ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1995, p. 750–753 Vol. 39, No. 3
0066-4804/95/$04.00+0

Comparison of Activities of Fluoroquinolones in Murine Macrophages Infected with *Mycobacterium tuberculosis*

PAMELA S. SKINNER, SYNTHIA K. FURNEY, DORIS A. KLEINERT, AND IAN M. ORME*

Mycobacteria Research Laboratories, Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

Received 29 August 1994/Returned for modification 10 November 1994/Accepted 2 January 1995

In this study the compounds levofloxacin and sparfloxacin, as well as three experimental compounds (AMQ2, AMQ4, and AMQ5), were compared with isoniazid and rifabutin in terms of their capacity to inhibit the intracellular growth of the drug-susceptible *Mycobacterium tuberculosis* strain Erdman and the isoniazid-resistant *katG* gene-negative strain 24 within monolayers of mouse bone marrow-derived macrophages. Both levofloxacin and sparfloxacin, as well as compound AMQ4, had substantial activity in this physiologically relevant model, further confirming the potential usefulness of this class of compounds in the therapy of tuberculosis.

Disease caused by *Mycobacterium tuberculosis* is becoming increasingly difficult to treat as a result of the large number of clinical isolates exhibiting some degree of resistance to conventional antimycobacterial drugs. Accordingly, the need for new agents with which to treat this disease is becoming imperative (1, 3, 7, 9, 17).

One class of compounds that has shown some promise is the fluoroquinolones. Of these, ofloxacin was the first to be used to treat tuberculosis (8, 21, 26, 28). More recently, other active compounds in this class, including the difluorinated quinolone sparfloxacin and levofloxacin (the optically active *l*-isomer of ofloxacin) have also been investigated (2, 4, 5, 10, 13, 14, 16, 18, 22, 23). This class of compounds has the useful property of concentration within host cells (6, 15, 27), including macrophages, where they are needed to give rise to mycobacterial destruction.

Because fluoroquinolones exhibit similar pharmacokinetics and tissue penetration in humans and mice, we reasoned that mouse macrophages might provide a useful screening model for these compounds, as has been convincingly demonstrated by Rastogi and his colleagues using isolates of *Mycobacterium avium* and *M. tuberculosis* (21, 23). In the present study, the capacities of sparfloxacin and levofloxacin to inhibit the growth of the virulent *M. tuberculosis* strain Erdman and the *katG* gene-negative isoniazid-resistant isolate strain 24 (29) in the murine bone marrow-derived macrophage model were determined and compared with the activities of other experimental compounds.

A panel of *M. tuberculosis* clinical isolates was collected from various sources throughout the world and is described in detail elsewhere (19). Each isolate was grown to mid-log phase in either glycerol alanine salts medium or Proskauer-Beck medium and stored in ampoules frozen at −70°C. Drug susceptibility profiles (MICs) were determined by a broth dilution method in which tissue culture wells were seeded with 10^5 bacilli in nutrient 7H9 broth containing a range of concentrations of drugs, with the capacity of the isolate to grow under such conditions determined visually 3 to 4 weeks later.

Bone marrow-derived macrophages were obtained from specific-pathogen-free C57BL/6 female mice purchased from the Jackson Laboratories, Bar Harbor, Maine. Mice were euthanized by exposure to CO_2, and femur bones were dissected out. Bones were trimmed at each end, and marrow was flushed out with a 26-gauge needle with 5.0 ml of Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum, 10% L-929 fibroblast conditioned supernatant (note: this medium contains colony-stimulating factors, but at con-

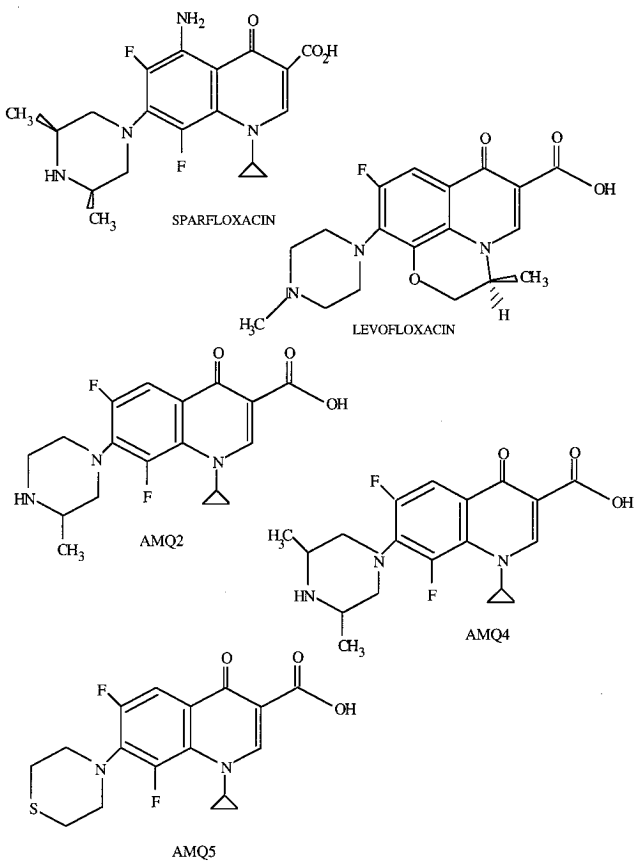

FIG. 1. Structures of fluoroquinolones used in this study.

* Corresponding author. Mailing address: Dept. of Microbiology, Colorado State University, Fort Collins, CO 80523. Phone: (303) 491-5777. Fax: (303) 491-1815.

TABLE 1. MICs for the panel of *M. tuberculosis* isolates

Strain	MIC (μg/ml) of[a]:									
	INH	ETHAM	RBT	RIF	CIPRO	SPAR	LEVO	AMQ2	AMQ4	AMQ5
Erdman	≤0.125	4	≤0.125	≤0.125	0.5	0.125	0.25	≤0.125	0.25	0.5
CSU11	2	4	≤0.125	≤0.125	0.25	≤0.125	0.25	0.25	≤0.125	2
CSU12	0.5	2	≤0.125	≤0.125	0.5	≤0.125	0.25	≤0.125	≤0.125	0.5
CSU15	4	4	≤0.125	0.25	0.25	≤0.125	≤0.125	≤0.125	≤0.125	0.25
CSU18	4	2	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	0.5
CSU20	≤0.125	2	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	0.25	1
CSU21	4	>8	8	>8	0.25	0.25	0.5	0.25	≤0.125	2
CSU22	>8	8	4	>8	0.5	≤0.125	0.5	0.5	≤0.125	1
CSU23	≤0.125	2	≤0.125	≤0.125	≤0.125	≤0.125	0.25	≤0.125	≤0.125	1
CSU25	≤0.125	2	≤0.125	≤0.125	0.25	≤0.125	≤0.125	0.25	≤0.125	0.5
CSU26	≤0.125	4	≤0.125	≤0.125	≤0.125	≤0.125	0.5	0.25	0.25	4
CSU27	≤0.125	2	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	2
CSU28	≤0.125	4	≤0.125	≤0.125	0.5	≤0.125	0.25	0.25	≤0.125	2
Strain 24	>8	8	≤0.125	≤0.125	0.5	0.125	0.125	0.25	0.25	1

[a] INH, isoniazid; ETHAM, ethambutol; RBT, rifabutin; RIF, rifampin; CIPRO, ciprofloxacin; SPAR, sparfloxacin; LEVO, levofloxacin.

centrations much lower than that needed to influence the growth of *M. tuberculosis* [20]), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer, nonessential amino acids, L-glutamine, and antibiotics. Cell suspensions were then washed twice and plated in 24-well tissue culture plates at a concentration of 10^5 cells per well in supplemented DMEM. Monolayers were then incubated at 37°C in 5% CO_2 with the medium changed every 3 days.

Macrophages were used 8 to 9 days later; they were infected with a 1.0-ml suspension containing 10^5 *M. tuberculosis* cells suspended in antibiotic-free DMEM and incubated as described above for 4 to 5 h. Wells were then thoroughly washed to remove extracellular bacteria and replaced with 1.0 ml of antibiotic-free DMEM containing different concentrations of the compound being tested. Each concentration of drug was run in triplicate wells. Control wells contained 1.0 ml of antibiotic-free DMEM without any drug. Wells were periodically observed under a microscope to check for cell viability or

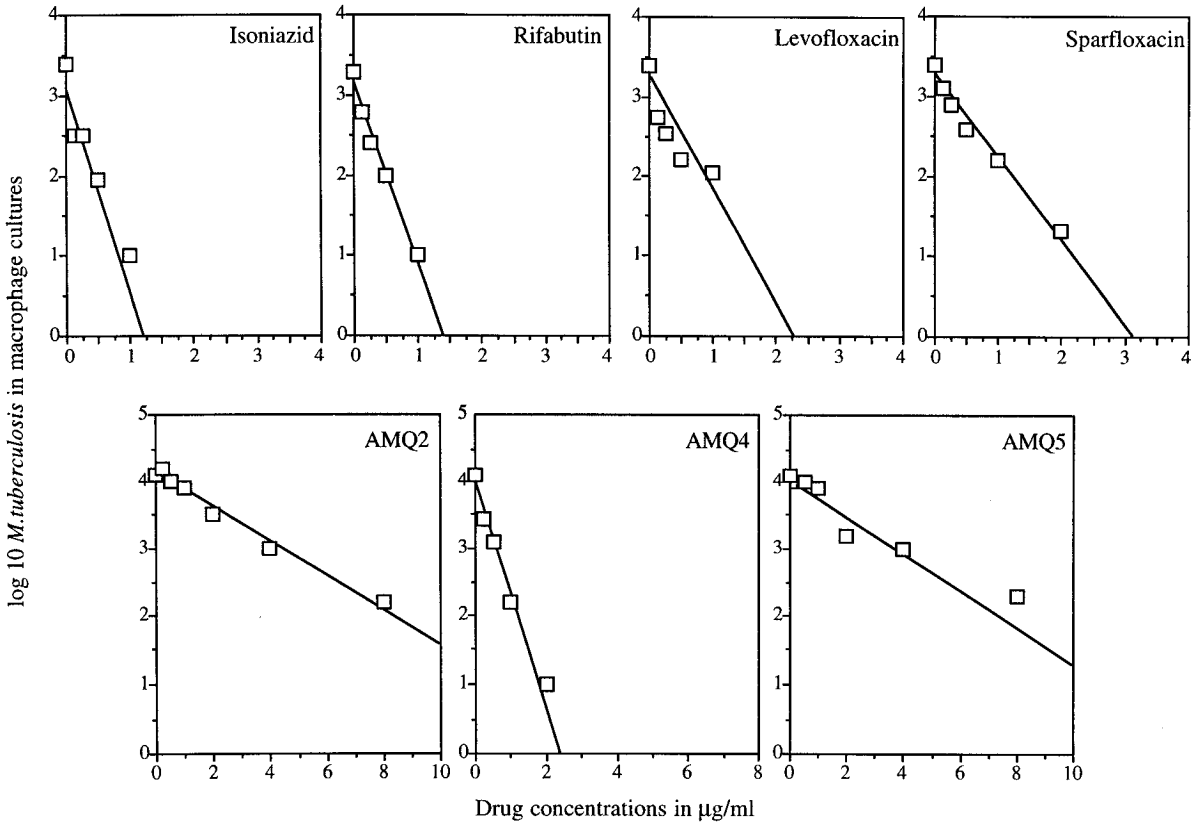

FIG. 2. Capacities of various compounds to inhibit the intracellular growth of *M. tuberculosis* Erdman in murine bone marrow-derived macrophage monolayers. Increasing concentrations of drug were added to triplicate cultures on day 0, and bacterial growth was assessed on day 8. Data are expressed as mean bacterial numbers; the standard errors of the mean are omitted and did not exceed 0.3.

detachment (none of the compounds used in this study had any discernible toxic effects). After 8 days of incubation at 37°C in 5% CO_2, each well was gently washed, and monolayers were then lysed with 0.1% saponin (Sigma, St. Louis, Mo.) dissolved in sterile water. Lysates were serially diluted in sterile saline and plated on nutrient 7H11 agar (Difco, Detroit, Mich.). Bacterial colony formation was enumerated after incubation of plates for 10 to 14 days at 37°C in humidified air. Data were expressed as the $\log_{10}$ mean numbers of bacteria in the triplicate cultures for each drug concentration; this information was entered into a simple computer graphics program (Cricket Graph; Cricket Software, Malvern, Pa.) where a curve fit line was established to define antimicrobial activity. The equation of this line was used to calculate the bactericidal concentration of drug giving a 2 log (99%) reduction in bacterial numbers (BC_{99}), as previously described (25).

Isoniazid was purchased from Sigma. Ethambutol was obtained from Lederle Laboratories, Pearl River, N.Y. Rifampin and rifabutin were obtained from Pharmacia Adria, Dublin, Ohio. Ciprofloxacin was obtained from Miles Laboratories, West Haven, Conn. Sparfloxacin was obtained from Parke-Davis, Ann Arbor, Mich. Levofloxacin was obtained from the R. W. Johnson Research Institute, Raritan N.J. Experimental AMQ compounds were kindly provided by Gilles Klopman, Case Western Reserve University, Cleveland, Ohio. Structures of these last compounds are shown in Fig. 1; synthesis and other characteristics of these compounds are described elsewhere (11, 12).

The MICs for the panel of *M. tuberculosis* isolates are shown in Table 1. The six fluoroquinolones tested all had consistently good activity, including against isolates with resistance to isoniazid and/or rifampin.

The experimental compounds AMQ2, AMQ4, and AMQ5, as well as sparfloxacin and levofloxacin, were then titrated in comparison with isoniazid and rifabutin in the mouse macrophage model. All seven compounds inhibited the growth of *M. tuberculosis* Erdman in this model (Fig. 2), although compounds AMQ2 and AMQ5 had considerably less activity than the others.

These experiments were then repeated, with sparfloxacin and levofloxacin titrated against the *katG* gene-negative isoniazid-resistant strain 24 (29). (This isolate has virulence in mice similar to that of *M. tuberculosis* Erdman [24] and was included in these studies because the majority of drug-resistant isolates now being encountered are isoniazid resistant [3].) As predicted, isoniazid was inactive, whereas both fluoroquinolones inhibited bacterial growth (Fig. 3).

By taking the data from the macrophage assays in which *M. tuberculosis* Erdman was used, we also estimated BC_{99}s for the test compounds (BC_{99} is an arbitary measure of potential therapeutic activity, as we have recently proposed [25]). The values obtained were as follows: isoniazid, 0.71 μg/ml; rifabutin, 0.91 μg/ml; levofloxacin, 1.45 μg/ml; sparfloxacin, 1.81 μg/ml; AMQ2, 8.02 μg/ml; AMQ4, 1.21 μg/ml; and AMQ5, 7.81 μg/ml. When tested against strain 24, values of 0.16 μg for rifabutin, 1.72 μg for levofloxacin, and 0.62 μg for sparfloxacin were determined. These data suggest that levofloxacin and sparfloxacin should have a real therapeutic effect in vivo, given that their BC_{99}s should be achievable in plasma on the basis of previous pharmacokinetic information (16, 22). The inference made here is based on results from two strains, however, and obviously a larger number of isolates would need to be examined in this assay.

A significant number of clinical isolates are resistant to isoniazid but remain susceptible to rifamycins and other classes of compounds (3, 9, 17). Given the noted ability of both rifabutin and the new fluoroquinolones to concentrate within the infected macrophage, combinations of these compounds might be useful and should be evaluated in animal models. In situations in which the isolate is also resistant to rifamycin, combinations of fluoroquinolones with pyrazinamide and streptomycin could be helpful, as a report by Lalande and colleagues (13) has recently demonstrated.

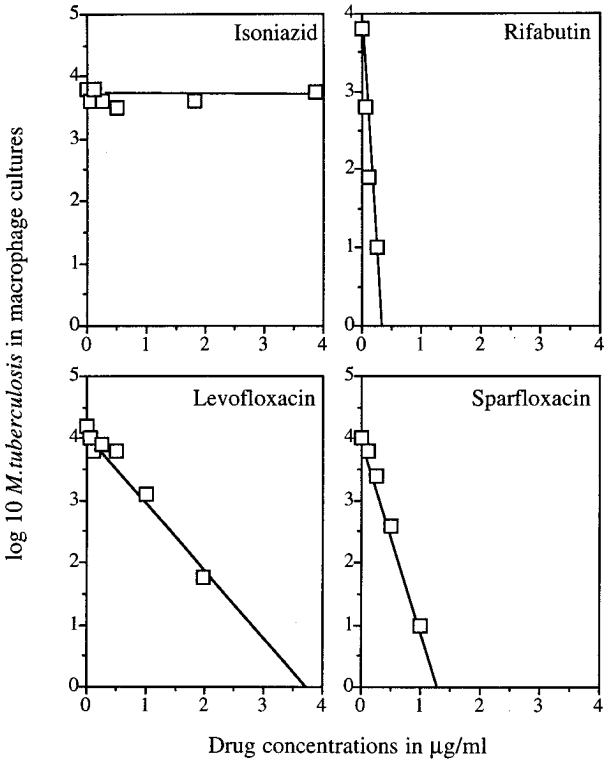

FIG. 3. Capacities of various compounds to inhibit the intracellular growth of the isoniazid-resistant *M. tuberculosis* strain 24 in murine bone marrow-derived macrophage monolayers. Increasing concentrations of drug were added to triplicate cultures on day 0, and bacterial growth was assessed on day 8. Data are expressed as mean bacterial numbers; the standard errors of the mean are omitted and did not exceed 0.3.

This study was supported by grant AI-30189 from the NIH and by a grant from Pharmacia Adria.

We thank Gilles Klopman for helpful advice and Douglas Young for providing strain 24.

REFERENCES

1. **Bloom, B. R., and C. J. L. Murray.** 1992. Tuberculosis: commentary on a reemergent killer. Science **257:**1055–1066.
2. **Chu, D. T. W., and P. B. Fernandes.** 1989. Structure-activity relationships of the fluoroquinolones. Antimicrob. Agents Chemother. **33:**131–135.
3. **Ellner, J. J., A. R. Hinman, S. W. Dooley, M. A. Fischl, K. A. Sepkowitz, M. J. Goldberger, T. M. Shinnick, M. D. Iseman, and W. R. Jacobs.** 1993. Tuberculosis symposium: emerging problems and promise. J. Infect. Dis. **168:**537–551.
4. **Franzblau, S. G., M. L. R. Parrilla, and G. P. Chan.** 1993. Sparfloxacin is more bactericidal than ofloxacin against *Mycobacterium leprae* in mice. Int. J. Lepr. **61:**66–69.
5. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.
6. **Gerding, D. N., and J. A. Hill.** 1989. Tissue penetration of the new quinolones in humans. Rev. Infect. Dis. **11:**S1046–S1058.
7. **Grosset, J.** 1989. Present status of chemotherapy for tuberculosis. Rev. Infect. Dis. **11:**S347–S352.
8. **Heifets, L. B., and P. J. Lindholm-Levy.** 1987. Bacteriostatic and bactericidal activities of ciprofloxacin and ofloxacin against *Mycobacterium tuberculosis*

and *Mycobacterium avium* complex. Tubercle **68:**267–276.
9. **Iseman, M. D.** 1993. Treatment of multi-drug resistant tuberculosis. N. Engl. J. Med. **329:**784–791.
10. **Ji, B., C. Truffot-Pernot, and J. Grosset.** 1991. In vitro and in vivo activities of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis*. Tubercle **72:** 181–186.
11. **Klopman, G., J. Li, S. Wang, A. J. Pearson, K. Chang, M. R. Jacobs, S. Bajaksouzian, and J. J. Ellner.** 1994. In vitro anti-*Mycobacterium avium* activities of quinolones: predicted active structures and mechanistic considerations. Antimicrob. Agents Chemother. **38:**1794–1802.
12. **Klopman, G., S. Wang, M. R. Jacobs, S. Bajaksouzian, K. Edmonds, and J. J. Ellner.** 1993. Anti-*Mycobacterium avium* activity of quinolones: in vitro activities. Antimicrob. Agents Chemother. **37:**1799–1806.
13. **Lalande, V., C. Truffot-Pernot, A. Paccaly-Moulin, J. Grosset, and B. Ji.** 1993. Powerful bactericidal activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. Antimicrob. Agents Chemother. **37:**407–413.
14. **Leysen, D. C., A. Haemers, and S. R. Pattyn.** 1989. Mycobacteria and the new quinolones. Antimicrob. Agents Chemother. **33:**1–5.
15. **Lockley, M. R., R. Wise, and Y. Dent.** 1984. The pharmacokinetics and tissue peneration of ofloxacin. J. Antimicrob. Chemother. **14:**647–652.
16. **Mor, N., J. Vanderkolk, and L. Heifets.** 1994. Inhibitory and bactericidal activities of levofloxacin against *Mycobacterium tuberculosis* in vitro and in human macrophages. Antimicrob. Agents Chemother. **38:**1161–1164.
17. **Murray, C. J. L., K. Styblo, and A. Rouillon.** 1990. Tuberculosis in developing countries: burden, intervention, and cost. Bull. Int. Union Tuberculosis Lung Dis. **65:**2–19.
18. **Nakamura, S., N. Kurobe, T. Ohue, M. Hashimoto, and M. Shimizu.** 1990. Pharmacokinetics of a novel quinolone, AT-4140, in animals. Antimicrob. Agents Chemother. **34:**89–93.
19. **Ordway, D. J., M. G. Sonnenberg, S. A. Donahue, J. T. Belisle, and I. M. Orme.** 1995. Drug-resistant strains of *Mycobacterium tuberculosis* exhibit a range of virulence for mice. Infect. Immun. **63:**741–743.
20. **Orme, I. M.** Unpublished data.
21. **Rastogi, N., and M. C. Blom-Potar.** 1990. Intracellular bactericidal activity of ciprofloxacin and ofloxacin against *Mycobacterium tuberculosis* H37Rv multiplying in the J774 macrophage cell line. Zentralbl. Bakteriol. **273:**195–199.
22. **Rastogi, N., and K. S. Goh.** 1991. In vitro activity of the new difluorinated quinolone sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* compared with activities of ofloxacin and ciprofloxacin. Antimicrob. Agents Chemother. **35:**1933–1936.
23. **Rastogi, N., V. Labrousse, K. S. Goh, and J. P. Carvalho de Souza.** 1991. Antimycobacterial spectrum of sparfloxacin and its activities alone and in association with other drugs against *Mycobacterium avium* complex growing extracellularly and intracellularly in murine and human macrophages. Antimicrob. Agents Chemother. **35:**2473–2480.
24. **Roberts, A. D., and I. M. Orme.** Unpublished data.
25. **Skinner, P. S., S. K. Furney, M. R. Jacobs, G. Klopman, J. J. Ellner, and I. M. Orme.** 1994. A bone marrow-derived murine macrophage model for evaluating efficacy of antimycobacterial drugs under relevant physiological conditions. Antimicrob. Agents Chemother. **38:**2557–2563.
26. **Tsukamura, M., E. Nakamura, S. Yoshii, and H. Amano.** 1985. Therapeutic effect of a new antibacterial substance ofloxacin (DL-8280) on pulmonary tuberculosis. Am. Rev. Respir. Dis. **131:**352–356.
27. **Van der Auwera, P., T. Matsumoto, and M. Husson.** 1988. Intraphagocytic penetration of antibiotics. J. Antimicrob. Chemother. **22:**185–192.
28. **Young, L. S., G. W. Berlin, and C. B. Inderlied.** 1987. Activity of ciprofloxacin and other fluorinated quinolones against mycobacteria. Am. J. Med. **82**(Suppl. 4A)**:**23–26.
29. **Zhang, Y., B. Heym, B. Allen, D. Young, and S. Cole.** 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature (London) **358:**591–593.