

Sparfloxacin, Ethambutol, and Cortisol Receptor Inhibitor RU-40 555 Treatment for Disseminated *Mycobacterium avium* Complex Infection of Normal C57BL/6 Mice

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Sparfloxacin (50 mg/kg of body weight given subcutaneously each day), alone or in combination with ethambutol (50 mg/kg given subcutaneously each day), was examined for its therapeutic efficacy against experimental infection induced with the *Mycobacterium avium* complex in normal C57BL/6 mice. In addition, the potential anti-infective role of RU-40 555 (100 mg/kg given intraperitoneally each day), a drug that inhibits the cortisol receptors, was examined in the same model. Treatments were started 24 h after intravenous bacterial challenge and were continued for 21 days. Compared with controls, sparfloxacin or ethambutol decreased the CFU counts in spleens and lungs ($P < 0.001$). The sparfloxacin plus ethambutol combination was more effective than sparfloxacin alone in spleens ($P < 0.001$) but not in lungs. The sparfloxacin plus ethambutol plus RU-40 555 combination was more effective than the sparfloxacin plus ethambutol combination in spleens and lungs ($P < 0.001$). Thus, in this model, RU-40 555 enhanced the antibacterial activities of the antibiotics tested. Results of the study showed that normal C57BL/6 mice infected with the *M. avium* complex can be used for the evaluation of antimicrobial agents.

Antimicrobial agents active in vitro against the *Mycobacterium avium* complex usually exhibit only bacteriostatic or poorly bactericidal activity against experimental *M. avium* complex infections in mice. These disappointing results correlate with the lack of elimination of the *M. avium* complex from patients with AIDS treated with various combinations of drugs (9, 19). Immunomodulators such as tumor necrosis factor may enhance the activity of an antibiotic against infections with the *M. avium* complex (3, 4). Thus, new drugs, either antibiotics or immunomodulators, are needed for the treatment of *M. avium* complex infections. The most promising antibiotics are new macrolides and new fluoroquinolones (4, 6, 11, 20, 21, 30, 31). Sparfloxacin is a fluoroquinolone that exhibits a good efficacy against the *M. avium* complex in vitro and in a model of intracellular infection within human macrophages (26, 31, 36). Ethambutol, which inhibits bacterial cell envelope synthesis, has been shown to be synergistic with fluoroquinolones against the *M. avium* complex (32). Mifepristone (RU-38 486; Roussel-UCLAF, Romainville, Seine-Saint Denis, France), an inhibitor of the progesterone receptors, is used in humans as a short course of treatment for early pregnancy interruption and for prolonged periods, with good tolerance, for the treatment of some cases of breast cancer, meningioma, and Cushing's syndrome (2, 27, 33, 34). It has been shown in animals that mifepristone is also an inhibitor of the cortisol receptors and could enhance the inflammatory reaction (5, 7, 8, 10, 12, 17, 18, 23-25, 28, 35). RU-40 555 (Roussel-UCLAF) is a molecule that differs from mifepristone only by a methyl radical, with RU-40 555 having a higher inhibiting activity against the cortisol receptors (37). The animal model usually used for the evaluation of antimicrobial agents against *M. avium* complex is the beige mouse model (15).

Since beige mice, which often develop spontaneous tumors (13), are expensive and not easily available, we studied the experimental infection of normal C57BL/6 mice with the *M. avium* complex in order to validate the feasibility of using this mouse strain for the evaluation of antimicrobial agents. The second aim of the present study was to evaluate the in vivo efficacy of sparfloxacin, alone or in combination with ethambutol, against experimental infection of C57BL/6 mice with the *M. avium* complex and to evaluate the potential anti-infective role of a drug that could enhance the inflammatory reaction, such as RU-40 555.

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MATERIALS AND METHODS

Microorganism. *M. avium* complex strain MO-1, which was previously used in a macrophage model to determine the intracellular antimycobacterial activity of sparfloxacin, was used in all experiments (31). This strain was isolated from the blood of a patient with AIDS and was used after a single culture on Mycobacteria 7H11 agar (Difco Laboratories, Detroit, Mich.) supplemented with Middlebrook OADC enrichment (Difco). One flat transparent colony was picked and cultivated at 37°C in Middlebrook 7H9 broth (Difco) supplemented with Middlebrook ADC enrichment (Difco). The bacterial suspension was adjusted to a density of 1 mg/ml with a turbidimeter (Institut Pasteur Production), and aliquots were frozen at -80°C.

Animal model. Experiments were performed with 7-week-old normal female C57BL/6 mice from Iffa Credo, L'Arbresle, Rhône, France. Mice were infected with 1.5×10^7 viable bacteria that were injected intravenously (i.v.) into the retroorbital plexus.

In a preliminary study, mice were kept untreated after the

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inoculation to evaluate the long-term spontaneous evolution of infection. Four randomly selected mice were sacrificed on days 1, 4, 7, 14, and 21 and months 1, 2, 3, and 6 after inoculation.

Because a chronic infection could be established in C57BL/6 mice, this model was used for evaluation of the activities of antimicrobial agents.

Antimicrobial agents. Sparfloxacin (Rhône DPC Europe, Antony, Hauts-de-Seine, France) and ethambutol (Lederle Laboratories, Oullins, Rhône, France) were provided by the respective manufacturers. Sparfloxacin was dissolved in 0.1 N NaOH and diluted in phosphate-buffered saline. Ethambutol was diluted in sterile water.

Immunomodulator. RU-40 555 (Roussel-UCLAF) was provided by the manufacturer and was suspended in carboxymethyl cellulose.

Antimicrobial susceptibility testing. The MICs of sparfloxacin and ethambutol were determined by the agar macrodilution method. Serial twofold dilutions of each antimicrobial agent were incorporated into 7H11 agar medium plated in quadrant petri dishes. The inoculum was made from a 7-day-old culture in Dubos-Tween medium, adjusted to 1 mg (wet weight) per ml, and diluted to 10^{-3} and 10^{-5} . From each dilution, 0.05 ml was plated onto one quadrant. Every assay was performed in duplicate. Plates were incubated at 37°C, and colonies were counted after 14 days of culturing. The lowest concentration of drug that inhibited more than 99% of the bacterial population was considered to be the MIC.

Administration of drugs. Mice were divided into eight groups. The sparfloxacin group received sparfloxacin, 50 mg/kg of body once daily in 0.2 ml given subcutaneously (s.c.). The ethambutol group received ethambutol, 50 mg/kg once daily in 0.1 ml given s.c. The RU-40 555 group received RU-40 555, 100 mg/kg once daily in 0.4 ml given intraperitoneally. The sparfloxacin-ethambutol group, the sparfloxacin-RU-40 555 group, the ethambutol-RU-40 555 group, and the sparfloxacin-ethambutol-RU-40 555 group received the same regimen of each drug as those given to the groups administered a single drug. Control groups received saline.

Treatments were started on day 1 after the inoculation and were continued for 21 days.

Quantitation of mycobacteria in spleen and lung. Treated and control mice were sacrificed at days 4, 7, 14, and 21 of treatment, 24 h after the last injection of drug(s). Control mice were also sacrificed at day 1, before treatment. At least four animals were sacrificed at each time point. The spleen and the right lung of each mouse were removed aseptically, weighed, and homogenized in sterile water with a glass homogenizer. Serial 10-fold dilutions were plated onto 7H11 agar (Difco) supplemented with OADC enrichment (Difco). After 14 days at 37°C, colonies were counted, and the number of CFU per gram of tissue was calculated.

Statistical analysis. Results were analyzed by Student's *t* test.

RESULTS

In vitro susceptibility to antimicrobial agents. For strain MO-1 of the *M. avium* complex, the MIC of sparfloxacin was 0.5 µg/ml and the MIC of ethambutol was 8 µg/ml.

Long-term evolution of infection in untreated mice. As shown on Fig. 1, the inoculation of C57BL/6 mice produced a chronic infection that did not kill the animals. As already described for nonimmunocompromised mice (14, 15), a transient pulmonary clearance of bacteria by control mice was observed during the first 4 days. After a 4-week plateau,

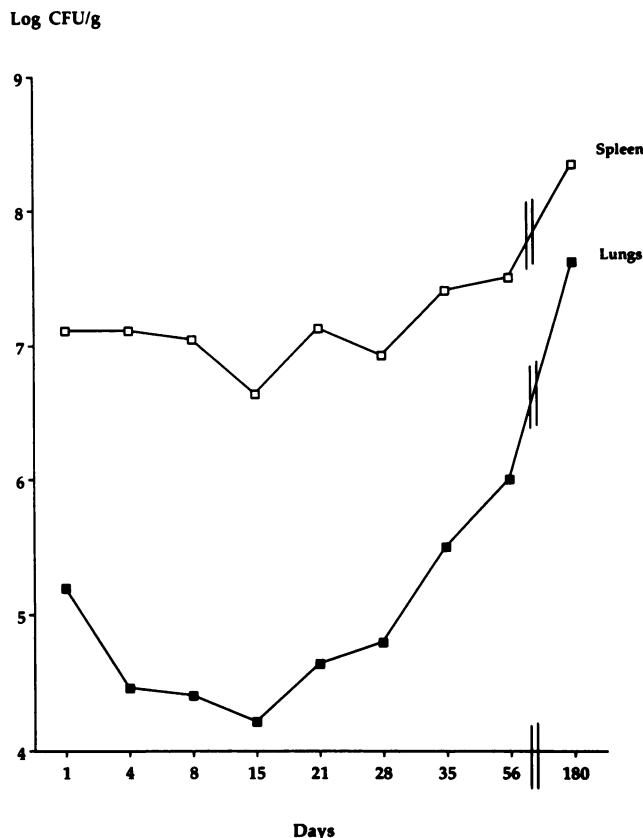


FIG. 1. Time course of CFU of the *M. avium* complex in the spleens and lungs of untreated C57BL/6 mice inoculated at day 0 with 1.5×10^7 CFU by intravenous injection. Spontaneous evolution during 6 months (days 1 to day 180).

the bacterial concentrations in the spleen and lung rose until the sixth month.

Effect of treatment on infection in mice. CFU counts in the spleen are shown in Fig. 2. Compared with control mice, sparfloxacin ($P < 0.001$), ethambutol ($P < 0.001$), or RU-40 555 ($P < 0.01$) decreased the CFU in the spleens of treated mice. The combinations of sparfloxacin plus RU-40 555 ($P < 0.001$) or sparfloxacin plus ethambutol ($P < 0.001$) were more effective than sparfloxacin alone in decreasing the CFU in the spleens. The combination of sparfloxacin plus RU-40 555 plus ethambutol ($P < 0.001$) was more effective than the two-drug combinations in decreasing the CFU in the spleens.

CFU counts in the lungs are shown in Fig. 3. Compared with control mice, sparfloxacin ($P < 0.001$) or ethambutol ($P < 0.001$) decreased the CFU in the lungs of treated mice. RU-40 555 alone did not decrease the CFU in the lungs. The combinations of sparfloxacin plus RU-40 555 or sparfloxacin plus ethambutol were as effective as sparfloxacin alone in decreasing the CFU in the lungs. The combination of sparfloxacin plus RU-40 555 plus ethambutol ($P < 0.001$) was more effective than the two-drug combinations in decreasing the CFU in the lungs.

DISCUSSION

The beige mouse model is usually used for in vivo studies of *M. avium* complex infections. Beige mice are mutants of C57BL/6 mice with decreased natural killer cell activity. In

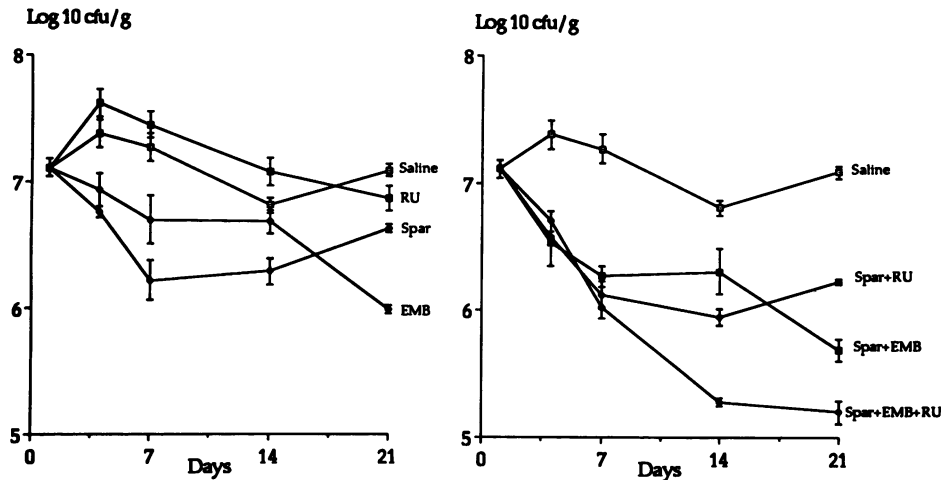


FIG. 2. Time course of CFU of the *M. avium* complex in the spleens of C57BL/6 mice treated with sparfloxacin (Spar; 50 mg/kg/day s.c.), ethambutol (EMB; 50 mg/kg/day s.c.), or RU-40 555 (RU; 100 mg/kg/day intraperitoneally). These drugs were used alone or in combination. Control mice were treated with saline.

beige mice, some selected strains of the *M. avium* complex may give a rapidly increasing level of infection and may cause the death of some animals (13, 15). In the present study, we showed that the CFU counts of the *M. avium* complex in organs of untreated wild-type C57BL/6 mice rose progressively, reaching a concentration greater than 10^8 CFU/g of spleen and greater than 10^7 CFU/g of lung after 6 months of infection, without the spontaneous clearance of bacteria that is observed in Swiss-Webster mice (13–15, 21). Because the death of animals is not an indispensable end point for the evaluation of drugs, our model with normal C57BL/6 mice is valid for the evaluation of antimicrobial agents.

In our study, sparfloxacin or ethambutol slightly decreased the CFU counts in the spleens and lungs of C57BL/6 mice infected with the *M. avium* complex. The efficacy of ethambutol was more pronounced after 2 weeks of treatment, as has already been described for the treatment of experimental tuberculosis (16). Our results confirm the moderate *in vivo* efficacy of sparfloxacin used as monotherapy

that was reported in the model of the beige mouse infected with the *M. avium* complex (22). Kolonoski et al. (22) used sparfloxacin at a dose of 25 mg/kg/day. The dose of sparfloxacin that we used (50 mg/kg/day) provides in mice a peak concentration in serum of 3 μ g/ml, when measured by high-pressure liquid chromatography (1, 1a). Thus, the peak level of sparfloxacin in serum was higher than the MIC for the strain used (0.5 μ g/ml). Rastogi et al. (32) reported that the *in vitro* susceptibility of the *M. avium* complex to fluoroquinolones is enhanced by inhibitors of bacterial cell envelope synthesis, such as ethambutol. Ethambutol (125 mg/kg/day) did not offer additional benefit to the rifabutin plus clofazimine combination in one study of infection in beige mice (15), whereas in another study, ethambutol (25 mg/kg per day), with a peak concentration in serum of 4 μ g/ml after 1 month of treatment, enhanced the activity of rifabutin in the spleen of TxCd4⁻ mice infected with the *M. avium* complex (13). TxCd4⁻ mice are C57BL/6 mice thymectomized at 4 weeks of age and treated intravenously with anti-CD4 antibody. In the present study, in which we

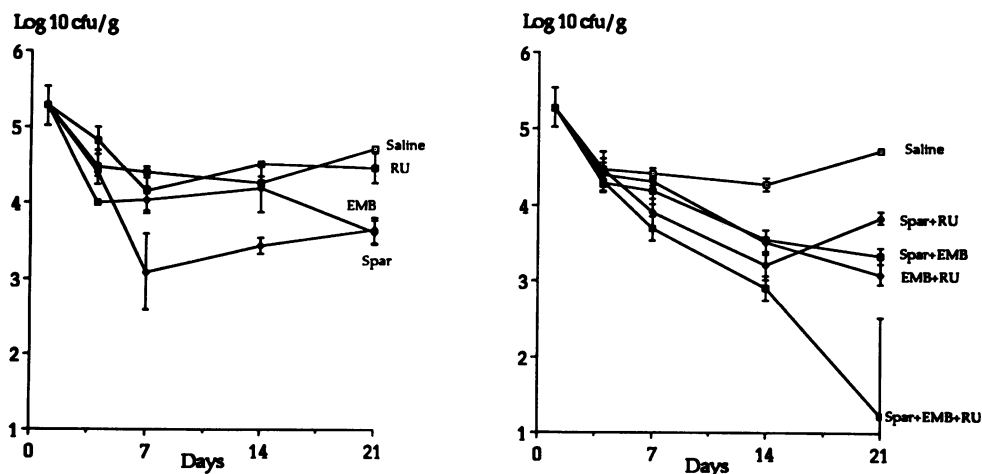


FIG. 3. Time course of CFU of the *M. avium* complex in the lungs of C57BL/6 mice treated with sparfloxacin (Spar; 50 mg/kg/day s.c.), ethambutol (EMB; 50 mg/kg/day s.c.), or RU-40 555 (RU; 100 mg/kg/day intraperitoneally). These drugs were used alone or in combination. Control mice were treated with saline.

used an intermediate dose of ethambutol (50 mg/kg/day), the sparfloxacin plus ethambutol combination was more effective than sparfloxacin alone in spleens but not in lungs. The effect of this antibiotic combination, however, was not frankly bactericidal, since the decrease in CFU counts was less than 1.5 log units in the spleens and lungs. The potential use of biologic response modifiers such as interleukin 2 or tumor necrosis factor for the treatment of *M. avium* complex infections in C57BL/6 mice has been reported previously (3, 4). Biologic response modifiers that increase the inflammatory reaction could enhance the in vivo efficacies of antimicrobial agents. Mifepristone can enhance the inflammatory reaction in animals by inhibiting the cortisol receptors (5, 8, 10, 12, 17, 18, 23–25, 28, 35). The mifepristone antagonization of the induction of latent Epstein-Barr virus infection by dexamethasone is the only anti-infectious activity of mifepristone that has been studied (7). Because mifepristone is mainly a progesterone receptor inhibitor, in the present experiments we used a very closely related molecule, RU-40555, whose inhibiting effect is mainly targeted against cortisol receptors. In opposition to mifepristone, RU-40555 has never been used in humans. Our results showed that RU-40555 alone slightly decreases the CFU counts in spleens but not in the lungs. More interestingly, compared with the controls, the addition of RU-40555 to the sparfloxacin plus ethambutol combination decreased the CFU counts by 2 log units in the spleens and by 3 log units in the lungs. In conclusion, the new fluoroquinolone sparfloxacin seems of interest for the treatment of *M. avium* complex infections, and RU-40555, which enhances the inflammatory reaction by inhibiting the cortisol receptors, could increase the immune reaction. These drugs should be studied further.

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