NOTES

Activities of Moxifloxacin Alone and in Combination with Other Antimicrobial Agents against Multidrug-Resistant *Mycobacterium tuberculosis* Infection in BALB/c Mice

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The activity of moxifloxacin was enhanced by the addition of ethionamide but not by that of cycloserine, thiacetazone, capreomycin, para-aminosalicylic acid, or linezolid in BALB/c mice infected with a strain of *Mycobacterium tuberculosis* resistant to isoniazid, rifampin, and six other drugs. These observations are important for the therapy of multidrug-resistant tuberculosis.

The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*, i.e., strains resistant at least to isoniazid (INH) and rifampin (RMP), poses serious problems for tuberculosis (TB) control and increases the demand for new therapies. Fluoroquinolone agents such as ciprofloxacin (CIP) and ofloxacin (OFL) are effective against TB, including disease caused by MDR strains (7, 16). Of the recently developed fluoroquinolones, moxifloxacin (M) showed potent activity against *M. tuberculosis* in mice (9, 11, 18) but no studies have been performed with difficult-to-treat strains resistant to INH, RMP, and several other drugs. We therefore tested the activity of M, alone and in two-drug combinations with other agents, including the traditional second-line drugs capreomycin (CM), cycloserine (CS), para-aminosalicylic acid (PAS), ethionamide (ETH), and thiacetazone (TC) (6) and oxazolidinones (linezolid [LNZ]) (3), in mice infected with an MDR *M. tuberculosis* strain also resistant to all of the other first-line antituberculosis drugs. In order to evaluate a possible enhancement of the activity of M, a low dose of the drug (30 mg/kg) (9) was used.

The MDR clinical isolate RM22, known to be resistant to RMP, INH, streptomycin (SM), ethambutol (EMB), pyrazinamide, and kanamycin (KM) (4), was used in this study. The mutation conferring resistance to RMP is TCG531TTG (Ser→Leu) in the *rpoB* gene (4, 15); the mutation conferring resistance to INH is AGC315ACC (Ser→Thr) in the *katG* gene (unpublished data). Comparative MICs of these and other drugs for isolate RM22 and for a susceptible *M. tuberculosis* strain, H37Rv (ATCC 27294), as determined by the twofold agar dilution technique on Middlebrook 7H11 agar (Difco Laboratories, Detroit, Mich.) (4–6, 13), are shown in

Table 1. INH, CM, CS, PAS, EMB, SM, KM (Sigma Chemical Co., St. Louis, Mo.), and CIP (Bayer, Milan, Italy) were dissolved in distilled water; RMP (Sigma), rifapentine (RPT) (Aventis Pharma, Vitry-Alfortville, France), and rifabutin (RFB) (Pharmacia & Upjohn, Kalamazoo, Mich.) were dissolved in ethanol; OFL (Sigma), sparfloxacin (SPX) (Aventis), and M (Bayer) were dissolved in 0.1 M NaOH; and TC, ETH (Sigma), and LNZ (Pharmacia) were dissolved in dimethyl sulfoxide.

Male BALB/c mice (Charles River, Calco, Como, Italy) were infected intravenously with 0.2-ml portions containing 5×10^5 CFU of isolate RM22. One day after the infection, four mice were sacrificed and the numbers of CFU in the spleens and lungs were determined (untreated day 1 control). Organs were removed and homogenized in Middlebrook 7H9 broth (Difco). To enumerate CFU, appropriate dilutions of the homogenates were plated onto Middlebrook 7H10 agar and colonies were counted after 3 to 4 weeks of incubation at 37°C under a humidified 5% CO₂ atmosphere. The remaining mice were allocated either to untreated groups or to various drug-treated groups (six mice per group). The following drugs were administered by gavage five times weekly: ETH at 100 mg/kg (10), CS at 300 mg/kg (10), TC at 60 mg/kg (8), PAS at 750 mg/kg (17), and LNZ at 50 mg/kg (3). CM was administered subcutaneously at 150 mg/kg (10). M was administered at 30 mg/kg (9), both alone and in combination with ETH, CS, TC, PAS, LNZ, and CM. Untreated mice were injected with saline. Thirty days postinfection, both untreated (untreated day 30 control) and treated mice were sacrificed and CFU counts and organ weights were determined; to reduce the carryover effect of drugs in the organs, mice were sacrificed from 3 to 4 days after the last dose was administered. The significance of the CFU count and organ weight differences was assessed by a two-tailed Student *t* test. $P \leq 0.01$ was considered significant.

Following infection with strain RM22, the log₁₀ CFU counts of the spleens increased from 3.27 ± 0.05 on day 1 to $5.57 \pm$

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TABLE 1. MICs and drug susceptibility profiles of *M. tuberculosis* RM22 and H37Rv

Drug	MIC ($\mu\text{g/ml}$) and drug susceptibility ^a	
	<i>M. tuberculosis</i> RM22 ^b	<i>M. tuberculosis</i> H37Rv
INH	16 (R)	≤ 0.125 (S)
RMP	>64 (R)	0.25 (S)
SM	32 (R)	1 (S)
EMB	15 (R)	0.47 (S)
RFB	32 (R)	≤ 0.125 (S)
RPT	32 (R)	≤ 0.125 (S)
KM	96 (R)	1.5 (S)
CIP	1 (S)	1 (S)
OFL	1 (S)	1 (S)
SPX	0.25	0.125
M	0.25	≤ 0.125
CM	5 (S)	2.5 (S)
CS	30 (S)	7.5 (S)
ETH	5 (S)	1.25 (S)
TC	≤ 0.156	0.156
PAS	4 (S)	0.25 (S)
LNZ	0.125	0.125

^a R, resistant; S, susceptible. The strains were considered resistant or susceptible to an individual drug on the basis of the reported critical concentrations in Middlebrook 7H11 agar (references 4 to 6 and 13); the critical concentration of RFB in Middlebrook 7H11 agar (0.5 $\mu\text{g/ml}$ [reference 13]) was tentatively used to discriminate strains resistant or susceptible to RPT.

^b The strain was also resistant to pyrazinamide (reference 4).

0.22 on day 30 (Table 2). No mortality was observed in either untreated or treated mice. The inactivity of PAS, TC, and CS alone (which are active against the strain *in vitro*) may be related to the capacity of these drugs to penetrate and persist

TABLE 2. CFU counts after 30 days of treatment of BALB/c mice infected with *M. tuberculosis* RM22 with M alone and in combination with other antimicrobial agents

Treatment (daily dose [mg/kg])	Log ₁₀ CFU count ^a (mean $\pm$ SD)	
	Spleen	Lung
Untreated day 1 control	3.27 $\pm$ 0.05	1.91 $\pm$ 0.20
Untreated day 30 control	5.57 $\pm$ 0.22	5.77 $\pm$ 0.12
M (30)	4.52 $\pm$ 0.25 ^b	3.04 $\pm$ 0.30 ^b
ETH (100)	2.36 $\pm$ 0.56 ^{b,c,e}	1.50 $\pm$ 0.15 ^{b,c,e}
M (30) + ETH (100)	1.55 $\pm$ 0.37 ^{b,c,d,e}	1.32 $\pm$ 0.16 ^{b,c,e}
CS (300)	5.26 $\pm$ 0.28	5.61 $\pm$ 0.24
M (30) + CS (300)	4.39 $\pm$ 0.18 ^b	3.71 $\pm$ 0.49 ^b
TC (60)	5.41 $\pm$ 0.33	5.58 $\pm$ 0.20
M (30) + TC (60)	4.52 $\pm$ 0.69 ^b	3.05 $\pm$ 0.53 ^b
CM (150)	4.71 $\pm$ 0.27 ^b	4.61 $\pm$ 0.21 ^b
M (30) + CM (150)	4.46 $\pm$ 0.19 ^b	2.90 $\pm$ 0.45 ^b
PAS (750)	5.31 $\pm$ 0.28	5.57 $\pm$ 0.27
M (30) + PAS (750)	4.79 $\pm$ 0.22 ^b	3.64 $\pm$ 0.35 ^b
LNZ (50)	4.65 $\pm$ 0.32 ^b	4.04 $\pm$ 0.29 ^b
M (30) + LNZ (50)	4.92 $\pm$ 0.22 ^b	2.83 $\pm$ 0.26 ^b

^a Mice were infected intravenously with 5×10^5 CFU of *M. tuberculosis* RM22, and treatment was begun on the day after infection. Data are the means of two experiments.

^b Significantly lower than the CFU counts in the untreated day 30 control mice ($P < 0.001$).

^c Significantly lower than the CFU counts in the mice treated with M alone ($P < 0.001$).

^d Significantly lower than the CFU counts in the mice treated with ETH alone ($P = 0.007$).

^e Significantly lower than the CFU counts in the untreated day 1 control mice ($P \leq 0.01$).

in mouse cells: PAS and TC are known to penetrate mouse cells poorly (12, 17), while the rate of CS excretion in mice is high (2, 14, 17). Some support for this view comes from the knowledge that CM, which is absorbed subcutaneously in mice (17), was effective both *in vivo* and *in vitro*. The good concordance of the *in vitro* and *in vivo* activities of ETH may also be due to the good distribution of the drug (17). Compared with the day 1 CFU count, significant bactericidal effects of ETH and M-ETH were observed. Of the individual drugs, ETH was the most effective on day 30, with a mean CFU count decrease of 3.21 log₁₀ in comparison with that of the untreated day 30 control. Addition of M significantly increased the activity of the M-ETH combination in comparison with that of the day 30 control, M alone, or ETH alone. All of the other M-containing combinations tested significantly reduced, by about 1 log₁₀, the CFU counts in comparison with that of the day 30 control, but none was either bactericidal or significantly more active than M alone.

A similar pattern was observed for the lungs. The organism efficiently multiplied in untreated control mice, with an increase in the log₁₀ CFU count from 1.91 ± 0.20 on day 1 to 5.77 ± 0.12 on day 30 (Table 2). Compared with the day 1 CFU counts, significant bactericidal effects of ETH and M-ETH were observed. Of the individual drugs, ETH was the most effective on day 30, with a mean CFU count decrease of 4.27 log₁₀, in comparison with that of the untreated day 30 control. The M-ETH combination showed significantly greater activity than the day 30 control or M alone. M-ETH was slightly more active than ETH alone, but not at a statistically significant level. All of the other M-containing combinations tested reduced by 2 to 3 log₁₀ the CFU counts in comparison with that of the day 30 control, but none was either bactericidal or significantly more active than M alone.

The weights of the spleens of untreated control mice increased from 80 ± 8.2 mg on day 1 to 193 ± 23 mg on day 30 ($P < 0.001$), and those of the lungs increased from 162.5 ± 9.6 mg on day 1 to 185.7 ± 26.4 mg on day 30. In general, the higher the CFU count, the greater the spleen weight. Compared with the weights of noninfected mice on day 30, ETH or M-ETH was the most active in preventing the development of splenomegaly, while no significant differences in lung weights in comparison with that of the control were observed in mice treated with any of the regimens tested.

These observations indicate that ETH was more active in both the lungs and the spleen than any of the other individual drugs tested. The activity of M was not enhanced by the addition of oxazolidinones (LNZ) or the traditional second-line anti-TB drugs, with the exception of ETH. These results are in keeping with the observations recently reported by other investigators (1), who showed that, in mice, ETH enhanced the anti-TB activity of another novel fluoroquinolone, i.e., gatifloxacin.

Overall, our study indicates that the combination of M with ETH merits further studies of its potential activity in the treatment of infection by MDR *M. tuberculosis* strains.

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