been reported. Since the in vitro effect of combinations of drugs can be additive, synergistic, or antagonistic (1), studies are needed to test the potential effect of combination therapy on intracellular MAC organisms.

The aims of this study were (i) to compare the in vitro intracellular activities of clarithromycin and erythromycin against MAC strains by using J774 cells and alveolar macrophages obtained from HIV-1-infected patients and (ii) to determine the effects of combinations of these drugs with other antimicrobial agents on intracellular MAC survival.

The four strains of MAC used in this study were isolated from patients with AIDS. Broth dilution MICs of clarithromycin for these strains ranged from 2 (two strains) to 8 (two strains) μg/ml, while MICs of erythromycin ranged from 8 (one strain) to 128 (three strains) μg/ml. Previous experiments using J774 cells showed that these four MAC strains, as well as all other strains of MAC tested so far, are able to grow inside J774 cells over a 7-day incubation period in the absence of drugs (15).

Drug susceptibility tests of MAC organisms inside J774 cells were performed as previously described (15). Briefly, 10^5 J774 cells in tissue culture flasks were infected with approximately 3 CFU of MAC organisms per cell, incubated at 35°C overnight, and then washed and treated with antimicrobial agents for 7 days. Macrophages in control flasks received no drugs. Clarithromycin, erythromycin, ethambutol, ciprofloxacin, and rifampin were tested at concentrations that are serum achievable (i.e., 2, 1, 4, 2, and 8 μg/ml, respectively). At time zero (t = 0) and after 7 days of incubation, duplicate sets of J774 cells were washed and lysed with 0.25% sodium dodecyl sulfate, and the lysates were plated on 7H10 agar to determine the number of CFU of MAC organisms present (15). Survival of MAC organisms in drug-treated macrophages was expressed as a percentage of the number of CFU of MAC organisms present inside untreated J774 cells at t = 0. Values less than 100% indicate a reduction in the intracellular survival of MAC organisms in the presence of drugs. In every experiment, CFU counts in drug-free controls increased during the 7-day incubation period.

The results of drug treatment experiments showed that clarithromycin had greater activity than erythromycin against the four MAC strains tested in J774 cells (Fig. 1).
While recovered from the bronchoalveolar lavage fluid of patients who had AIDS or were at risk for AIDS as part of a diagnostic procedure for the detection of Pneumocystis carinii. The procedure has been described in detail elsewhere (16). Briefly, macrophages in the lavage fluid (approximately 10^6) were washed, infected with 3 to 5 CFU of MAC organisms per cell, and incubated in tissue culture flasks at 35°C overnight. Macrophages were washed to remove non-adherent cells and bacteria and then were treated with drugs for 48 to 72 h. Concentrations of drugs were as listed above. Macrophages in control flasks received no drugs. At the time of the addition of drugs (t = 0) and after 48 to 72 h of incubation, macrophages in duplicate sets of flasks were washed, detached by trypsinization, counted, and lysed. The lysates were then plated as described above. The numbers of CFU of MAC organisms per macrophage in control flasks at t = 0 and those in drug-treated and control flasks after 48 to 72 h of incubation were calculated. The results of drug treatment are expressed as a percentage of the number of CFU of MAC organisms per macrophage present in control flasks (16). Values less than 100% indicate a reduction in the intracelular survival of MAC organisms due to drugs.

The data obtained by using patient alveolar macrophages (Fig. 2) were similar to those obtained by using the J774 cell line (Fig. 1), with clarithromycin alone showing significantly greater intracellular activity against MAC strains than erythromycin alone (P < 0.05). Clarithromycin alone had mycobactericidal activity against two strains (mean survival for these strains = 45%) and had a bacteriostatic effect on one strain in alveolar macrophages. Erythromycin alone had no mycobactericidal activity but had bacteriostatic activity against one of the strains. The addition of ethambutol enhanced the intracellular activity of each drug inside alveolar macrophages. The combination of clarithromycin plus ethambutol had mycobactericidal activity against all four MAC strains (mean survival = 50%), while erythromycin plus ethambutol was mycobactericidal for two (mean survival = 39%) of the four strains. The three-drug combination of clarithromycin plus ethambutol plus rifampin was mycobactericidal for all four strains (mean survival = 16%), while erythromycin plus ethambutol plus rifampin was mycobactericidal for three strains (mean survival = 31%) and bacte-

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FIG. 1. Intracellular activity of erythromycin (●) versus that of clarithromycin (▲) against MAC strains inside mouse J774 cells. Drug treatment was for 7 days and was with serum-achievable concentrations. Each point in a column represents a different strain of MAC. Abbreviations: ERY, erythromycin; CLAR, clarithromycin; EMB, ethambutol; RIF, rifampin; CIP, ciprofloxacin.

Clarithromycin alone reduced the intracellular survival of three strains (mean survival of these strains = 34%) and was partially inhibitory for one strain, while erythromycin alone reduced the intracellular survival of only one strain (survival = 84%). The difference between these two drugs was statistically significant (P < 0.05). The addition of ethambutol enhanced the activity of each drug. The combination of clarithromycin plus ethambutol had mycobactericidal activity against all four MAC strains (mean survival = 30%), while erythromycin plus ethambutol was mycobactericidal for three strains (mean survival = 42%) and was partially inhibitory for one strain. The three-drug combinations of clarithromycin plus ethambutol plus rifampin and erythromycin plus ethambutol plus rifampin were mycobactericidal for all four strains (mean survival = 13 and 12%, respectively). Replacing rifampin with ciprofloxacin in either three-drug combination, however, did not improve killing of MAC strains, and in the case of one strain it had a deleterious effect on killing. In no case was the difference between clarithromycin and erythromycin statistically significant when these drugs were compared in drug combination experiments.

The above experiments were repeated with alveolar macrophages from HIV-1-infected individuals. Macrophages were recovered from the bronchoalveolar lavage fluids of patients who had AIDS or were at risk for AIDS as part of a diagnostic procedure for the detection of Pneumocystis carinii. The procedure has been described in detail elsewhere (16). Briefly, macrophages in the lavage fluid (approximately 10^6) were washed, infected with 3 to 5 CFU of MAC organisms per cell, and incubated in tissue culture flasks at 35°C overnight. Macrophages were washed to remove non-adherent cells and bacteria and then were treated with drugs for 48 to 72 h. Concentrations of drugs were as listed above. Macrophages in control flasks received no drugs. At the time of the addition of drugs (t = 0) and after 48 to 72 h of incubation, macrophages in duplicate sets of flasks were washed, detached by trypsinization, counted, and lysed. The lysates were then plated as described above. The numbers of CFU of MAC organisms per macrophage in control flasks at t = 0 and those in drug-treated and control flasks after 48 to 72 h of incubation were calculated. The results of drug treatment are expressed as a percentage of the number of CFU of MAC organisms per macrophage present in control flasks (16). Values less than 100% indicate a reduction in the intracelular survival of MAC organisms due to drugs.

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FIG. 2. Intracellular activity of erythromycin (●) versus that of clarithromycin (▲) against MAC strains inside alveolar macrophages obtained from HIV-1-infected individuals. Drug treatment was for 48 to 72 h with serum-achievable concentrations. Each point in a column represents a different strain of MAC. See the legend to Fig. 1 for abbreviations.
riostatic for one strain. In no case was the difference between clarithromycin and erythromycin statistically significant when these drugs were used in combination with other antimicrobial agents. As was observed in the J774 cell experiments, the substitution of ciprofloxacin for rifampin in the three-drug combinations sometimes had a deleterious effect on the killing of MAC strains in alveolar macrophages.

The results obtained in this study give evidence that clarithromycin is more active than erythromycin against MAC strains inside cells of the mouse macrophage-derived cell line J774 and inside alveolar macrophages from HIV-1-infected individuals. These results support previous in vitro and in vivo data which indicated that clarithromycin had potential therapeutic benefits in treating MAC infection (2, 3, 9–11). Our data from experiments using alveolar macrophages from HIV-1-infected individuals suggest, however, that clarithromycin alone may not be efficacious against all strains of MAC and that combination therapy may be required in some cases. Furthermore, it is interesting that although erythromycin alone has poor intracellular activity against MAC, the three-drug combination of erythromycin plus ethambutol plus rifampin had mycobactericidal activity against all four MAC strains inside J774 cells and against three of the four tested strains inside alveolar macrophages. Even though clarithromycin alone is clearly superior to erythromycin alone, the data suggest that erythromycin deserves further study, since it has considerable intracellular activity against most MAC strains when it is used in combination with other antimicrobial agents.

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