J. Pharm. Pharmacol. 1997, 49 (Suppl. 1): 31–36

Mechanisms of Tuberculosis Chemotherapy

D. A. MITCHISON

St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

In the two decades after the first clinical trials of streptomycin in 1948, the main emphasis in the development of effective chemotherapy for tuberculosis was in preventing the emergence of drug resistance by using combinations of two or three drugs. In the next twenty years, the emphasis changed to shortening the duration of treatment from the earlier 12–24 months to the present 6 months. At the same time, increasing attention was paid to improving compliance, since failure in compliance, rather than inadequate drugs, is the main reason for the appearance of multidrug resistant *Mycobacterium tuberculosis*. We looked for drug combinations that had the greatest bactericidal activity to be used in short-course regimens and for the best methods for full supervision of the patient's drug taking.

Phases of Chemotherapy

Bactericidal activity is complex. There appear to be two loosely separated phases of chemotherapy. In the first phase, tubercle bacilli in the lesions of patients with pulmonary tuberculosis lie tightly packed in very large numbers in a narrow zone close to the air–caseum interface (Canetti 1955). Since caseum is composed of dead cells, the bacilli are usually some distance from living macrophages and are extracellular. They resemble bacteria in a laboratory culture. As chemotherapy proceeds, the necrotic caseum is coughed up, and the residual bacilli lie closer to living cells including macrophages. It is reasonable to suppose that they are now often phagocytosed and that part, but not necessarily all, of the population is intracellular.

The Early Phase

In the first phase, bactericidal activity in the lesions can be measured by successive counts of colony forming units (CFU) in sputum collections (Jindani et al 1980). Killing takes place rapidly during the first 2–4 days and then progressively slows down during the next 12 days. The slowing rate of kill can be explained by the presence in lesions of a mixture of rapidly growing organisms which are exponentially killed, as occurs in log-phase cultures (Fig. 1a), together with organisms that are metabolically less active and therefore killed less quickly, as in stationary-phase cultures (Fig. 1b). If all lesional bacilli were rapidly growing and were eliminated exponentially, it would only take about 14 days to sterilize lesions. Unfortunately, effective sterilization takes about 6 months because of the presence of persisting metabolically inactive bacilli.

The Late Sterilizing Phase

In the late phase of chemotherapy the main concern is with elimination of the persisting bacilli. What causes their metabolic inactivity? Bacterial pheromones are sometimes responsible for preventing growth beyond a critical density of organisms (Wirth et al 1996), but the close packing of the bacilli in lesions suggests that there is no similar bar to a local high density of tubercle bacilli. The location of the bacilli close to the air–caseum interface gives a clue, suggesting lack of oxygen as the main reason rather than lack of carbon or nitrogen nutrients. Further evidence for the critical role of oxygen tension is shown by the paucity of bacilli in closed caseous lesions that do not open to the air. We need to know more about tubercle bacilli surviving under microaerophilic or anaerobic conditions and their response to antibacterial drugs,

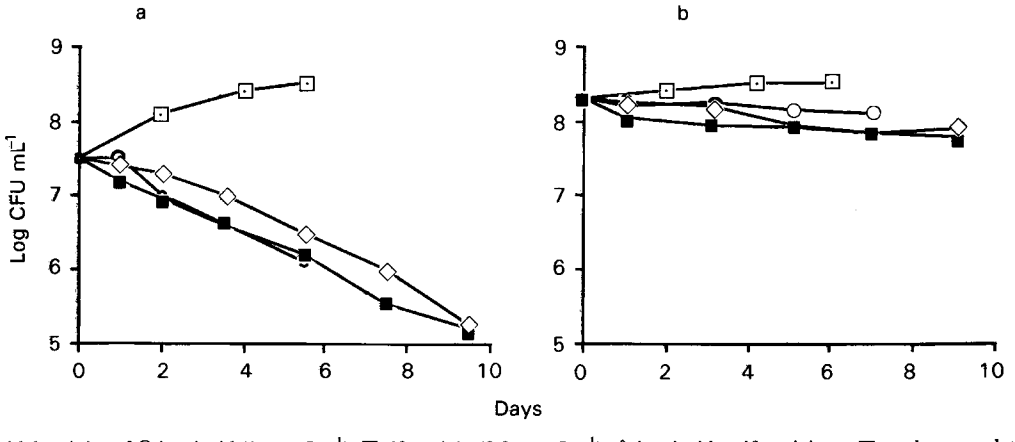

FIG. 1. Bactericidal activity of ○ isoniazid (1 μg mL^{-1}), ■ rifampicin (0.2 μg mL^{-1}), ◇ isoniazid + rifampicin, or ⊡ no drug on a late log-phase culture (a) and a stationary-phase culture (b) of *M. tuberculosis* H37Rv.

since they are an important key to improving the speed of response to chemotherapy. While low oxygen tension may be of critical importance in limiting growth of extracellular bacilli as, for instance, in persisters in the walls of cavities in human lesions, other factors, including host defence mechanisms, may inhibit the growth of intracellular bacilli which may be more common in, for example, experimental murine infections and in new areas of tuberculous pneumonitis in human lungs.

Metronidazole

Wayne and colleagues have shown that cultures of *M. tuberculosis* grown in the depths of liquid medium adapt to microaerophilic conditions (Wayne 1994; Wayne & Sramek 1994; Wayne & Hayes 1996) and these findings have been confirmed by Yan-Min Hu in our laboratory (unpublished data). When a log-phase culture was placed in strictly anaerobic conditions, the organisms were killed. However, if the culture had been left incubating until it reached the stationary phase, it could be transferred to anaerobic conditions without loss of viability. These stationary cultures became sensitive to metronidazole. Fig. 2 shows that metronidazole, even at the high concentration of 256 μg mL^{-1}, had no effect on the growth of log-phase *M. tuberculosis*. However, a stationary culture, left incubating for 30 days in 7H9 Tween–albumin medium, was slowly killed by 64 μg mL^{-1} metronidazole (Fig. 3), and when the culture was transferred to strict anaerobic conditions in a jar, the culture was killed by 16 μg mL^{-1} metronidazole (Fig. 4), a concentration that can be achieved clinically. The change in metronidazole activity when the culture was placed in the anaerobic jar probably indicates that growth in the depths of liquid medium was microaerophilic rather than anaerobic. We do not as yet know whether metronidazole might turn out to be of value as an additional sterilizing drug with specific activity against persisters.

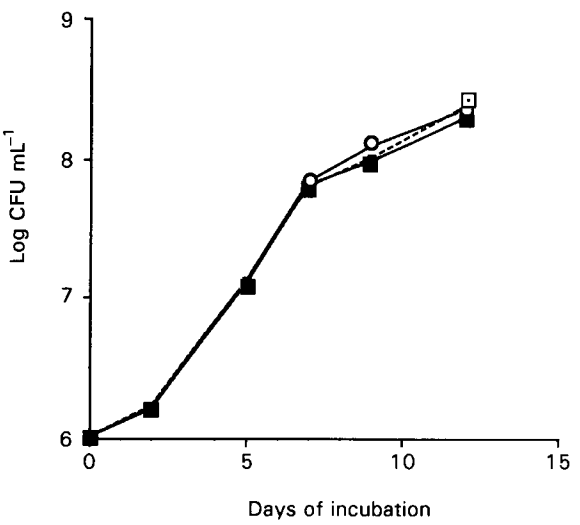

FIG. 2. Absence of activity of metronidazole on log-phase culture of *M. tuberculosis* H37Rv. ⊡ Control, ○ metronidazole (128 μg mL^{-1}), ■ metronidazole (256 μg mL^{-1}).

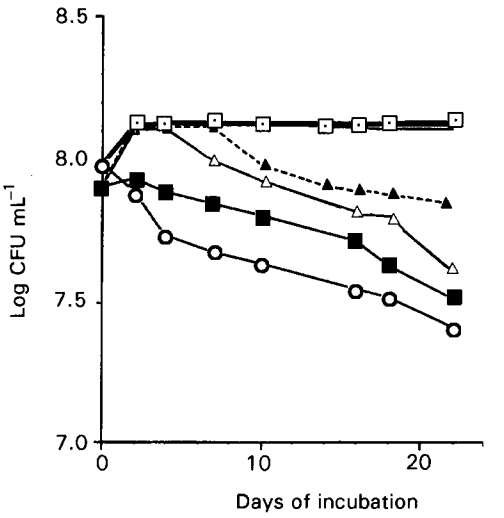

FIG. 3. Bactericidal activity of ▲ metronidazole (64 μg mL^{-1}) on stationary culture of *M. tuberculosis* H37Rv. Effects of ⊡ control and other concentrations of metronidazole; −2, 4, 8, 16 and 32 μg mL^{-1}, △ 128 μg mL^{-1}, ■ 256 μg mL^{-1}, ○ 512 μg mL^{-1} are also shown.

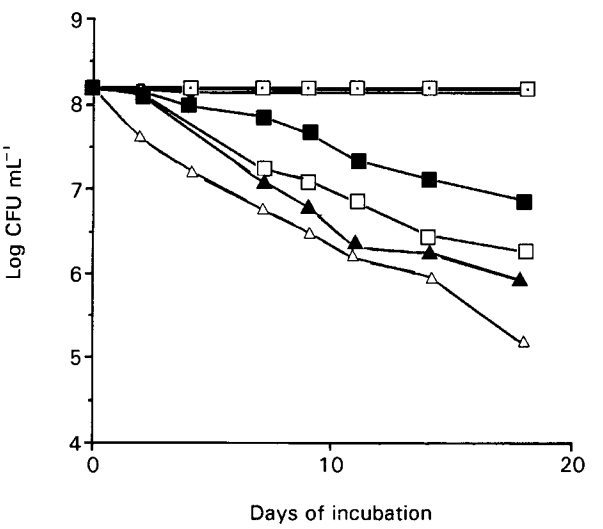

FIG. 4. Bactericidal activity of ■ metronidazole (16 μg mL^{-1}) on anaerobic culture of *M. tuberculosis* H37Rv. Effects of ⊡ control and other concentrations of metronidazole; — 0, 2, 4 and 8 μg mL^{-1}, □ 32 μg mL^{-1}, ▲ 64 μg mL^{-1}, △ 128 μg mL^{-1} are also shown.

Examination of Drug Action on Stationary (Microaerophilic) Cultures

For many years we have been testing the activity of drugs against stationary-phase as well as log-phase cultures for the reason that the stationary-phase tests might show activity against persisters and thus indicate the drug's role in shortening chemotherapy (Dickinson & Mitchison 1987, 1990). This concept has been strengthened by the similar recommendation made recently by Wayne & Hayes (1996). A recent example explores the bactericidal activities of the quinolone, ofloxacin, and a mixture of sulbactam, which inhibits mycobacterial penicillinase, and ampicillin, in comparison with isoniazid and rifampicin (Herbert et al 1996). Fig. 5 shows the activity of these drugs against *M. tuberculosis* in 7H9 medium

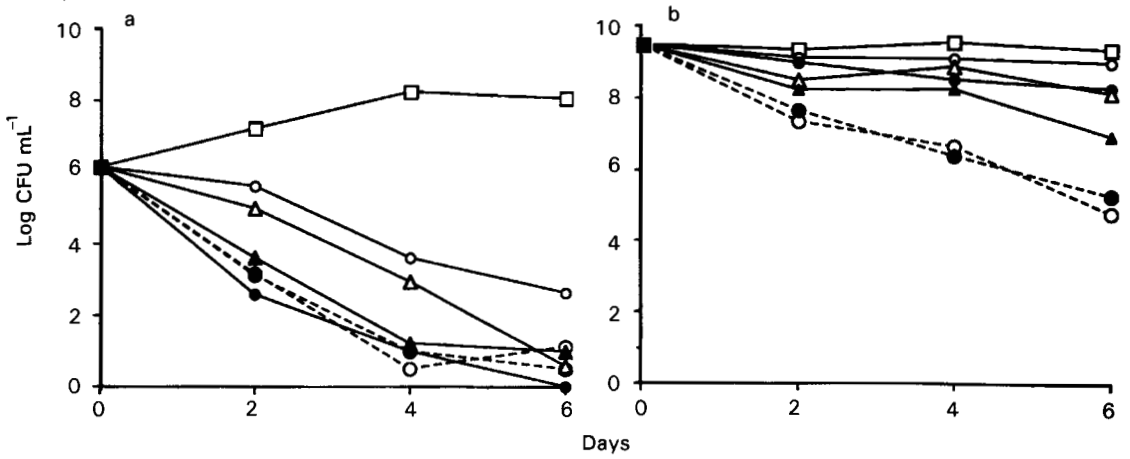

FIG. 5. Bactericidal activity of isoniazid, rifampicin, ofloxacin and sulbactam-ampicillin against log-phase (a) and stationary-phase (b) cultures of *M. tuberculosis* H37Rv. □ No drug, - -○- - isoniazid (1 μg mL^{-1}), - -●- - rifampicin (1 μg mL^{-1}), ○ ofloxacin (1.25 μg mL^{-1}), ● ofloxacin (5 μg mL^{-1}), △ sulbactam + ampicillin (8 μg mL^{-1}), ▲ sulbactam + ampicillin (15 μg mL^{-1}).

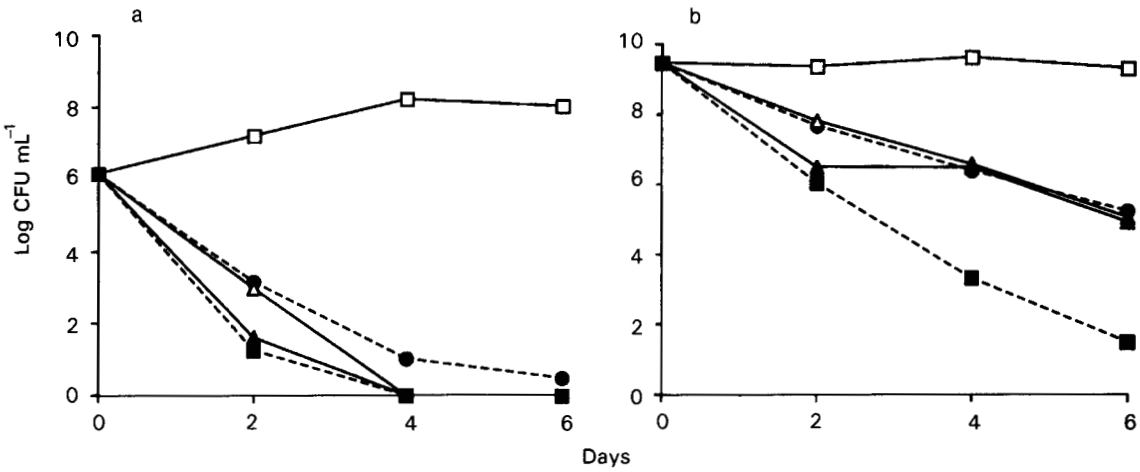

FIG. 6. Activity of ofloxacin + rifampicin on log-phase (a) and stationary-phase (b) cultures of *M. tuberculosis* H37Rv. □ No drug, ● rifampicin (1 μg mL^{-1}), ■ isoniazid + rifampicin (1 μg mL^{-1}), △ rifampicin + ofloxacin (1.25 μg mL^{-1}), ▲ rifampicin + ofloxacin (5 μg mL^{-1}).

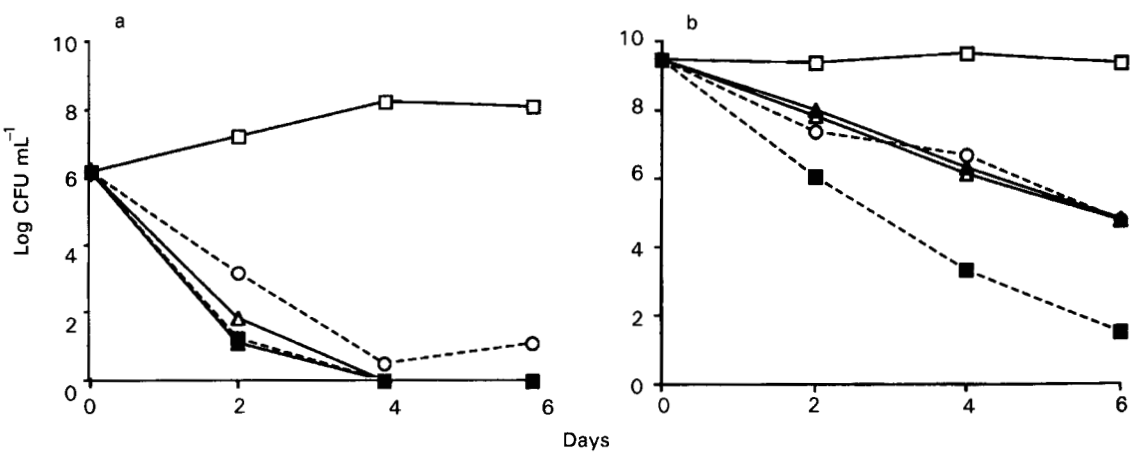

FIG. 7. Activity of sulbactam-ampicillin + isoniazid on log-phase (a) and stationary-phase (b) cultures of *M. tuberculosis* H37Rv. □ No drug, ○ isoniazid (1 μg mL^{-1}), ■ isoniazid + rifampicin (1 μg mL^{-1}), △ isoniazid + sulbactam-ampicillin (8 μg mL^{-1}), ▲ isoniazid + sulbactam-ampicillin (15 μg mL^{-1}).

either in the log phase of growth or in a 30-day stationary phase. The higher concentrations of ofloxacin (5 μg mL^{-1}) and sulbactam–ampicillin (15 μg ampicillin mL^{-1}) were as bactericidal as isoniazid or rifampicin against the log-phase culture but were much less bactericidal than either isoniazid or rifampicin against the stationary-phase culture. Fig. 6 shows that addition of ofloxacin to rifampicin in the log-phase culture synergizes the bactericidal activity to approximate equality with isoniazid–rifampicin, but no corresponding synergism occurs in the stationary-phase culture. Fig. 7 shows similar findings with sulbactam–ampicillin. The considerable bactericidal activity of ofloxacin and sulbactam–ampicillin against log-phase cultures, but the much lower activity against stationary-phase cultures suggests that, though either of these drugs might be useful in the early phase of chemotherapy, they would be of little value in the late sterilizing phase.

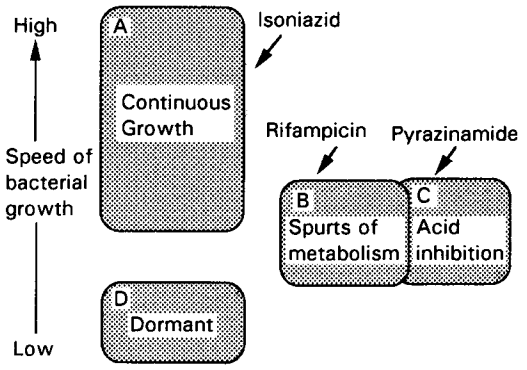

FIG. 8. Special populations hypothesis for the sterilizing activity of rifampicin and pyrazinamide. Actively growing bacilli (A) are killed by isoniazid though other drugs—rifampicin, streptomycin or ethambutol—may act if the infecting strain is isoniazid-resistant. Semi-dormant bacilli with spurts of metabolism (B) are selectively killed by rifampicin, and those in very acid environment (C) are selectively killed by pyrazinamide.

Current Drugs

Early studies in experimental murine tuberculosis established that combinations of isoniazid, streptomycin and *p*-aminosalicyclic acid reduced the numbers of viable bacilli in the organs, but were unable to sterilize them. However, when either rifampicin (Grosset 1978) or pyrazinamide (McCune et al 1956) were given with isoniazid, negative cultures were obtained from the organs, and these two drugs are therefore known as potent sterilizing drugs. In the treatment of human tuberculosis the addition of either rifampicin or pyrazinamide to a basic regimen of daily isoniazid and streptomycin substantially reduced the relapse rate after the end of chemotherapy (Table 1), thus confirming their role as sterilizing drugs (East African/British Medical Research Council 1974). As a result of this study and subsequent clinical trials under the auspices of the Medical Research Council in East Africa, Madras, Hong Kong and Singapore, current standard short-course chemotherapy is a 6-month regimen of isoniazid and rifampicin with the addition of pyrazinamide for the first 2 months (a fourth drug, usually ethambutol, may be given initially with the aim of reducing the chances of drug resistance emerging). The modes of action of these three essential drugs are shown diagramatically in Fig. 8. Actively growing bacilli are killed by isoniazid. Rifampicin and pyrazinamide kill specific populations of semi-dormant bacilli.

Early Bactericidal Activity

The initial rapid kill of actively growing bacilli takes place during the first few days. The role of different drugs at this early stage has been measured as their early bactericidal activity (EBA) (Jindani et al 1980; Sirgel et al 1993; Botha et al 1996). The EBA is obtained by performing CFU counts, using selective culture medium, on overnight collections of sputum just before the start of treatment with the drug concerned and then at daily or 2-day intervals thereafter. Since the rate of kill of different drugs and different doses of the same drug varied during the first 2 days of treatment but did not vary appreciably thereafter, the EBA is defined as the fall in $\log_{10}$ CFU counts (mL sputum)$^{-1}$ day^{-1} during the first 2 days. Values of the EBA obtained with usual dose sizes of the main antituberculosis drugs are set out in Table 2.

Isoniazid has the highest EBA of about 0·6, its high activity is little influenced by the presence of other drugs, including rifampicin and pyrazinamide, given at the same time. Rifampicin has a lower EBA. The EBA of pyrazinamide is so low as to be difficult to distinguish from no treatment at all.

Sterilizing Activity of Rifampicin

Rifampicin starts to kill after only a short exposure lasting about 30 min whereas isoniazid often only starts killing after about 24 h. This is the probable reason why rifampicin is such an effective sterilizing drug. It can kill when dormant bacilli start to metabolize as a result of only brief stimuli such as a temporary rise of temperature from 8°C to 37°C, provision of additional oxygen or addition of small volumes of fresh culture medium (Dickinson & Mitchison 1981).

Sterilizing Activity of Pyrazinamide

In both the mouse (McCune et al 1956) (Fig. 9) and in human pulmonary tuberculosis (Mitchison 1985), pyrazinamide is only very slowly bactericidal to begin with, but, unlike other

Table 1. East African/British Medical Research Council short course chemotherapy study.

Regimen (6 months)	Total patients	Relapses 12 months after treatment (%)
Streptomycin + isoniazid	114	27
Streptomycin + isoniazid + thiacetazone	102	22
Streptomycin + isoniazid + pyrazinamide	167	8
Streptomycin + isoniazid + rifampicin	156	3

Table 2. Early bactericidal activity of antituberculosis drugs.

Drug	Dose size	Early bactericidal activity (fall in CFU (mL sputum)$^{-1}$ day^{-1}) Nairobi*	South Africa**
None	–	−0·02	−0·02
Pyrazinamide	2 g	0·04	0·004
Streptomycin	1 g	0·07	–
Rifampicin	10–12 mg kg^{-1}	0·19	0·20
Ethambutol	25 mg kg^{-1}	0·31	0·25
Isoniazid	300 mg	0·72	0·50
Rifater†	–	0·60	

†Rifater = pyrazinamide + rifampicin + isoniazid. *n = 4–8; **n = 9–12.

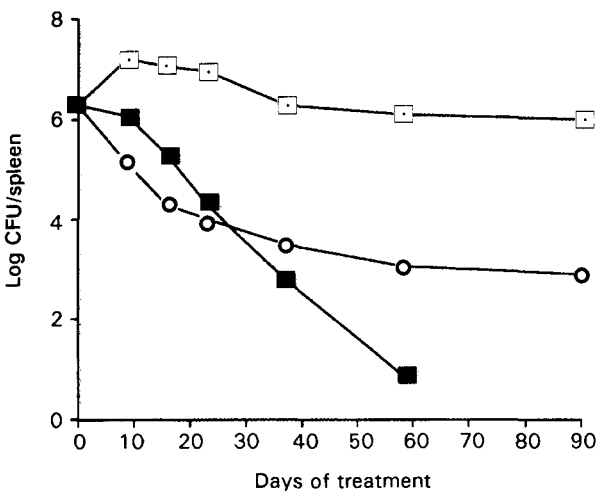

FIG. 9. Sterilizing activity of ■ isoniazid and pyrazinamide compared with ○ streptomycin, *p*-amino salicylic acid and isonazid and ⊡ control in experimental murine tuberculosis. Redrawn from McCune et al (1956).

drugs, the killing continues at the same rate throughout treatment and does not slow down as occurs with other drugs. Thus, during the first few days it kills much more slowly than isoniazid, but after about a month it has overtaken the curve due to isoniazid. Eventually, only negative organ cultures were obtained from mice treated with the combination of high dosage pyrazinamide and isoniazid for 12 weeks. There is good evidence that pyrazinamide given for the first 2 months in the treatment of pulmonary tuberculosis reduces the eventual relapse rate and allows shortening of the duration of treatment from 9 months to 6 months. Thus, there is strong evidence that pyrazinamide is an effective sterilizing drug in the mouse and in humans. There is, however, little known about why it is a good sterilizing drug. Pyrazinamide is converted to the active moiety pyrazinoic acid (Konno et al 1967) by the bacterial amidase of *M. tuberculosis*, whose gene pncA has recently been discovered (Scorpio & Zhang 1996). It is only active at an acid pH of about 5·6 or less (McDermott & Tompsett 1954). Although it inhibits growth of *M. tuberculosis* in macrophages or macrophage-derived cell lines, it never appears to kill (Crowle et al 1986; Rastogi et al 1988; Dhillon & Mitchison 1989). Furthermore, the microenvironment in the vesicles in which *M. tuberculosis* resides is probably not acid and inhibition depends on the accumulation of pyrazinoic acid. These observations and the evidence that pyrazinamide is only effective during the first 2 months of treatment when bacilli are mainly extracellular strongly suggests that the bacilli against which it acts are extracellular in lesions made acid by local inflammation. Because of the local acidity, these bacilli are only just metabolizing and are therefore unaffected by isoniazid but may be killed by pyrazinamide. The difficulty with this explanation is that killing in culture is almost as difficult to demonstrate as killing of bacilli in cell lines. At the very acid pH of 4·8, Heifets & Lindholm-Levy (1992) demonstrated active killing, but it must seem unlikely that a pH value as low as 4·8 is actually encountered in lesions. Further work on pyrazinamide is needed to explain its characteristic slow but continued bactericidal activity, its requirement for an acid pH, and the nature of the bacterial population that it can kill more effectively than other antituberculosis drugs.

Conclusions

Antituberculosis chemotherapy could be improved by drugs that are particularly bactericidal against persisting bacilli. The two mechanisms suggested for inhibition of bacterial growth, and therefore the creation of persisters, seem to be microaerophilic conditions inhibiting extracellular bacilli in cavity walls and host defence mechanisms slowing intracellular growth. Recognition of the role of microaerophilic conditions leads to the need to test new antituberculosis drugs against stationary (microaerophilic) cultures so as to discover additional drugs that might be active against persisters. The interaction between host immunity and the action of drugs needs further exploration. In our experience activation of murine macrophages by γ-interferon did not alter the rate at which intracellular *M. tuberculosis* was killed by isoniazid or rifampicin (Khor et al 1986) but the more effective BCG-induced immunity decreased the bactericidal activity of isoniazid in mice and increased the activities of isoniazid and rifampicin in the guinea-pig (Dhillon & Mitchison 1989).

References

Botha, F. J. H., Sirgel, F. A., Parkin, D. P., Van de Wal, B. W., Donald, P. R., Mitchison, D. A. (1996) Early bactericidal activity of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater) in patients with pulmonary tuberculosis. S. Afr. Med. J. 86: 155–158

Canetti, G. J. (1955) The Tubercle Bacillus in the Pulmonary Lesions of Man; Histobacteriology and its Bearing on the Therapy of Pulmonary Tuberculosis. Springer, New York, 62–68

Crowle, A. J., Sbarbaro, J. A., May, M. H. (1986) Inhibition by pyrazinamide of tubercle bacilli within cultured human macrophages. Am. Rev. Respir. Dis. 134: 1052–1055

Dhillon, J., Mitchison, D. A. (1989) Influence of BCG-induced immunity on the bactericidal activity of isoniazid and rifampicin in experimental tuberculosis of the mouse and guinea-pig. Br. J. Exp. Pathol. 70: 103–110

Dickinson, J. M., Mitchison, D. A. (1981) Experimental models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis. Am. Rev. Respir. Dis. 123: 367–371

Dickinson, J. M., Mitchison, D. A. (1987) In vitro observations on the suitability of new rifamycins for the intermittent chemotherapy of tuberculosis. Tubercle 68: 183–193

Dickinson, J. M., Mitchison, D. A. (1990) In vitro activities against mycobacteria of two long-acting rifamycins, FCE22807 and CGP40469A (SPA-S-565). Tubercle 71: 109–115

East African/British Medical Research Council (1974) Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet ii: 237–40

Grosset, J. (1978) The sterilizing value of rifampicin and pyrazinamide in experimental short course chemotherapy. Bull. Int. Un. Tuberc. 53: 5–12

Heifets, L., Lindholm-Levy, P. (1992) Pyrazinamide sterilizing activity in vitro against semidormant *Mycobacterium tuberculosis* bacterial populations. Am. Rev. Respir. Dis. 145: 1223–1225

Herbert, D., Paramasivan, C. N., Venkatesan, P., Kubendiran, G., Prabhakar, R., Mitchison, D. A. (1996) Bactericidal action of ofloxacin, sulbactamampicillin, rifampicin and isoniazid, on logarithmic and stationary phase cultures of *M. tuberculosis*. Antimicrob. Agents Chemother. 40: 2296–2299

Jindani, A., Aber, V. R., Edwards, E. A., Mitchison, D. A. (1980) The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am. Rev. Respir. Dis. 121: 939–949

Khor, M., Lowrie, D. B., Coates, A. R. M., Mitchison, D. A. (1986) Recombinant interferon-gamma and chemotherapy with isoniazid and rifampicin in experimental murine tuberculosis. Br. J. Exp. Pathol. 67: 587–596

Konno, K., Feldmann, F. M., McDermott, W. (1967) Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am. Rev. Respir. Dis. 95: 461–469

McCune, R. M., Feldmann, F. M., Lambert, H. P., McDermott, W. (1956) The fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculous infection to the latent state by the administration of pyrazinamide and a companion drug. J. Exp. Med. 104: 763–802

McDermott, W., Tompsett, R. (1954) Activation of pyrazinamide and nicotinamide in acidic environments *in vitro*. Am. Rev. Tuberc. 70: 748–754

Mitchison, D. A., (1985) The action of antituberculosis drugs in short-course chemotherapy. Tubercle 66: 219–225

Rastogi, N., Potar, M.-C., David, H. L. (1988) Pyrazinamide is not effective against intracellularly growing *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 32: 287

Scorpio, A., Zhang, Y. (1996) Mutations in *pncA*, a gene encoding pyrazinamidasenicotinamidase, cause resistance to antituberculous drug pyrazinamide in tubercle bacillus. Nature Med. 2: 662–667

Sirgel, F. A., Botha, F. J. H., Parkin, D. P., Van de Wal, B. W., Donald, P. R., Clark, P. K., Mitchison, D. A. (1993) The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts. J. Antimicrob. Chemother. 32: 867–75

Wayne, L. G. (1994) Dormancy of *Mycobacterium tuberculosis* and latency of disease. Eur. J. Clin. Microbiol. Infect. Dis. 13: 908–914

Wayne, L. G., Sramek, H. A. (1994) Metronidazole is bactericidal to dormant cells of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 38: 2054–2058

Wayne, L. G., Hayes, L. G. (1996) An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. Infect. Immun. 64: 2062–2069

Wirth, R., Muscholl, A., Wanner, G. (1996) The role of pheromones in bacterial interactions. Trends Microbiol. 4: 96–100