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NOTES

Rapid In Vivo Screening of Experimental Drugs for Tuberculosis Using Gamma Interferon Gene-Disrupted Mice

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We have developed a rapid new in vivo method for screening experimental drugs for their activity against *Mycobacterium tuberculosis* by using the gamma interferon gene-disrupted (GKO) C57BL/6 mouse. Due to the rapid growth of the infection, statistical differences indicating positive efficacy of active compounds can be seen after only 8 days of treatment. To validate this model, several fluoroquinolones, including ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin, were tested in parallel.

Therapy for tuberculosis is arduous due to its long duration and multidrug regimens. Therefore, there is an increasing demand for the development of new compounds and screening of existing compound libraries in order to treat tuberculosis. As part of the overall research effort at the National Institutes of Health, the National Institute of Allergy and Infectious Diseases has established a screening program at several institutions to efficiently screen large numbers of compounds for possible activity against *Mycobacterium tuberculosis*. The compounds are received as part of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), which provides no-cost screening of compounds from industrial and academic sources. To date, over 60,000 compounds have been provided to the program. About 0.3% of the compounds which performed well during the extensive initial testing in in vitro assays are subsequently tested in mouse models (more information on the program and assays used can be found at www.taacf.org). In order to screen a larger number of compounds against tuberculosis in animals, we have developed a rapid new in vivo method.

Mouse models have been extensively used for preclinical assessment of experimental compounds against tuberculosis (2, 6, 7, 9, 10). In intravenous infection models, Swiss outbred mice are infected with *M. tuberculosis* with $\sim 5 \times 10^6$ bacteria and treated once daily with various dosages for 28 days starting on the day after infection (4, 11, 12, 14, 15). More recently, we have developed a second model in which inbred C57BL/6 mice are infected with 50 to 100 CFU by low-dose aerosol and treated daily for 35 to 45 days starting 20 days postinfection when the bacterial load reached its peak (13). In both models, the drug regimen lasts 1 to 1.5 months of daily therapy. In an attempt to shorten this process, we have developed a novel short-term in vivo model for the rapid screening of experimental compounds.

This model uses the highly susceptible gamma interferon gene-disrupted (GKO) C57BL/6 mouse (3, 5, 8, 18) and was performed as follows. Eight- to ten-week-old female specific-pathogen-free C57BL/6-Ifngtm1ts (GKO) mice (Jackson Laboratories, Bar Harbor, Maine) were exposed to a low-dose aerosol infection with *M. tuberculosis* in a Glas-Col inhalation exposure system as previously described (13). The virulent *M. tuberculosis* strain Erdman (TMCC 107) has been used as the standard strain for drug testing in animal models in this laboratory (13). One day postinfection, three mice were sacrificed to verify bacterial uptake of 50 to 100 CFU per mouse. Every treatment group consisted of five mice for every following time point. Treatment was started 18 days after infection and lasted up to 28 days postinfection. One control group of infected mice was sacrificed at the start of treatment. A second group of infected but untreated mice was sacrificed after the cessation of treatment. The quinolones were suspended in 5% ethanol for treatment, and isoniazid (INH) was dissolved in distilled water. All compounds were administered by oral gavage in eight treatments for 5 days/week. The quinolones were given at 100, 200, and 400 mg/kg of body weight, while INH was administered at 25 mg/kg. After completion of therapy, the mice were sacrificed by CO_2 inhalation. Spleens and left lungs were aseptically removed and disrupted in a tissue homogenizer. The number of viable organisms was determined by serial dilution of the homogenates on nutrient Middlebrook 7H11 agar plates (GIBCO BRL, Gaithersburg, Md.). The plates were incubated at 37°C in ambient air for 4 weeks prior to the counting of viable *M. tuberculosis* colonies (CFU). The viable counts were converted to logarithms, which were then evaluated by multiple-comparison analysis of variance by a one-way Dunnett test. Differences were considered significant at the 95% level of confidence.

Initial experiments were designed to establish optimal conditions for drug testing in the GKO model in order to obtain reproducible results with minimal standard deviations. The age of the mice, the time of the start of chemotherapy, and its duration were found to be important parameters. Reproducibility of the GKO model was demonstrated by four independent experiments using INH at 25 mg/kg as a control com-

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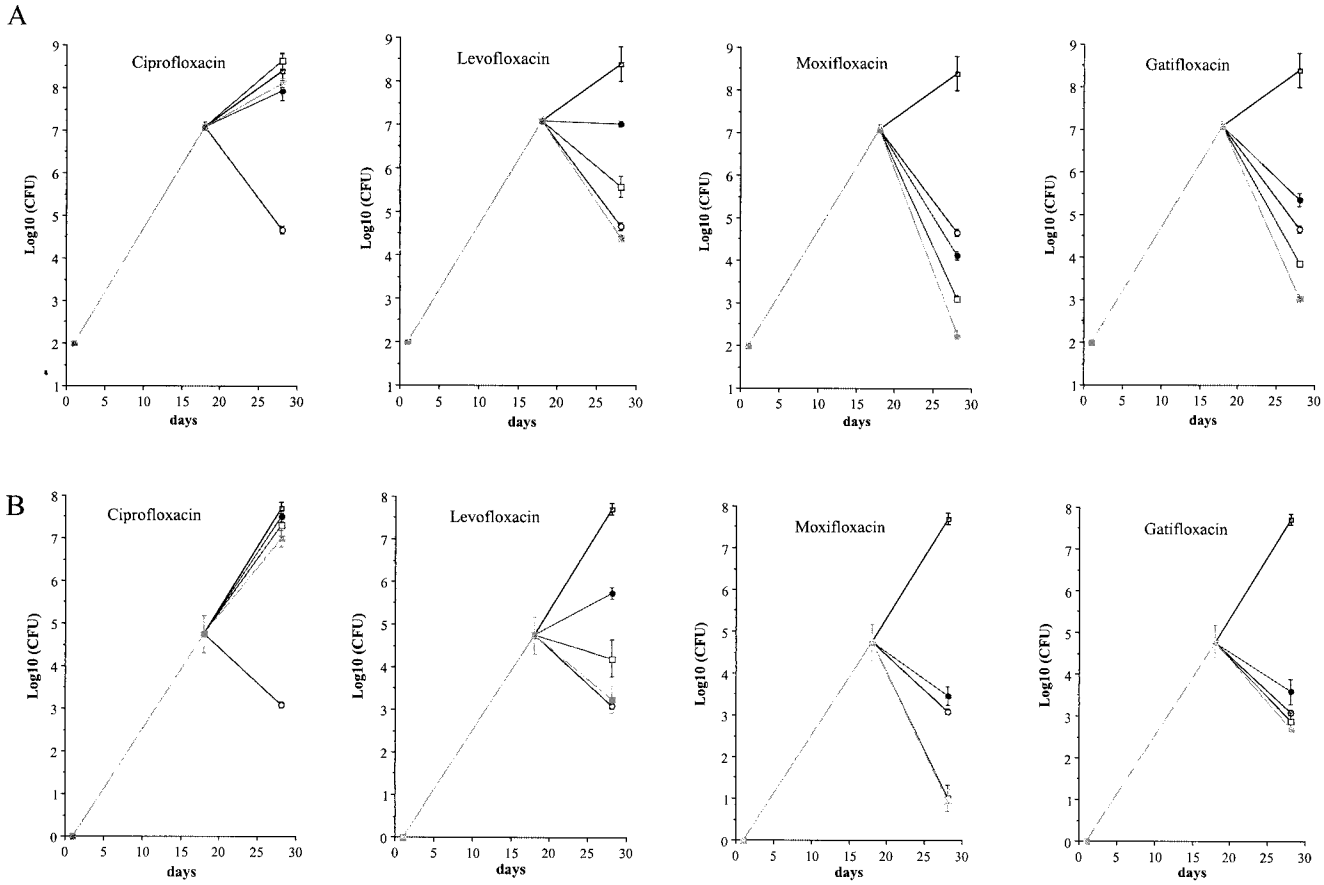

FIG. 1. Numbers of viable *M. tuberculosis* organisms in lungs (A) and spleens (B) of infected mice after once-daily treatment with fluoroquinolones at 100 (●), 200 (□), and 400 mg/kg (■) or INH at 25 mg/kg (○) for 8 treatment days. Infected untreated mice from control groups (⊡) were sacrificed at the start and end of the treatment period. The results are means ± standard deviations of data from five mice per group. Results were statistically significant relative to results for the untreated controls for levofloxacin, moxifloxacin, gatifloxacin, and INH in spleens and lungs for all concentrations tested ($P < 0.01$); ciprofloxacin showed a statistically significant result only in spleen at 400 mg/kg ($P < 0.01$).

pound. INH gave reductions in bacterial loads of 3 to 4 logs in lungs and 2.5 to 4.5 logs in spleens relative to those of the untreated controls (detailed data: reductions of 3.69, 2.92, 3.27, and 3.48 $\log_{10}$ CFU in lungs and 4.62, 2.62, 4.23, and 4.28 $\log_{10}$ CFU in spleens). In order to validate this model, several quinolones, including ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin, were studied side by side. At the start of treatment, the CFU in the lungs of the mice reached 7.1 logs, and the number increased to 8.4 logs in the untreated control mice at the end of the experiment. At all concentrations tested, ciprofloxacin did not significantly reduce the bacterial load in lungs relative to that of the untreated controls ($P > 0.05$), and it showed a statistical significant reduction in the bacterial number in the spleen only at 400 mg/kg ($P < 0.01$). All other quinolones tested were shown to be effective against the *M. tuberculosis* infection, each in a dose-dependent manner. Statistically significant reductions in bacterial counts in lungs were observed for levofloxacin, moxifloxacin, and gatifloxacin for all concentrations tested ($P < 0.01$) (Fig. 1). In the spleens of the GKO mice, a similar trend was observed, with a statistically significant reduction in viable bacteria for the same treatment groups ($P < 0.01$) (Fig. 1). The treatment with moxifloxacin resulted in the strongest bactericidal effect, with a reduction of the bacterial load in the lungs of more than 6 logs at 400 mg/kg. All of the tested concentrations of moxifloxacin were more active than INH at 25 mg/kg ($P < 0.01$) (Fig. 1). Gatifloxacin was slightly less active than moxifloxacin in this model. Gatifloxacin at 100 mg/kg showed activity similar ($P > 0.05$) to that of INH at 25 mg/kg, while higher concentrations of gatifloxacin (200 and 400 mg/kg) were significantly more active ($P < 0.01$) (Fig. 1). Levofloxacin at 400 mg/kg had antibacterial efficacies in spleens and lungs similar to that of INH at 25 mg/kg ($P > 0.05$) (Fig. 1).

The efficacy of experimental compounds against *M. tuberculosis* is usually demonstrated through a series of in vitro assays followed by further testing of the most promising compounds in animal models. However, the ability of the in vitro assessments and the use of intracellular assays to accurately predict clinical efficacy is limited (1). On the other hand, animal models have accurately predicted the clinical efficacy of antituberculosis therapy and have proven to be essential in the evaluation of new drug regimens (2, 6, 7, 9). For the animal models currently used for drug screens, there are several limiting factors, including the fact that they are time-consuming and ex-

pensive and often require a substantial amount of compound. We describe here a novel short-term mouse model which requires only 8 days of treatment and therefore allows for rapid testing of compounds against *M. tuberculosis*. This new drug screening model uses the GKO C57BL/6 mouse, which is highly susceptible to *M. tuberculosis* infection. Histopathology and immune responses in the GKO mice after infection with *M. tuberculosis* are well documented by this laboratory and others (3, 5, 8, 18). GKO mice were infected via low-dose aerosol, and after 18 days, drug treatment was started for 8 treatment days. Due to the rapid growth of the bacteria in the GKO mice in the untreated controls, positive drug activity could be seen after only a short period of treatment. Reproducibility of the GKO model was demonstrated by four independent experiments using INH as a control. As an example of the use of this model, several fluoroquinolones, including ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin, were tested in parallel. A dose of 400 mg/kg of moxifloxacin in mice (17) was recently shown to have an area under the concentration-time curve equivalent to that of the human dosage of 7.5 mg/kg, or 400 mg per day based on pharmacokinetic analysis (16, 19). Therefore, for all fluoroquinolones, a range of concentrations up to 400 mg/kg was tested. Gatifloxacin and moxifloxacin at 400 mg/kg were the most active in this model, with large reductions in the bacterial load (5.4 and 6.1 logs). Levofloxacin at 400 mg/kg reduced the bacterial load by 3.5 logs, which was similar to the result of INH at 25 mg/kg. Prior testing by others of the clinically available fluoroquinolones yielded results against *M. tuberculosis* similar to the data described here (11, 12, 15).

When the activity of a single compound in an in vivo model is studied, the sensitivity of the GKO model compares favorably to that of most mouse models currently used. In our previously described aerosol infection model using the wild-type C57BL/6, the mice are infected with low-dose aerosol, and treatment is started 21 days postinfection and lasts for 30 to 45 days (13). If we compare the activity of INH given at 25 mg/kg in both models, we see a far more pronounced effect in the GKO mice due to the uncontrollable bacterial growth in this mouse strain. In wild-type C57BL/6 mice, INH reduces the bacterial load, with a reduction of 1 to 1.5 log CFU in lungs after 30 or 45 days of treatment (data obtained under NIH contract), whereas in the GKO model, a reduction of more than 3 logs is observed in lungs relative to the untreated controls after only 8 days of treatment (obtained in four independent experiments). Moreover, due to the short duration of treatment in the GKO model, the variance in bacterial numbers from mice within a treatment group remains very small. In a GKO model with only five mice per treatment group, a reduction in CFU of 0.28 log was statistically significant, whereas for the standard model, statistical significance was achieved only at a reduction of 0.6 log with the same number of animals (after statistical analysis of three experiments each) (data not shown). In addition, the GKO model is cost effective and less labor intensive due to its short duration, and it requires far less compound than other currently used mouse models. The GKO model does not, however, replace mouse models using long-term treatment regimens, since important features, such as sterilizing activity of a clinical compound, relapse of infection, and toxicity when administered long term, cannot be studied in this model. Therefore, we propose this model as a rapid way to test the activity of single experimental compounds against *M. tuberculosis* in a first-line in vivo screen.

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