ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2001, p. 3229–3230 Vol. 45, No. 11
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.11.3229–3230.2001

Efficacies of Clarithromycin Regimens against *Mycobacterium xenopi* in Mice

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Received 11 April 2001/Returned for modification 4 June 2001/Accepted 6 August 2001

Mice were infected intravenously with 3.5×10^7 CFU of *Mycobacterium xenopi* and treated with various clarithromycin-containing regimens or left untreated for 4 weeks. All nine of the clarithromycin-containing regimens reduced the CFU counts to the levels below the pretreatment values, indicating that these regimens had a bactericidal effect on *M. xenopi* in mice. The rifampin-isoniazid-ethambutol regimen was significantly less bactericidal than clarithromycin alone or clarithromycin-containing combined regimens.

Mycobacterium xenopi is a slow-growing, scotochromogenic acid-fast bacillus with optimal growth at approximately 43°C (10). It is an opportunistic mycobacterium that may cause chronic pulmonary disease, and it is second to *Mycobacterium avium* complex as a cause of nontuberculosis mycobacterial lung disease in parts of Canada, the United Kingdom, and other areas of Europe (1). The organism frequently colonizes human immunodeficiency virus-infected patients, and significant infection due to *M. xenopi* may occur in patients with more advanced human immunodeficiency virus infection (2).

M. xenopi is significantly less susceptible than other mycobacteria to the first-line antituberculosis drugs (3), and in general, the clinical response of patients with *M. xenopi* infection to antituberculosis chemotherapy is poor (2, 7). Therefore, more effective treatment is urgently needed. Among the newer antimicrobials, although clarithromycin (CLR) is virtually inactive against *M. tuberculosis* both in vitro and in vivo (9), its MIC for *M. xenopi* is rather low (5, 7), and it is active against *M. xenopi* infection in mice (5). Furthermore, there have been reports that *M. xenopi* infection was successfully controlled in patients being treated with drug combinations containing CLR (6, 7). Nevertheless, all of this information was derived from very preliminary studies (5) or from single case reports (6, 7). Obviously, more studies are needed for further evaluation of the potential role of CLR in the treatment of *M. xenopi* infection. Based on the concept that, similar to the treatment of other mycobacterial infections, *M. xenopi* infection probably requires multidrug therapy, we carried out a study in which the therapeutic effects of various CLR-containing combined regimens were tested in the mouse model and compared with those of CLR alone or the combination rifampin-isoniazid-ethambutol (RIF-INH-EMB). The following drugs were used in combination with CLR: ofloxacin (OFX), levofloxacin (LVX), gemifloxacin (GMX), moxifloxacin (MXX), sparfloxacin (SPX), ciprofloxacin (CIP), amikacin (AMK), RIF, INH, and EMB.

The MIC of CLR against the strain used in this study was 0.12 μg/ml, as determined by an established method (4) with 10% oleic acid–albumin–dextrose–catalase-enriched 7H11 agar medium. This MIC is higher than those previously published (5, 7). The MICs of other antimicrobials against strain Elb 3441 are as follows: RIF, 0.5 μg/ml; INH, 0.5 μg/ml; EMB, 16 μg/ml; AMK, 4 μg/ml; OFX, 2 μg/ml; LVX, 1 μg/ml; CIP, 2 μg/ml; SPX, 0.25 μg/ml; GMX, 4 μg/ml; and MXX, 0.25 μg/ml.

One hundred twenty-eight 4-week-old, female Swiss mice were infected intravenously with 0.5 ml of diluted Dubos broth culture containing 3.5×10^7 CFU of *M. xenopi* strain Elb 3441, which was isolated from the sputum sample of a patient with *M. xenopi* pulmonary infection. The next day after infection (day 1), 10 mice were sacrificed to provide the pretreatment values of the mean spleen weight and mean numbers of CFU per spleen and lung. The remaining mice were randomly allocated to an untreated control group of 10 mice and 10 treated groups of 8 to 10 mice each. Each of the treated groups was administered one of the 10 regimens shown in Table 1. Treatment began on day 1 and was given five times weekly for 4 weeks. Except for AMK, which was given by subcutaneous injection, all of the remaining drugs were administered by an esophageal cannula (gavage). As routinely applied in mouse experiments, the drugs were given at the following dosages: CLR, 200 mg/kg of body weight; OFX, 300 mg/kg; LVX, 150 mg/kg; CIP, 300 mg/kg; SPX, 100 mg/kg; MXX, 100 mg/kg; GMX, 150 mg/kg; AMK, 100 mg/kg; RIF, 10 mg/kg; INH, 25 mg/kg; and EMB, 100 mg/kg.

The severity of infection and the effectiveness of treatment were assessed according to the mean spleen weight and mean number of CFU per spleen and lung by methodologies described elsewhere (4). Unlike in studies of *M. tuberculosis* infection in mice, *M. xenopi* infection does not kill the mice nor produce a gross lesion in the lung; therefore, survival rate and gross lung lesion cannot be applied as parameters. A regimen was considered bactericidal if the mean numbers of CFU per organ were significantly smaller in the treated group than those in the pretreatment group.

The Mann-Whitney test was used to compare groups. Because all treatment groups were compared in the first analysis to the control group in order to assess the efficacy of each regimen and in the second analysis were compared to the

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TABLE 1. Mean spleen weight and number of CFU per spleen and lung of mice after intravenous infection with 3.5×10^7 CFU of *M. xenopi*

Group[a] (no. of mice)	Spleen wt (mg)	Mean CFU ($\log_{10}$)	
		Spleen	Lung
Pretreatment (10)	115 ± 18	6.30 ± 0.28	5.18 ± 0.17
Untreated control (10)	458 ± 16	6.25 ± 0.20	5.18 ± 0.38
CLR alone (10)	153 ± 35	4.04 ± 0.36	2.75 ± 0.66
CLR-OFX (10)	112 ± 25	3.55 ± 0.34	2.79 ± 0.21
CLR-OFX-AMK (10)	127 ± 30	3.46 ± 0.58	2.34 ± 0.21
CLR-LVX (10)	143 ± 39	5.01 ± 0.10	2.89 ± 0.39
CLR-SPX (10)	114 ± 14	4.71 ± 0.07	2.38 ± 0.31
CLR-CIP (10)	108 ± 20	4.16 ± 0.40	3.26 ± 0.22
CLR-MXX (10)	99 ± 22	4.97 ± 0.13	2.48 ± 0.57
CLR-GMX (8)	84 ± 19	5.24 ± 0.07	3.40 ± 0.20
CLR-OFX-RIF-INH-EMB (9)	90 ± 51	4.78 ± 0.39	3.19 ± 0.21
RIF-INH-EMB (8)	245 ± 73	5.62 ± 0.14	3.78 ± 0.21

[a] Except for the pretreatment values, which were obtained from mice sacrificed on day 1 after inoculation, the remaining results were obtained from mice sacrificed on day 30. Treatment began on day 1 and was administered five times weekly for 4 weeks.

CLR-alone group in order to assess the efficacy of CLR-containing regimens, the critical value of z set at 1.96 for a type 1 error of 5% was adjusted to account for multiple comparisons. The appropriate critical value was calculated by using $z_{\alpha 2(k-1)}$ instead of $z_{\alpha 2}$, with k being the number of groups. The critical values for a significant difference with a type 1 error of 5% were 2.77 in the first analysis, corresponding to a P value of 0.0028, and 2.73 in the second analysis, corresponding to a P value of 0.0032 (8).

As shown in Table 1, the mean spleen weight increased significantly in the untreated group, from 115 ± 18 mg on day 1 to 458 ± 16 mg on day 30 ($P < 0.0001$), similar to that observed in other mycobacterial infection, such as *M. tuberculosis* or *M. avium* complex infection in mice. On the other hand, the mean spleen weight in mice treated with CLR alone was significantly smaller than that in the untreated control ($P < 0.0001$) and did not differ significantly from the pretreatment value. While the value in mice treated with RIF-INH-EMB was smaller than that in untreated control mice and greater than the pretreatment value or the value in mice treated with CLR alone, none of the differences has attained statistical significance. Despite the fact that the mean spleen weights of the eight groups treated with CLR-containing combined regimens were always smaller than those in mice treated with CLR alone, the differences did not attain statistical significance.

The next day after intravenous infection of 3.5×10^7 CFU of *M. xenopi*, the mean number of CFU reached 6.30 ± 0.28 $\log_{10}$ per spleen and 5.18±0.17 $\log_{10}$ per lung. However, as shown in Table 1, no further increase in the bacterial population was observed in either organ of the untreated group during the following 4 weeks. The mean numbers of CFU per spleen or lung in all treated groups were significantly smaller than the pretreatment values or those in the untreated control group ($P < 0.0001$). Among the treated groups, the mean number of CFU per organ was significantly smaller in mice treated with CLR alone than in mice treated with RIF-INH-EMB ($P < 0.0001$); however, none of the values for mice treated with various CLR-containing combined regimens was significantly smaller than those in mice treated with CLR alone.

Our results demonstrated that, in immunocompetent mice, 10^5 to 10^6 CFU may be found in the spleen or lung 24 h after inoculation with 10^7 CFU of *M. xenopi*. Because the mean numbers of CFU remained virtually unchanged during the following 4 weeks, indicating that, during that period, *M. xenopi* remained viable and constant in number, this methodology is therefore suitable for testing in vivo activity against *M. xenopi*. The finding that the mean numbers of CFU per organ of these mice were at least at the same level as those recovered from beige mice (7) suggests that immunocompetent mice, which are widely available and much cheaper than beige mice, are equally suitable for testing in vivo activity against *M. xenopi*.

The results of our experiment clearly demonstrated that treatment with CLR alone or the combination of RIF-INH-EMB was able to prevent the development of splenomegaly caused by *M. xenopi* infection and significantly reduced the bacterial population of *M. xenopi* in the organs. However, such effects were significantly stronger with CLR alone than with the combination of RIF-INH-EMB. Nevertheless, among the eight groups treated with various CLR-containing combined regimens, none of these parameters was significantly smaller than those in mice treated with CLR alone, indicating that none of the components in the combined regimens—whether a fluoroquinolone, an aminoside, or the combination of RIF-INH-EMB—has significantly improved the effect of CLR in preventing the development of splenomegaly or in killing *M. xenopi*. Consequently, the search for a more effective CLR-based combined regimen should continue.

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