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Clarithromycin Is Inactive against *Mycobacterium tuberculosis*

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When 10% oleic acid-albumin-dextrose-catalase-enriched Mueller-Hinton agar medium was employed, the MICs of clarithromycin (CLARI) at which 50 and 90% of 12 strains of *Mycobacterium tuberculosis* were inhibited were 64 and >128 μg/ml, respectively, which are significantly greater than the achievable peak CLARI concentrations in serum and in lung tissue in humans. In two different mouse experiments, 4 to 6 weeks of treatment with CLARI at 200 mg/kg of body weight six times weekly produced neither bactericidal nor bacteriostatic effects against *M. tuberculosis*. Therefore, we conclude that CLARI as a single drug is inactive against *M. tuberculosis*.

For patients infected with multidrug-resistant *Mycobacterium tuberculosis*, only a limited number of alternative chemotherapeutic regimens are available; none of them is very effective, and the mortality with the available regimens is high (8, 15). Therefore, new antituberculosis drugs with bactericidal mechanisms different from those of the presently available agents, i.e., isoniazid (INH), rifampin (RMP), pyrazinamide, ethambutol, and streptomycin, are urgently needed. Because clarithromycin (CLARI) displays powerful bactericidal activities against *Mycobacterium leprae* in mice (11) and in humans (9) and is by far the most active agent against disseminated *Mycobacterium avium* complex infection in beige mice (10) and in AIDS patients (4), naturally one wonders whether CLARI would also be active against *M. tuberculosis*. As the available information regarding the activity of CLARI against *M. tuberculosis* is rather limited (1, 2, 12) and the evidence concerning its in vivo activity remains inconclusive (12), we have therefore tested its in vitro and in vivo activities against *M. tuberculosis.*

The MICs of CLARI against 12 strains of drug-susceptible *M. tuberculosis*, including strain H37Rv and 11 clinical isolates, were determined. Organisms were grown in Dubos medium (Diagnostics Pasteur) for 7 days at 37°C, and the resulting suspension was adjusted with normal saline to match the turbidity of *Mycobacterium bovis* BCG standard (1 mg/ml). The adjusted suspension was further diluted to 10^{-5} mg/ml, and suspensions at 10^{-3} and 10^{-5} mg/ml, at a volume of 0.05 ml of suspension per quadrant, were plated in duplicate onto 10% oleic acid-albumin-dextrose-catalase-enriched Mueller-Hinton agar (Difco Laboratories) (13). The agar plates contained either serially twofold diluted CLARI, with final concentrations ranging from 128 to 0.5 μg/ml, or no drug, as a control. The plates were incubated at 37°C for 6 weeks, and the MIC was defined as the lowest concentration of CLARI which inhibited ≥99% of growth compared with that on drug-free agar plates. The MICs for the 12 strains ranged from 16 to >128 μg/ml, and the MICs at which 50 and 90% of these strains were inhibited were 64 and >128 μg/ml, respectively. Even the MIC at which 50% of the tested strains were inhibited was significantly greater than the achievable peak CLARI concentrations in serum or lung tissue in humans (5, 7), indicating that the strains were naturally resistant to CLARI.

In the first in vivo experiment, 45 female Swiss mice were each inoculated intravenously with $10^{4.40}$ CFU of H37Rv. Ten mice each were sacrificed on day 1 and day 14 after inoculation for enumeration of CFU in the spleens, and the remaining 25 mice were allocated randomly to three groups: 10 mice each in the untreated control and CLARI-treated groups and 5 mice in the RMP-treated group. Treatments were begun on day 14 and continued for 6 weeks; drugs were administered six times weekly through an esophageal cannula (gavage) with CLARI at 200 mg/kg of body weight or RMP at 10 mg/kg per dose. In terms of area under the concentration-time curve, the 200-mg/kg dose of CLARI in mice is equivalent to the 2,000-mg/kg dose of CLARI in humans (unpublished data), which is the highest clinically tolerated dose that may be administered to humans. All mice were sacrificed 48 h after administration of the last dose of the treatment. For the enumeration of CFU, the spleens were removed aseptically and homogenized (6), and three or four appropriate dilutions of the suspension prepared from each spleen were plated at least in triplicate onto Löwenstein-Jensen medium. The results of the cultures were recorded after incubation at 37°C for 6 weeks, and bactericidal activity of the treatment was defined as a significant decrease from the pretreatment value in the mean number of CFU in the treated mice, i.e., the value on day 14 after inoculation. Since the inoculum size was small, it only caused a chronic, self-limited, and nonfatal infection in immunocompetent mice as previously observed (3). As shown in Fig. 1, the mean number of CFU ($\log_{10}$) in the spleens of untreated mice progressively increased from day 1 to day 14 ($P < 0.01$) and then remained at the same level and even slightly decreased on day 56 ($P < 0.05$), i.e., at the end of 6 weeks of treatment in the treated groups; by day 56, while the mean number of CFU in RMP-treated mice was very much smaller than the pretreatment value ($P < 0.01$), that for the CLARI-treated mice did not differ significantly from the pretreatment value or from that of control mice sacrificed concomitantly, indicating that 6 weeks of treatment with CLARI was inactive against *M. tuberculosis* in a model of chronic infection.

In the second in vivo experiment, 64 female Swiss mice were each inoculated intravenously with $10^{6.85}$ CFU of H37Rv. After 10 mice were sacrificed on day 1, the remaining mice were allocated randomly to an untreated control group of 24 mice and two treated groups of 15 mice each. The treated mice were gavaged six times weekly for 4 weeks with one of two treatments, either CLARI at 200 mg/kg or INH at 25 mg/kg, and all surviving mice were sacrificed 48 h after administration of the last dose of the treatment. As reported earlier (3), the large inoculum size caused an acute and fatal infection even in

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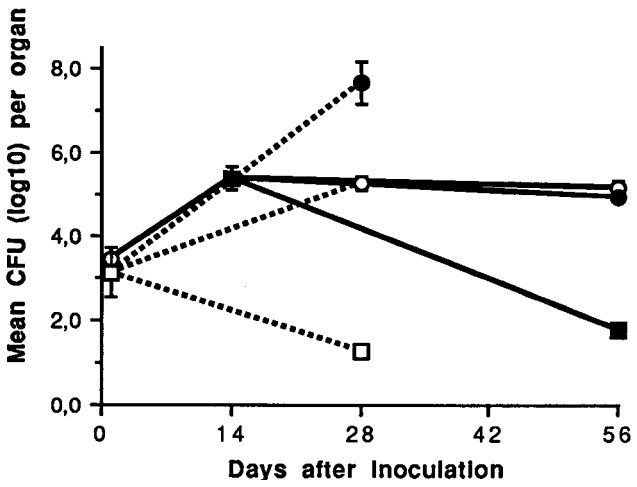

FIG. 1. Growth curves of *M. tuberculosis* in the spleens (experiment [Exp] I; solid lines) or lungs (Exp II; dotted lines). Mice were each inoculated intravenously with either $10^{4.40}$ (Exp I) or $10^{6.85}$ (Exp II) CFU of strain H37Rv, and treatments were begun on day 14 and continued for 6 weeks (Exp I) or begun on day 1 and continued for 4 weeks (Exp II). Drugs were administered six times weekly through an esophageal cannula, with CLARI at 200 mg/kg (●), RMP at 10 mg/kg (■), or INH at 25 mg/kg (□) per dose. All surviving mice were sacrificed 48 h after administration of the last dose of the treatment, and the CFU were enumerated on Löwenstein-Jensen medium. The error bars represent the standard deviations of the enumerations. ○, control.

immunocompetent mice, and therefore by day 30, 21 (87.5%) of the 24 untreated control mice and 4 (26.7%) of the 15 CLARI-treated mice died whereas no mortality was observed for mice treated with INH. The mortality rate of CLARI-treated mice was significantly lower than that of the controls ($P < 0.01$) but higher than that of INH-treated mice ($P = 0.05$). Among the mice that were sacrificed on day 30, the mean spleen weights ± standard deviations for untreated control and CLARI-treated mice were, respectively, 748 ± 221 and 623 ± 150 mg, both of which are very much greater than the pretreatment value of 117 ± 26 mg ($P < 0.01$) though the two of them did not differ significantly from each other ($P > 0.05$); the mean spleen weight for INH-treated mice was 146 ± 39 mg, which is only slightly greater than the pretreatment value ($P < 0.05$) and significantly lower than those for control and CLARI-treated mice. The severity of gross lung lesions was scored from 0 to 2+, with the latter referring to a lung extensively infiltrated with tubercles (14). By day 30, lesions with a severity of 2+ were encountered in all surviving mice of the control and CLARI-treated groups, whereas no gross lung lesion was observed in mice that had been treated with INH. With respect to the enumeration of CFU in the lungs, the mean numbers of CFU in untreated control and CLARI-treated mice on day 30 were significantly greater than the pretreatment value ($P < 0.01$), while the mean number in the INH-treated group was significantly smaller than the pretreatment value ($P < 0.01$) (Fig. 1). The CFU counts in control mice were probably grossly underestimated on day 30 because of the high mortality rate in the group. In brief, although 4 weeks of CLARI treatment had reduced the mortality rate caused by *M. tuberculosis* infection, it was unable to prevent the development of splenomegaly or gross lung lesions or to inhibit the multiplication of *M. tuberculosis.*

Because the MICs of CLARI against *M. tuberculosis* were very high compared with the achievable peak concentrations in serum and lung tissue in humans and because 4 to 6 weeks of treatment with high doses of CLARI produced neither bactericidal nor bacteriostatic effects, we conclude that CLARI is inactive against *M. tuberculosis.*

Although it was claimed recently that CLARI displayed in vitro synergistic effects with other drugs, resulting in a 4- to 32-fold reduction in the MICs of INH, RMP, and ethambutol against multidrug-resistant strains of *M. tuberculosis* (2), the clinical relevance of such synergistic effects remains unclear; in addition, the in vitro synergism has not been confirmed by other investigators (1). The in vivo experiment is probably the most reasonable approach to demonstrate whether or not such an inactive agent may display synergistic effects with other antituberculosis drugs.

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