Therapy of Multidrug-Resistant Tuberculosis: Lessons from Studies with Mice

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The activities of antituberculosis agents were evaluated in a murine tuberculosis model using a drug-resistant isolate. A multidrug-resistant clinical isolate from a recent outbreak of tuberculosis in the New York State correctional system was used for infection. Approximately 10^7 viable *Mycobacterium tuberculosis* ATCC 49967 (strain CNL) organisms were given intravenously to 4-week-old female outbred mice. Treatment was started 1 day after infection and given for 4 weeks. Spleens and lungs were homogenized, and viable cell counts were determined. Statistical analysis indicated that ethionamide, sparfloxacin, ofloxacin, capreomycin, clarithromycin, and clofazimine are active in the murine test system with this multidrug-resistant tuberculosis isolate. Sparfloxacin is the most active quinolone. Despite in vitro resistance, isoniazid has moderate activity. In vitro susceptibility data coupled with evaluation of agents against drug-resistant isolates in the murine system should provide information necessary to design clinical trials for treatment of infections with these organisms.

Since 1985, a resurgence of tuberculosis has occurred in the United States, due in part to the human immunodeficiency virus pandemic (2, 6, 14). Institutional and community-based outbreaks of multidrug-resistant tuberculosis (MDR-TB) have occurred with high mortality rates in immunocompromised individuals (3, 13, 26).

During the period from August to October 1991, an outbreak of MDR-TB among inmates in New York State correctional facilities resulted in nosocomial transmission to inpatients and health care workers at SUNY Health Science Center in Syracuse, N.Y. (5). Seventy-four health care workers converted their tuberculin skin tests; one inpatient hospitalized during the outbreak and two health care workers subsequently developed active pulmonary MDR-TB (5, 9). Management of individuals with active MDR-TB has been difficult, and appropriate preventive chemotherapy for health care workers who converted their skin test remains undefined (4, 12).

The purpose of the present study was to evaluate the comparative activities of currently available agents and agents in preclinical development in a murine model of multidrug-resistant tuberculosis, using the outbreak isolate.

MATERIALS AND METHODS

Drugs. Clarithromycin (CLA) and temafloxacin (TEM) were provided by Abbott Laboratories, Abbott Park, Ill. Rifabutin (RBT) was provided by Adria Laboratories, Dublin, Ohio. Clofazimine (CFZ) was provided by Ciba-Geigy Pharmaceuticals, Summit, N.J. Ofloxacin (OFL) was provided by R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J. Rifapentine (RPT) was provided by Merrell Dow Research Institute-Lepetit Research Center, Gerenzano, Italy. Sparfloxacin (SPA) was provided by Parke-Davis, Ann Arbor, Mich. Capreomycin (CAP), cycloserine (CS), ethionamide (ETA), isoniazid (INH), pyrazinamide (PZA), and rifampin (RIF) were obtained from Sigma Chemical Co., St. Louis, Mo.

CLA, TEM, OFL, SPA, and ETA were dissolved in absolute ethanol with subsequent dilution in distilled water

prior to administration. RBT, CFZ, RPT, and RIF were dissolved in dimethyl sulfoxide with subsequent dilution in distilled water. The final concentration of ethanol or dimethyl sulfoxide in drug preparations was 0.5%. CAP, CS, INH, and PZA were dissolved in distilled water. Drugs were freshly prepared each morning prior to administration.

Isolate. *Mycobacterium tuberculosis* ATCC 49967, strain CNL, was obtained as a clinical isolate from a patient with AIDS at SUNY Health Science Center. MICs of all antimicrobial agents except PZA were determined in modified Middlebrook 7H10 broth (7H10 agar formulation with agar and malachite green omitted), pH 6.6, supplemented with 10% Middlebrook OADC (oleic acid-albumin-dextrose-catalase) enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80 (28). MICs of PZA were determined in 7H10 agar, pH 5.8, supplemented with 10% Middlebrook OADC enrichment (28). Comparative MICs (in micrograms per milliliter) for strain CNL and a susceptible *M. tuberculosis* strain, H37Rv (ATCC 25618), determined concurrently, are provided in Table 1.

Medium. The organism was grown in modified Middlebrook 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker for 6 days. The culture suspension was diluted in 7H10 broth to yield 100 Klett units/ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.), or approximately 5×10^7 CFU/ml. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% OADC enrichment. The plates were incubated at 37°C for 4 weeks prior to counting.

Infection studies. The infection studies were performed in three separate experiments. Four-week-old female outbred CD-1 mice (Charles River, Wilmington, Mass.) were infected intravenously through a caudal vein. Each mouse received approximately 10^7 viable organisms suspended in 0.2 ml of 7H10 broth. There were 8 to 10 mice per group. A control group of infected but untreated mice was compared with treated groups of mice. Treatment was started 1 day after infection and given for 4 weeks. All agents except CAP were administered by gavage. CAP was administered subcutaneously. Dose volumes were 0.2 ml.

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TABLE 1. MICs of antimicrobial agents against *M. tuberculosis* CNL and *M. tuberculosis* H37Rv

Agent	MIC (μg/ml) against:	
	CNL	H37Rv
INH	1	0.125
RIF	64	0.125
PZA	>256	32
ETA	2	4
CS	8	8
CAP	4	4
RBT	>64	0.25
RPT	64	0.25
CLA	2	4
OFL	0.5	0.125
TEM	0.125	0.25
SPA	2	1
CFZ	0.06	0.5

TABLE 2. Difference in mean log CFU for spleens and lungs in various treatment groups compared with those in untreated control mice

Agent and concn (mg/kg)	Difference in mean log CFU from control	
	Spleen	Lung
SPA, 150	-3.41	-2.04
OFL, 300	-2.08	-1.58
TEM, 150	-1.00	-1.00
INH, 25	-2.08	-1.65
INH, 75	-1.86	-2.19
ETA, 125	-2.58	-2.96
ETA, 50	-0.41	-0.43
PZA, 150	-0.26	-0.27
CAP, 150	-1.44	-0.94
CS, 300	-0.58	-0.36
CFZ, 20	-2.00	-2.96
CLA, 200	-1.63	-0.97
RIF, 20	+0.15	+0.08
RBT, 20	+0.02	-0.33
RPT, 20	-0.02	-0.38

Animals were sacrificed by cervical dislocation 3 to 5 days after the last dose of drug. Spleens and lungs were aseptically removed and ground in a tissue homogenizer. The number of viable organisms was determined by titration on 7H10 agar plates.

Statistical evaluation. The viable cell counts were converted to logarithms which were then evaluated with one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the Tukey honestly significant difference test (20) to make pairwise comparisons among means. The results of the statistical evaluations are summarized in the following section.

RESULTS

Infection study 1 (Fig. 1 and Table 2). TEM, SPA, or CAP (150 mg/kg of body weight), OFL or CS (300 mg/kg), or ETA (125 mg/kg) was given 5 days per week for 4 weeks to female mice which had been infected with 1.6×10^7 viable *M. tuberculosis* organisms. Treatment with TEM, SPA, CAP, OFL, or ETA reduced cell counts in spleens and lungs compared with those in mice given no treatment ($P < 0.01$). Of the quinolones, SPA was more active than TEM or OFL

($P < 0.01$). Treatment with CS did not reduce cell counts in spleens or lungs compared with those in mice given no treatment ($P > 0.05$).

Infection study 2 (Fig. 2 and Table 2). INH (25 mg/kg 5 days/week or 75 mg/kg 3 days/week) was given to female mice which had been infected with 7.0×10^6 viable *M. tuberculosis* organisms. Other treatment groups received PZA or SPA (150 mg/kg) or CLA (200 mg/kg) 5 days/week. Treatment with INH (at either dose), CLA, or SPA reduced cell counts in spleens and lungs compared with those in mice given no treatment ($P < 0.01$). The difference in cell counts between mice given 25 mg of INH per kg and mice given 75 mg of INH per kg was significant for the lungs ($P < 0.01$) but not for the spleens ($P > 0.05$). Treatment with PZA did not reduce organ cell counts compared with those in mice given no treatment ($P > 0.05$).

Infection study 3 (Fig. 3 and Table 2). RIF, RBT, RPT, or CFZ (20 mg/kg) or ETA (50 or 125 mg/kg) was given 5 days/week to female mice which had been infected with 1.7×10^7 viable *M. tuberculosis* organisms. Treatment with CFZ or ETA at 125 mg/kg reduced cell counts in spleens and
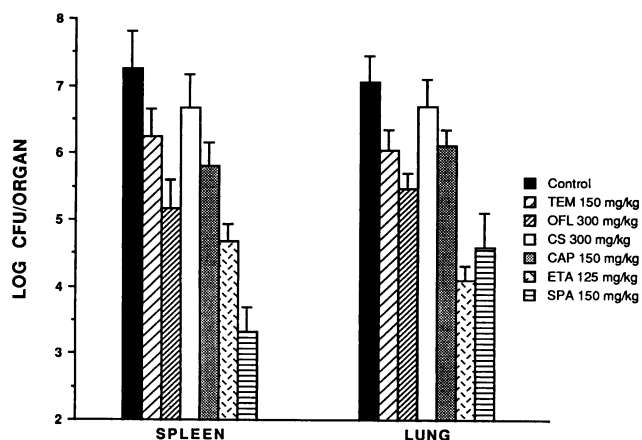

FIG. 1. Activity of new quinolones CS, CAP, and ETA against *M. tuberculosis* CNL in mice. The results are means for 8 to 10 mice per group. Error bars indicate standard deviations.
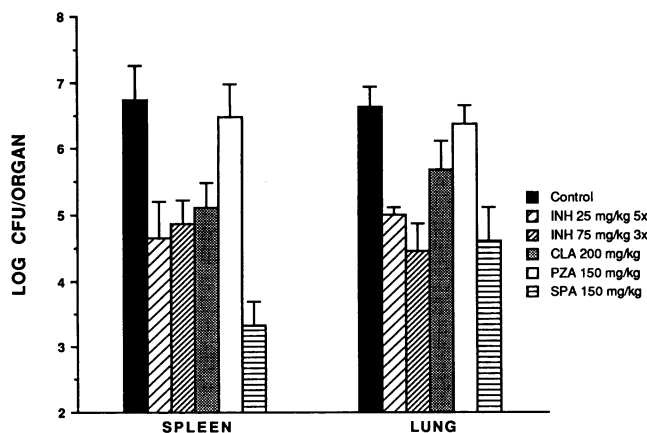

FIG. 2. Activity of INH (at two dosages), CLA, PZA, and SPA against *M. tuberculosis* CNL in mice.
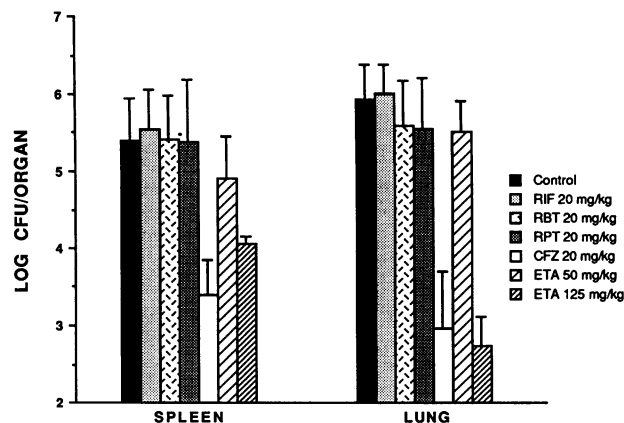
FIG. 3. Activity of RIF, RBT, RPT, CFZ, and ETA (at two concentrations) against *M. tuberculosis* CNL in mice.

lungs compared with those in mice given no treatment ($P < 0.01$). Treatment with any rifamycin or ETA at 50 mg/kg did not reduce organ cell counts compared with those in mice given no treatment ($P > 0.05$).

DISCUSSION

Despite the in vitro resistance, INH had moderate activity against this "resistant" strain in the murine test system. However, INH is substantially more active in vivo against a susceptible *M. tuberculosis* strain. Using similar experimental methods and the 4-week treatment period, INH results in approximately a 2-log-greater reduction in organ cell counts for H37Rv (ATCC 25618) than for *M. tuberculosis* CNL (21). Enhanced activity might be expected if a higher INH dose were given on an intermittent basis (75 mg/kg three times per week); however, there was only a modest difference between numbers of organisms in the lungs of mice treated with the higher and lower INH doses.

PZA was inactive against this isolate, as predicted by the results of our in vitro susceptibility testing. Discordant MIC results have been reported for this isolate by other laboratories (5); however, susceptibility testing for PZA is technically difficult (15, 16).

Of current second-line antituberculous agents, CAP had modest activity and CS had no activity in the murine test system. The relative activity of CS may be underestimated because of the high rate of CS excretion in the mouse (8, 25). Perhaps two- or three-times-per-day dosing of CS would better approximate the human pharmacokinetics of this agent. ETA had moderate activity against the drug-resistant isolate, although this agent is often poorly tolerated in humans because of dose-related gastrointestinal side effects (10). ETA administered at a lower dose in the murine test system had limited activity.

Newer quinolones were active in the murine test system of drug-resistant tuberculosis. SPA was the most active quinolone tested. The area under the concentration-time curve for mice dosed with SPA at 100 mg/kg approximates that for humans following a single 400-mg oral dose (23, 24). SPA was more active than OFL, a finding obtained by Ji et al. with a murine tuberculosis model using a drug-susceptible isolate (19).

CLA had modest activity in the murine test system; however, it was relatively less active against organisms in the lungs than against organisms in the spleens. This same

trend has been noted for CLA activity against *Mycobacterium avium* complex in the beige mouse (22). CLA was subsequently evaluated against a drug-susceptible isolate of *M. tuberculosis* in a similar murine system (21). There was little activity against organisms in spleens or lungs; therefore, this agent should probably not be used for the treatment of tuberculosis.

As reflected in the in vitro susceptibility results, there was complete cross-resistance between RIF and the newer rifamycins against this isolate in vivo, perhaps due to the high level of rifamycin resistance. It may be useful to assess in vivo activity of the newer rifamycins against *M. tuberculosis* isolates with lower levels of in vitro resistance. Improved understanding of the correlation between in vitro and in vivo rifamycin activity will help to clarify the potential role of RBT or RPT in the treatment of RIF-resistant tuberculosis (7, 11, 17).

CFZ was active in the murine test system, although its place in the treatment of tuberculosis is not clear. CFZ has promising in vitro activity against *M. tuberculosis*, with MICs in the range of 0.1 to 10 μg/ml, depending on the pH of the media (1). This agent has activity in mouse and guinea pig models of tuberculosis, but not in a rhesus monkey model of infection (27). Assessment of activity in human clinical disease is necessary to judge the role of CFZ as an antituberculosis agent.

The organism used in this study was found to be resistant to INH and ETA and susceptible to PZA by other investigators (4). It is likely that quantitative susceptibility testing of "resistant" isolates would provide information useful in designing treatment regimens for individuals infected with these organisms. Individuals may benefit from therapy with INH and ETA when isolates with low-level resistance occur. Individuals would derive little if any benefit from therapy with rifamycins when isolates with high-level resistance occur.

Management of drug-resistant tuberculosis, particularly in individuals with human immunodeficiency virus infection, is difficult and associated with a high risk of treatment failure (4, 18). There has been limited study of drug-resistant tuberculosis in animal systems. Quantitative susceptibility testing coupled with in vivo study of multidrug-resistant isolates in murine test systems will improve our understanding of the relationship between these parameters of antimicrobial activity. The lesson from this initial study is that our current breakpoint susceptibility testing method is satisfactory in predicting susceptible isolates but provides inadequate information with regard to "resistant" isolates. Further evaluation of the use of antimycobacterial agents against drug-resistant isolates will provide data that will be useful for the design of clinical trials and/or algorithms for treatment of infection with these organisms. The most effective approach to understanding the biology of drug-resistant tuberculosis is the study of these organisms both in vitro and in vivo.

REFERENCES

1. **Acharya, B. K., J. M. Robson, and F. M. Sullivan.** 1959. Antituberculous activity of a phenazine derivative (B663) Am. Rev. Tuberc. **80:**871–875.
2. **Brudney, K., and J. Dobkin.** 1991. Resurgent tuberculosis in New York City: human immunodeficiency virus, homelessness, and the decline of tuberculosis control programs. Am. Rev. Respir. Dis. **144:**745–749.
3. **Centers for Disease Control.** 1991. Transmission of multidrug-resistant tuberculosis from an HIV-positive client in a residential substance-abuse treatment facility—Michigan. Morbid.

Mortal. Weekly Rep. **40**:129–131.

4. **Centers for Disease Control.** 1992. Management of persons exposed to multidrug-resistant tuberculosis. Morbid. Mortal. Weekly Rep. **41**:61–71.

5. **Centers for Disease Control.** 1992. Transmission of multidrug-resistant tuberculosis among immunocompromised persons in a correctional system—New York, 1991. Morbid. Mortal. Weekly Rep. **41**:507–509.

6. **Chaisson, R. E., and G. Slutkin.** 1989. Tuberculosis and human immune deficiency virus infection. J. Infect. Dis. **159**:96–100.

7. **Chan, S. L., W. W. Yew, W. K. Ma, D. J. Girling, V. R. Aber, D. Felmingham, B. W. Allen, and D. A. Mitchison.** 1992. The early bactericidal activity of rifabutin measured by sputum viable counts in Hong Kong patients with pulmonary tuberculosis. Tubercle Lung Dis. **73**:33–38.

8. **Coletsos, P. J.** 1970. Experimental comparative study of the concentration of cycloserine in the serum and viscera in conventional laboratory animals and in the monkey. Scand. J. Respir. Dis. Suppl. **71**:S40–S50.

9. **Cynamon, M. H., M. S. DeStefano, and S. P. Klemens.** 1992. Therapy of multidrug-resistant tuberculosis: lessons from mice, abstr. 4–14. Program Abstr. 1992 World Congr. Tuberc., Bethesda, Md.

10. **Davidson, P. T., and H.-Q. Le.** 1992. Drug treatment of tuberculosis—1992. Drugs **43**:651–673.

11. **Dickinson, J. M., and D. A. Mitchison.** 1987. *In vitro* activity of new rifamycins against rifampicin-resistant *M. tuberculosis* and MAIS-complex mycobacteria. Tubercle **68**:177–182.

12. **Dooley, S. W., W. R. Jarvis, W. J. Martone, and D. E. Snider, Jr.** 1992. Multidrug-resistant tuberculosis. Ann. Intern. Med. **117**:257–259.

13. **Fischl, M. A., G. L. Daikos, R. B. Uttamchandani, R. B. Poblete, J. N. Moreno, R. R. Reyes, A. M. Boota, L. M. Thompson, T. J. Cleary, S. A. Oldham, M. J. Saldana, and S. Lai.** 1992. Clinical presentation and outcome of patients with HIV infection and tuberculosis caused by multiple-drug-resistant bacilli. Ann. Intern. Med. **117**:184–190.

14. **Fischl, M. A., R. B. Uttamchandani, G. L. Daikos, R. B. Poblete, J. N. Moreno, R. R. Reyes, A. M. Boota, L. M. Thompson, T. J. Cleary, and S. Lai.** 1992. An outbreak of tuberculosis caused by multiple-drug-resistant tubercle bacilli among patients with HIV infection. Ann. Intern. Med. **117**:177–183.

15. **Hawkins, J. E.** 1984. Drug susceptibility testing, p. 179–180. *In* G. P. Kubica and L. G. Wayne (ed.), The Mycobacteria: a sourcebook. M. Dekker, Inc., New York.

16. **Heifets, L. B.** 1991. Drug susceptibility in the management of chemotherapy of tuberculosis, p. 97–101. *In* L. B. Heifets (ed.),

Drug susceptibility in the chemotherapy of mycobacterial infections. CRC Press, Inc., Boca Raton, Fla.

17. **Hong Kong Chest Service/British Medical Research Council.** 1992. A controlled study of rifabutin and an uncontrolled study of ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to isoniazid, streptomycin and rifampicin. Tubercle Lung Dis. **73**:59–67.

18. **Iseman, M. D., and L. A. Madsen.** 1989. Drug-resistant tuberculosis, p. 341–353. *In* D. E. Snider (ed.), Clinics in chest medicine, vol. 10. Mycobacterial diseases. The W. B. Saunders Co., Philadelphia.

19. **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.

20. **Kirk, R. E.** 1986. Experimental design: procedures for the behavioral sciences. Brooks-Cole Publishing Co., Belmont, Calif.

21. **Klemens, S. P., and M. H. Cynamon.** Unpublished data.

22. **Klemens, S. P., M. S. DeStefano, and M. H. Cynamon.** 1992. Activity of clarithromycin against *Mycobacterium avium* complex infection in beige mice. Antimicrob. Agents Chemother. **36**:2413–2417.

23. **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.

24. **Montay, G., R. Bruno, J. J. Thebault, J. C. Vergniol, D. Chassard, M. Ebmeier, and J. Gaillot.** 1990. Dose-dependent pharmacokinetic study of sparfloxacin (SPX) in healthy young volunteers, abstr. 1248. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother.

25. **Panà, C., and L. Lenzini.** 1970. Experimental research on cycloserine. Scand. J. Respir. Dis. Suppl. **71**:S71–S76.

26. **Pearson, M. L., J. A. Jereb, T. R. Frieden, J. T. Crawford, B. J. Davis, S. W. Dooley, and W. R. Jarvis.** 1992. Nosocomial transmission of multidrug-resistant *Mycobacterium tuberculosis*: a risk to patients and health care workers. Ann. Intern. Med. **117**:191–196.

27. **Schmidt, L. H.** 1959. Observations on the prophylactic and therapeutic activities of 2-(p-chloroanilino)-5-(p-chlorophenyl)-3,5-dihydro-3(isopropylimino)phenazine(B.663). Bull. Int. Union Tuberc. **30**:316–321.

28. **Vestal, A. L.** 1969. Procedures for the isolation and identification of mycobacteria, p. 113–115. *In* Public Health Service publication no. 1995. Laboratory Division, National Communicable Disease Center, Atlanta.