

Growing Points in Leprosy Research

(4) Recent Advances in the Chemotherapy of Leprosy

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Recent advances in the chemotherapy of human and experimental leprosy are reviewed. These advances are beginning to place the treatment of leprosy on an objective bacteriological and pharmacological basis. The relevance of these recent studies to designing more effective and more conveniently administered regimens for the successful treatment of lepromatous leprosy is discussed.

Introduction

Several recent reviews provide excellent testimony to the decisive contribution made during the past decade by the mouse footpad model to advances in the treatment of leprosy (Shepard, 1969*a*, Rees and Weddell, 1970; Rees, 1971; Shepard, 1971*a, b*; Pattyn, 1972; Rees and Waters, 1972; Rees, 1973). It has provided methods for assessing the efficacy of established drugs and of successfully screening new compounds for potential use in the treatment of human leprosy. Through its use the chemotherapy of leprosy is at last beginning to be placed on an objective bacteriological and pharmacological basis.

For an anti-leprosy drug to be effective in man, well tolerated doses must produce concentrations in the body that at least temporarily exceed its minimal inhibitory concentration against *Myc. leprae*. The administration of each successive effective dose to a patient results in some viable drug-sensitive leprosy bacilli ceasing to multiply and in others being killed. The relative importance of these two effects depends on whether the drug is primarily bacteriostatic or bactericidal. The cell-mediated defence mechanisms of the body can also kill leprosy bacilli, although their ability in this respect decreases progressively as one moves from the tuberculoid to the lepromatous end of the clinical spectrum of leprosy. As effective treatment is continued the proportion of viable *Myc. leprae* steadily falls. In lepromatous patients this proportion can be estimated by inoculation into normal mice, inocula containing as few as 0.1% viable bacilli being infectious. By employing the mouse as an *in vivo* culture system to detect viable *Myc. leprae*, reductions of up to 99% can be measured in the numbers of viable bacilli in lepromatous patients. However this end-point may still be equivalent to as many as 10^7 viable *Myc. leprae* and if treatment were terminated at this point relapse would almost certainly occur. Non-viable leprosy bacilli gradually become morphologically degenerate so that the bacterial killing can be monitored indirectly by determining the rate of fall of the morphological index. Although such a procedure is relatively simple to carry out and gives

immediate results, it is only about a tenth as sensitive as the mouse inoculation method. Furthermore, by its very nature, it necessarily underestimates both the absolute and relative rates of killing of *Myco. leprae* by actively bactericidal drugs. The acid-fast fragments of morphologically degenerate *Myco. leprae* are then slowly removed, a process followed by measuring the rate of fall of the bacterial index. Although such a measure may be fundamentally correlated with the remission of the lepromatous patient's histological and clinical symptoms, it is inherently incapable of distinguishing between the relative efficacies of different antileprosy drugs.

Significant numbers of naturally drug-resistant leprosy bacilli are however to be expected among the large bacterial populations harboured by patients with untreated lepromatous leprosy. In these patients the number of viable *Myco. leprae* may total 10^{10} - 10^{11} . In tuberculosis the proportion of naturally resistant bacilli can vary from about 1 in 10^6 to 1 in 10^8 according to the drug studied. When only a single drug is given the drug-resistant leprosy bacilli may ultimately multiply to such an extent that the patient relapses and inocula may once more become capable of infecting mice. Their multiplication in the mouse will however only be prevented, if at all, by giving much higher dietary concentrations of the drug than are required to suppress the growth of strains of *Myco. leprae* derived from untreated patients.

Treatment of Leprosy

DAPSONE

Feeding 0.0001% dapsone (DDS) in the diet to mice results in continuous plasma concentrations of about 0.01 $\mu\text{g/ml}$ and completely inhibits the multiplication of strains of *Myco. leprae* from untreated patients. Since multiplication was not inhibited when the dose was lowered to 0.00001% it was estimated that the minimal inhibitory concentration of dapsone against *Myco. leprae* was between 0.0001 and 0.01 $\mu\text{g/ml}$ (Shepard, McRae and Habas, 1966; Rees, 1967*a, b*; Shepard, 1967*a*; Shepard, Tolentino and McRae, 1968; Ellard *et al.*, 1971; Ozawa, Shepard and Karat, 1971). The minimal inhibitory concentration of dapsone against one strain of *Myco. leprae* has been determined with greater precision in Lewis rats and shown to lie between 0.0015 and 0.004 $\mu\text{g/ml}$ (Peters *et al.*, 1972*b*). Kinetic studies in the mouse have consistently demonstrated the ability of dapsone to cause significant growth delays, but the results have been interpreted by some as due to the induction of prolonged bacteriostasis and by others as the result of limited bacterial killing (Shepard, 1967*a, b*; 1969*b*; Holmes, 1972; Holmes and Hilson, 1972; Levy, 1972). However the ability of doses of 0.01% dapsone given once-weekly or 0.1% given every 14 days in preventing the multiplication of *Myco. leprae* (Rees cited Shepard, 1967*a*; Pattyn and Saerens 1974), despite its rapid elimination in the mouse (Ellard *et al.*, 1971; Ozawa, Shepard and Karat, 1971; Levy *et al.*, 1972*a*), strongly suggests that dapsone is capable of inducing prolonged bacteriostasis in *Myco. leprae*.

Previous colorimetric methods for the determination of dapsone based on reaction with *p*-dimethylamino-benzaldehyde or on diazotisation followed by coupling with *N*-1-naphthyl-ethylene-diamine, were too insensitive and unspecific to determine accurately its plasma concentrations. Highly specific fluorimetric methods have however been recently developed that are capable of measuring plasma dapsone concentrations of down to 0.01 $\mu\text{g/ml}$ (Glazko *et al.*, 1968; Ellard and Gammon, 1969; Peters, Gordon and Colwell, 1970) or even to 0.001 $\mu\text{g/ml}$

(Murray, Gordon and Peters, 1971). A gas-chromatographic procedure has also been described for dapsone (Burchfield *et al.*, 1973), while a simple urine-test method has been developed which has revealed the potential seriousness of irregular self-administration of the drug by out-patients (Ellard, Gammon and Harris, 1974; Ellard *et al.*, 1974b).

Dapsone is rapidly and completely adsorbed in man. The only metabolite demonstrated so far in the plasma is monoacetyl-dapsone (MADDS) and slow or rapid acetylators of dapsone can be distinguished according to the ratios of acetylated to free drug found in the plasma (Gelber *et al.*, 1971; Peters *et al.*, 1972a; Ellard *et al.*, 1974c). It has not however been possible to establish whether this metabolite has intrinsic anti-leprosy activity because it is rapidly and completely deacetylated in the mouse (Levy *et al.*, 1972a). Dapsone is eliminated relatively slowly in man. The dapsone half-lives of patients can differ significantly (range 14-53 h), but are not related to their acetylator phenotype (Peters *et al.*, 1972a; Gelber and Rees, 1973; Ellard, Gammon and Harris, 1974).

The treatment of lepromatous patients with doses of dapsone ranging from as little as 1 mg a day to 300 mg twice a week results in the proportion of viable leprosy bacilli falling to less than 1% of the initial value over a period of about 3 months and in morphological indices falling to baseline values within 4½-6 months (Waters and Pettit, 1965; Pettit and Rees, 1967; Shepard, Tolentino and McRae, 1968; Pearson and Pettit, 1969; Ellard *et al.*, 1971).

Small numbers of viable *Myco. leprae* do however persist, often in preferred sites such as peripheral nerve or striated muscle (Pearson, Rees and Weddell, 1970), despite many years of continuous dapsone treatment (Waters *et al.*, 1973). Since these persisting bacilli may retain their sensitivity to dapsone (Waters *et al.*, 1973), and since experimental studies in the mouse, rat, dog and sheep have demonstrated that dapsone readily penetrates these and other tissues (Francis, 1953; Shepard and Chang, 1964; Peters, 1973; Weddell *et al.*, 1974), it is probable that these bacilli had remained dormant throughout the treatment period. Perhaps the sites in which they were situated had protected them from the extremely limited cell-mediated defence mechanisms displayed against *Myco. leprae* by lepromatous patients. These findings readily explain why lepromatous patients must be treated for so many years if permanent cures are to be achieved.

Relapses due to the appearance of dapsone-resistant leprosy bacilli were first demonstrated in Malaya some 10 years ago (Pettit and Rees, 1964). Other dapsone-resistant strains of *Myco. leprae* have since been isolated from relapsed lepromatous patients from many different parts of the world (Pettit, Rees and Ridley, 1966, Rees, 1967a, b; Shepard, Levy and Fasal, 1969). During the past 10 years some 2.5% of the lepromatous patients who began treatment with dapsone in Malaya from 1949 to 1963 have relapsed with proven dapsone-resistant leprosy after from 6 to 26 years of treatment (Meade *et al.*, 1973). Pharmacological studies indicate that among these patients relapse with dapsone-resistant *Myco. leprae* was not associated with either the rate of acetylation of dapsone or its elimination from the body (Ellard *et al.*, 1972; Gelber and Rees, 1973).

ACEDAPSONE

The demonstration that two-monthly injections of 6 mg/kg acedapsone (*N,N'*-diacetyl-dapsone, DADDS), a slow-release form of dapsone, suppressed the multiplication of *Myco. leprae* in the mouse (Shepard, 1967a) led to its evaluation for the treatment of human leprosy. Doses (225 mg) of acedapsone given

intramuscularly once every 11 weeks enable dapsone plasma concentrations of between about 0.03-0.06 $\mu\text{g/ml}$ to be maintained (Glazko *et al.*, 1968; Ozawa, Shepard and Karat, 1971; Russell *et al.*, 1973), and are therapeutically effective although the initial rate of fall in the number of viable *Myco. leprae* is rather slower than when 50 mg dapsone is given daily (Shepard, Levy and Fasal, 1968, 1972a).

LONG-ACTING SULPHONAMIDES

These compounds are only weakly active against *Myco. leprae* (Ellard, Gammon and Rees, 1970), and are inactive against dapsone-resistant strains (Adams and Waters, 1966; Rees, 1967a; Pattyn *et al.*, 1972). Since they are considerably more expensive than dapsone their continued use in the treatment of leprosy appears unjustified.

RIFAMPICIN

The sensitivity of *Myco. leprae* to rifampicin was first demonstrated in the mouse by Rees, Pearson and Waters, (1970), its minimal inhibitory concentration being about 0.3 $\mu\text{g/ml}$ (Holmes and Hilson, 1972). Subsequent kinetic studies revealed rifampicin's powerful bactericidal activity, infections apparently being sterilized by feeding 0.01% of the drug for 30 days (Holmes, 1972; Holmes and Hilson, 1972), 0.03% for 2 days (Shepard *et al.*, 1971), by giving 2 doses of 25 mg/kg separated by 70 days or a single 40 mg/kg dose (Shepard, Levy and Fasal, 1972b, c). In lepromatous patients the bactericidal activity of 600 mg rifampicin daily was equally impressive. Morphological indices fell to base-line values within 4 weeks (Rees, Pearson and Waters, 1970) and after only 3-7 days treatment viable bacilli could no longer be recovered (Shepard, Levy and Fasal, 1972b, c). Similar results were obtained when a single dose of 1500 mg rifampicin was given (Levy, Shepard and Fasal, 1973). Rifampicin is excellently absorbed in man, and its pharmacology has recently been studied using extremely sensitive microbiological methods (Dickinson *et al.*, 1974).

CLOFAZIMINE

Although the multiplication of *Myco. leprae* is prevented by feeding mice 0.0001% clofazimine (Shepard, 1969c), its minimal inhibitory concentration cannot be estimated because of the marked accumulation of the drug by reticulo-endothelial cells (Barry, 1969). In man the therapeutic response obtained with daily doses of 200-300 mg clofazimine is similar to that achieved with dapsone (Pettit and Rees, 1966; Pettit, Rees and Ridley, 1967; Levy, Shepard and Fasal, 1972b). Clofazimine also aids the control of *erythema nodosum leprosum* (Browne, 1965; Helmy, Pearson and Waters, 1972), although many light-skinned patients find the marked skin pigmentation caused by prolonged treatment unacceptable. Intermittent clofazimine treatment is highly successful in mice (Shepard *et al.*, 1971; Banerjee and Hilson, 1973), but results in man have been disappointing (U.S. Leprosy Panel/Leonard Wood Memorial, 1972). Its pharmacology in both mouse and man appears complex (Banerjee *et al.*, 1974; Levy, 1974).

THIAMBUTOSINE—OTHER DIPHENYL THIOUREAS—THIACETAZONE

Thiambutosine (Ciba 1906; *p*-butoxy-*p'*-dimethylamino-diphenyl-thiourea), thiocarlide (Isoxyl; *p,p'*-diisoamyloxy-diphenyl-thiourea), a number of other

diphenylthioureas and thiacetazone have all been shown to prevent the multiplication of *Myco. leprae* in the mouse, although their minimal inhibitory concentrations have yet to be determined (Pattyn and Royackers, 1965; Rees, 1965, 1967b; Gaugas, 1967; Shepard, 1967b; Hilson, Banerjee and Holmes, 1971; Pattyn and Wagner, 1972). These drugs are well tolerated in man, and showed initial promise in the treatment of human leprosy, but after 2-4 years' treatment relapses occurred due to the appearance of drug resistant *Myco. leprae* (Lowe, 1954; Davey, 1958, 1960; Quyen, Buu-Hoi and Xuong, 1960; Griffiths, 1965; Leading Article, 1965; Rees, 1965; Garrod and Ellard, 1968; East African/British Medical Research Council, 1970; Miller *et al.*, 1970). Cross-resistance is shown to these drugs by both *Myco. leprae* and *Myco. tuberculosis* suggesting they have a common mode of action (Rees, 1967a, b). Studies against *Myco. tuberculosis* indicate that they are likely to be purely bacteriostatic (Dickinson and Mitchison, 1966a, b). Specific chemical methods have yet to be developed to enable the plasma concentrations of the diphenyl thioureas to be determined. Pharmacological studies indicate that thiacetazone is well absorbed in man (Ellard *et al.*, 1974a), but both thiambutosine and thiocarlide are poorly absorbed (Ellard and Naylor, 1961; Emerson and Nicholson, 1965).

ETHIONAMIDE

This drug displays significant bactericidal activity against *Myco. leprae* in the mouse but is unfortunately not well tolerated in man (Fox *et al.*, 1969; Shepard, 1969b, c, 1972; Rollier and Rollier, 1972). More sensitive analytical methods are needed to enable its minimal inhibitory concentration against *Myco. leprae* to be determined.

Discussion

Regimens, if they are to be of a widespread use in the treatment of leprosy, need to be highly effective, cheap, easily administered and of low toxicity. The accepted practice of treating patients with tuberculoid and borderline leprosy with dapsone alone is convenient and successful. However in lepromatous patients, with large populations of leprosy bacilli and poor immunological response, there is a strong case for commencing treatment with combined chemotherapy in the hope of preventing subsequent relapse through the appearance of drug-resistant *Myco. leprae* (Rees, 1973). Obviously one of the drugs given must be dapsone. The recommended oral dose is 50-100 mg daily, since lower doses are no less toxic (Pearson and Pettit, 1969; Shepard, Levy and Fasal, 1972a; Pearson and Helmy, 1973; Russel *et al.*, 1973) and may encourage the growth of dapsone-resistant mutants of *Myco. leprae*. If at all possible these daily dapsone doses should be given under strict supervision since experience with other diseases demonstrates how irregular self-medication can be (Fox, 1962, 1968, 1972). An alternative treatment procedure, which might be of considerable value in urban situations, would be to give out-patients weekly supervised doses of 300 mg dapsone orally. If regularly taken such doses should maintain continuously inhibitory dapsone concentrations, and all multiplication of dapsone-sensitive *Myco. leprae* would probably be prevented even if the occasional dose were missed. Once and twice-weekly treatment schedules have been successfully used for tuberculosis patients in the Third World (Fox, 1968, 1971, 1972).

The companion drug of choice is rifampicin. However in view of its extremely

high cost the amount of rifampicin treatment that can be given is very limited. The results already obtained nevertheless indicate that as few as 3-7 daily doses of 600 mg rifampicin or even a single dose of 1500 mg rifampicin can reduce the number of viable *Myco. leprae* in lepromatous patients to less than 1% of their original total (Shepard, Levy and Fasal, 1972b, c; Levy, Shepard and Fasal, 1973).

Unfortunately the mouse footpad inoculation method is incapable of detecting the lethal action of further rifampicin doses so that their immediate benefit cannot be evaluated. However since studies with *Myco. tuberculosis* indicate that rifampicin only kills growing bacilli (Dickinson, Jackett and Mitchison, 1972), and since the great majority of such bacilli are probably killed by the first few doses of rifampicin, it would appear that continuing rifampicin treatment for more than a few weeks would be unlikely to justify the cost incurred. Thiambutosine at a dose of 1500 mg daily might also be employed as a companion drug for dapsone, but it would not be expected to aid the killing of *Myco. leprae* substantially. For the treatment of patients with proven dapsone-resistant strains of *Myco. leprae* combinations of rifampicin, thiambutosine or clofazimine are recommended.

When treatment has been continued to the stage that only the occasional viable leprosy bacillus can be detected, oral dapsone therapy might be conveniently replaced by intramuscular injections of 225 mg acedapsone given once every 3 months and continued until all signs of active leprosy have disappeared and a permanent cure seemed to have been achieved.

The knowledge gained from recent controlled clinical trials and experimental studies in the mouse should be of considerable assistance in designing regimens that are less likely to fail through the appearance of dapsone-resistant strains of *Myco. leprae*. Whether rifampicin will be more effective than dapsone in eliminating persisting leprosy bacilli remains to be established. If it were at all active in this respect it might significantly reduce the numbers of years treatment required to achieve the permanent cure of lepromatous patients. Ultimately however, despite the immense difficulties involved, the success of this and of any other new treatment procedure must be established by means of controlled clinical trials of many years duration and involving many patients.

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