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Editorial

RECENT ADVANCES IN THE CHEMOTHERAPY OF LEPROSY

The classical strategy of leprosy control, based on early detection and effective chemotherapy, is likely to remain unchanged for many years.[1,2] Effective chemotherapy will still be necessary for the treatment of millions of leprosy patients even after a leprosy vaccine with proved effectiveness becomes available.

The most important progress made in the history of leprosy control was the development and implementation of multidrug therapy (MDT) for both paucibacillary* (PB) and multibacillary* (MB) leprosy. Due to MDT, new hopes of controlling leprosy as a major public health problem have been raised. Around the topic of MDT we are trying to review the recent advances in the chemotherapy of leprosy.

Basic concept of multidrug therapy

By the end of the 1970s it was clear that attempts to control leprosy by life-long dapsone monotherapy were failing, because of the rapid increase of dapsone-resistance. Multiple surveys conducted in a number of leprosy-endemic areas clearly demonstrated that both secondary and primary dapsone-resistant leprosy have been found wherever they have been sought;[1,3] the prevalence of secondary dapsone-resistance increased with time if dapsone monotherapy continued, even if patients were treated regularly and were well supervised; the prevalence of primary dapsone-resistant leprosy, which was virtually nonexistent before 1977,[4] had become alarmingly high, about one-third of newly diagnosed MB patients were resistant, although the majority of the primary resistance was of a low degree.[5–7] It must be assumed that primary dapsone resistance, unlike secondary resistance, occurs in at least as high a proportion of PB leprosy as of MB leprosy. By analogy with what was known about the treatment of tuberculosis, it gradually became understood that selection of resistant mutants by monotherapy was involved in the emergence of drug resistance in MB leprosy; and the only way to prevent

* To classify leprosy patients into PB and MB leprosy is essentially an operational categorization for purposes of MDT [1] The WHO Expert Committee (1988) has recommended that : (1) PB leprosy includes only smear-negative indeterminate (I), polar–tuberculoid (TT) and borderline–tuberculoid (BT) cases in the Ridley–Jopling classification; or indeterminate (I) and tuberculoid (T) cases in the Madrid classification. Any case belonging to these types but showing smear positivity will be classified as MB; and (2) MB leprosy includes all midborderline (BB), borderline–lepromatous (BL) and polar lepromatous (LL) cases in the Ridley–Jopling classification or borderline (B) and lepromatous (L) in the Madrid classification, as well as any other smear-positive types [8]

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the emergence of dapsone resistance and the spread of the dapsone-resistant leprosy was to use multidrug therapy. It was recommended that combined therapy with rifampicin and dapsone should be used for all PB leprosy; and at least two additional drugs should be combined with dapsone for the treatment of MB leprosy, and one of the two additional drugs should always be rifampicin because of its great potency.[1] Another problem that was particularly worrying was that the persisting viable, drug-susceptible *Mycobacterium leprae* (persisters) had been detected in MB patients even after treatment of many years.[1] It was feared that after the therapy had been withdrawn, persisters would cause relapse in a large proportion of patients, and it was hoped that the multidrug therapy might be able to eliminate such persisting organisms.

During the dapsone-monotherapy era, poor compliance with the patients' self-administration of dapsone had been a serious problem in most leprosy control programmes; typically, only about half of the prescribed doses were ingested,[9] and irregular treatment appeared to predispose the development of dapsone resistance.[1] The unsupervised, self-administered chemotherapy simply could not be enforced for long periods of time. Many control programmes continued to have high drop-out rates. To improve the compliance and the case-holding, MDT should be administered only for a limited period of time, and whenever feasible the regimens should contain a high degree of supervised drug administration.

Based on these reasons, and the knowledge regarding the sizes and the compositions of the bacterial populations in PB and MB leprosy, the efficacies of available antileprosy drugs and the immunological status of the patients, the following standard MDT regimens were recommended by the WHO Study Group:[1]

PB leprosy—Rifampicin 600 mg once-monthly for 6 months, supervised, plus dapsone 100 mg (1–2 mg/kg body weight) daily, self-administered, for 6 months.

MB leprosy—Rifampicin 600 mg once-monthly, supervised, plus dapsone 100 mg daily, self-administered, plus clofazimine 300 mg once-monthly, supervised, and 50 mg daily, self-administered. The duration of treatment should be at least two years and be continued, wherever possible, up to smear negativity. Where clofazimine is totally unacceptable owing to the coloration of skin lesions that it caused, its replacement by 250–375 mg self-administered daily doses of ethionamide/protionamide should be considered.

The two standard regimens have been endorsed by the WHO Expert Committee on Leprosy (1988),[8] except that substitution of ethionamide/protionamide for clofazimine was not recommended because of potential serious toxic side-effects.

Overall achievements of multidrug therapy

Since the recommendation of MDT in 1982, many leprosy endemic countries have accepted and introduced, or are in the process of introducing, the MDT regimens. By the end of 1989, 1·8 million patients in the world were being treated with MDT and 900,000 patients had completed their treatment.[10] In 1988, for the first time in the history of leprosy control, the number of registered leprosy cases showed a slight decline, to 5·1 million,[8] and the number of registered cases further declined to 3·9 million by the end of

1989.[10] Although such decline could be partly due to the discharging of inactive patients during screening of all cases at the preparatory phase of implementing MDT,[11] there is no doubt that the effectiveness of MDT and shortening of the duration of treatment also played an important role.

Both regimens are very effective, as the clinical response during treatment is satisfactory, and so far the relapse rate after stopping treatment is low: in control programmes, less than 0·1% per year among 85,000 PB cases and less than 0·06% per year among 22,000 MB cases;[10] in field trials, 4·17 per 1000 person years in PB leprosy during the first year after completion of MDT,[12] and no relapse in more than 8000 person years of follow-up in MB cases.[13] It is important to point out that no relapse due to a drug-resistant strain, especially resistant to rifampicin, has yet been reported. With respect to the persisters, the results of a clinical trial has suggested that they were present in virtually all MB patients and did not respond to any of the 5 rifampicin-containing combined regimens;[14] the very low relapse rate in MB leprosy after stopping MDT strongly indicated that the presence of persisters did not carry a high risk of relapse following the termination of treatment. In Karigiri, India, the incidence rate in a population of 35,000 has been monitored annually since 1980 and MDT was introduced into the area in 1982. The incidence rate was 1·46/1000 during 1982–84 and 0·85/1000 in 1985–87; the 40% reduction suggested that effective interruption of transmission may be achieved much earlier by community-wide treatment of patients with MDT than with dapsone monotherapy.[15]

Both MDT regimens were well tolerated by the patients except that the coloration of skin caused by clofazimine has been a problem in certain light-skinned patients,[17] and side-effects were extremely rare and mild. High motivation of patients as well as health workers has also been reported; in terms of attendance rate, the regularity of treatment has been excellent.[8]

The needs for improved multidrug regimens

In order to minimize relapse after stopping treatment, it was recommended that the duration of MDT for MB leprosy should be at least two years, because it was thought that this is the minimum period of treatment to ensure the elimination of drug-resistant mutants, especially rifampicin-resistant mutants, and to reduce the number of drug-susceptible viable organisms to a low level which will not cause an unacceptable relapse rate. A recent clinical trial demonstrated that the number of viable organisms have been reduced to no more than 10^6 after a single dose of rifampicin.[14] By definition, the response of the dapsone- and clofazimine-resistant mutants to rifampicin should be the same as drug-susceptible organisms and to be killed rapidly during the course of rifampicin treatment. The major problem is that the necessary duration of treatment with MDT to eliminate rifampicin-resistant mutants is still unknown. Failure to isolate rifampicin-resistant mutants from nude mice infected with a large number of *M. leprae* suggested that the frequency of such mutants might be smaller than 10^{-7} (unpublished observation), thus, the average size of the rifampicin-resistant *M. leprae* in an untreated lepromatous patient is no more than 10^4. However, rifampicin-resistant mutants can only be killed by dapsone plus clofazimine in the combined regimens. A clinical trial is being undertaken with lepromatous patients aiming to assess the necessary duration of treatment for the

killing of 4 logs of *M. leprae* by dapsone plus clofazimine. The other approach is to investigate the efficacy, measured by relapse rate, of fixed duration (two years) MDT among previously untreated MB patients though field trials.[13,17] Before such information becomes available, there is no justification in reducing the duration of the current MDT regimen for MB leprosy to less than 2 years in any control programme. From an operational point of view, the recommended duration is still relatively long and the monthly supervised treatment for 24 months or even longer cannot always be applied in certain areas. It is likely that the coverage of MDT may be significantly improved if the duration of MDT can be substantially reduced.

There are more complaints of the MDT regimen for PB leprosy. The major concern is the persistence of active lesions at the end of 6 months treatment, which ranged from 4·3[12] to 27·8%.[18] Although the active lesions may gradually resolve and finally disappear within 1 to 2 years after stopping MDT,[17] the details of such regression have not yet been well documented. A certain number of health workers and patients wish to continue the treatment until the lesions are inactive. It was claimed that the inactivation rate of the lesions had significantly improved and that relapse after stopping treatment might be prevented if the 6 months MDT was continued by an additional 6 months dapsone monotherapy,[18,19] but the observations still require further investigation and independent verification. Another concern is reversal reaction, especially the late reaction which appears after stopping MDT, because it may cause irreversible deformities. The incidence rate of late reversal reaction ranged from 5%[12] to 9%.[19] Due to a lack of well documented baseline data and well planned controlled studies, no definite conclusion can be drawn as to whether or not reversal reaction has become more common since the introduction of MDT. The claim that an additional 6 months' dapsone treatment might prevent the late reversal reaction[19] should be verified. The question that remains to be answered is whether or not the inactivation of PB lesions and the development of reversal reaction are correlated with the bactericidal effect of chemotherapy. It is desirable to develop regimen(s) which may accelerate the inactivation rate of lesions and reduce the reversal reaction, although the approaches may not necessarily be chemotherapy.

The need for new bactericidal drugs against *M. leprae*

One of the basic concepts of MDT is that the treatment be administered for only a limited period, and therefore only bactericidal drugs should be considered as candidates for MDT regimens.[1] To date only four antileprosy drugs, i.e. dapsone, rifampicin, clofazimine and thioamide (ethionamide/protionamide), with bactericidal activities that act by different mechanisms, are available. Because thioamides may cause hepatotoxicity especially when combined with rifampicin, neither thioamide should be used as a component of MDT under field conditions unless absolutely necessary.[8] Consequently, apart from the combination of rifampicin, dapsone and clofazimine, the choice for an alternative MDT regimen for MB leprosy is practically nil. If a MB patient does not accept clofazimine because of skin coloration, he/she has no chance to be treated with MDT. In addition, in view of the fact that both dapsone and clofazimine are only weak bactericides against *M. leprae*, it is very unlikely that the current composition of MDT allows for a substantial reduction in the duration of treatment for MB leprosy without introducing another strong bactericidal drug into the regimen. Furthermore, rifampicin

was employed in certain areas for the treatment of leprosy before the introduction of MDT, usually for patients who had relapsed after prolonged dapsone monotherapy, and adding rifampicin to such patients probably represented a change of dapsone monotherapy to monotherapy with rifampicin. We have observed that at least 5% (22 out of 404) of such patients have relapsed with secondary rifampicin resistance, and 90% of the rifampicin-resistant strains were also resistant to dapsone.[20] The treatment of these doubly resistant patients is extremely difficult. A recent study demonstrated that close to 30% of patients in Karigiri, one of the best leprosy control programmes in the world and where clofazimine is well accepted by the patients because of their dark-skin, did not take their prescribed dapsone and clofazimine properly,[9] suggesting that, simply because of noncompliance, it is still possible for rifampicin resistance to be developed in a programme where MDT has been implemented. Although the magnitude of this threat to the success of MDT is still unclear, the problem of rifampicin resistance must not be ignored. For these reasons, new antileprosy drugs with bactericidal mechanisms entirely different from those of existing drugs are urgently needed.

Ideally, a new antileprosy compound should possess the following characteristics: strong bactericidal mechanisms against *M. leprae* without antagonism to available drugs; safe and well accepted by the patients; can be administered orally, and its pharmacokinetic properties allow the treatment to be given no more than once daily. The prospect is bleak for a compound if it requires to be given by injection, because it is difficult to implement under field conditions, and the recent epidemiological trend of HIV infection further hampers the application of multiple-injection treatment in rural areas.

Strategies of developing new drugs

Before 1960, the only approach to the search for an antileprosy drug was to conduct clinical trials. The progress was extremely slow, and the results were difficult to interpret. Now, there are two different approaches for the development of new antileprosy drugs: 1, synthesis of new compounds; and 2, the screening of existing compounds.

With the development of experimental infection in armadillo[21] and the method of purification of *M. leprae* from infected armadillo tissue,[22] a limited amount[23] of purified *M. leprae* has been obtained for studies of the metabolism and physiology of *M. leprae*. The scientific progress in the understanding of the metabolism and physiology of *M. leprae*,[23–26] and the techniques for producing and studying the structures of the potential target enzymes have made it possible to exploit such knowledge and capabilities for synthesizing new compounds in a systematic fashion—the process becoming known as rational drug development. In order to provide a large amount of *M. leprae*-derived enzymes that are supposed to be the targets of antileprosy drugs for screening, the genes encoding the dihydrofolate reductase, RNA polymerase and DNA gyrase are being cloned, and new inhibitors of several target enzymes are being synthesized. Unfortunately, rational drug design for leprosy is certainly not a high priority of the pharmaceutical industry because of the lack of commercial viability.

The laboratory screening of antileprosy drugs was started only after the mouse footpad model of *M. leprae* infection[27] became available. Huge amounts of different classes of antimicrobials are being developed every year. The recent discovery of strong bactericidal activities of pefloxacin,[28,29] ofloxacin,[30,31] minocycline[32] and clarithromycin[33]

clearly demonstrate that the screening of existing compounds is still the most practical approach. Of course, random screening is not possible due to limited facilities and it is also uneconomic. The candidates should focus on compounds which show strong activity, in terms of MICs, against Gram-positive organisms and particularly against cultivable mycobacteria. Favourable pharmacokinetic properties, e.g. better absorption rate or longer half-life, are also critical in selecting the analogues of active compounds, e.g. newer fluoroquinolones and macrolides, for screening.

Methods in screening antileprosy drugs

The mouse footpad technique is by far the only universally accepted experimental system for the study of drug activity against *M. leprae*. Three methods are employed for drug screening by this technique: 1, the continuous method;[34] 2, the kinetic method;[35,36] and 3, the proportional bactericidal method.[37] The methods and their applications have recently been reviewed.[38] All of the effective antileprosy drugs have been demonstrated to exert at least bacteriostatic activity in this system, whereas no compound shown to be inactive in mice has been demonstrated to exert definite therapeutic effects in leprosy patients. Nevertheless, the mouse footpad technique possesses several disadvantages. It is time consuming, requires many mice and gramme amounts of the compounds to be tested, and can therefore be employed to investigate only limited numbers of compounds that represent a very few selected classes.

The search for active compounds representing a wide variety of classes requires a rapid primary screening method that will yield results within days or, at most, a few weeks, and that requires only milligrammes rather than gramme amounts of the tested compounds. Ideally, drugs should be screened *in vitro*.

Despite the fact that *M. leprae* cannot be cultivated *in vitro*, and taking the advantage that viable organisms still retain many of their metabolic functions for a limited period of time outside the host,[39] within the last decade many systems have been reported to be capable of rapidly demonstrating the *in vitro* activity of a compound to kill or to impair the key metabolic processes of *M. leprae*. These techniques include the use of radiolabelled substrates to investigate the incorporation by *M. leprae* of dihydroxyphenylalanine (DOPA),[40] thymidinein,[41,42] hypoxanthine[43,44] and uracil;[45] measurement of changes of the adenosine triphosphate (ATP) content of *M. leprae*[46] and the changes of intrabacterial ratio of sodium and potassium;[47,48] changes of Fc receptors and sialic acid on the surface of macrophages[45] and changes of the ratio of cholesterol to cholesterol esters within macrophages[49] which have phagocytosed *M. leprae*; and changes of staining by fluorescein diacetate and ethidium bromide.[45,50] Recently three different *in vitro* screening systems have been developed by the scientists at Carville. It has been reported that the incorporation of ^{14}C-palmitic acid into phenolic glycolipid-1 (PGL-1), the *M. leprae*-specific antigen, has been suppressed in the presence of all known antileprosy drugs as well as other compounds in both intracellular[51] and extracellular *M. leprae*.[52,53] Another method is based on the measurement of intracellular ATP of *M. leprae* incubated in an axenic modified Dubos medium. Over 25 antimicrobial agents have been evaluated, and, except dapsone, 3 of the 4 most commonly used antileprosy drugs (rifampicin, clofazimine and ethionamide) demonstrated activity in this system.[54] The system appeared suitable for assessing comparative activity of new structural analogues of

clofazimine.[54,55] The third method is based on the measurement, by radiorespirometry with the Buddemeyer-type counting system[56] or with the BACTEC 460 system,[57] of the oxidation of ^{14}C-palmitic acid to $^{14}CO_2$ by *M. leprae*. It was reported that these are the simplest systems described to date and all established antileprosy drugs displayed significant activities by the method.[56,57] Its simplicity has facilitated screening of a variety of macrolides,[33] fluoroquinolone derivatives[58] and clofazimine derivatives.[55,59]

Because so many *in vitro* screening methods have been reported, it is of great urgency that highly specific and sensitive methods be identified. It is possible that in future more than one method will be employed simultaneously, such as a combination of palmitic acid oxidation and ATP measurements.[33,56] The evaluation of the methods must be conducted 'blindly', by testing a series of coded compounds by the respective investigators who developed the techniques. At the same time, independent assessments and verifications by other investigators are also needed.

The *in vitro* method may serve the purpose of primary drug screening and in the detection of secondary drug resistance,[42,56,57] but certainly the compounds found to be active *in vitro* should not yet be tested in humans. Besides the study of pharmacokinetics and toxicities, the activity against *M. leprae* must be firmly established in the mouse footpad system prior to the initiation of a clinical trial.

New drugs with bactericidal activity against *M. leprae*

FLUOROQUINOLONES

The fluoroquinolones inhibit bacterial DNA gyrase, a target which had never been exploited in leprosy chemotherapy. In view of the strong activities against Gram-positive microorganisms and the pharmacokinetic properties, we have tested, in the mouse footpad system, the activities against *M. leprae* of three major commercially available derivatives: ciprofloxacin, pefloxacin and ofloxacin. It turned out that ciprofloxacin was inactive even in the dosage of 150 mg/kg daily by continuous method, mainly because of its unfavourable pharmacokinetic properties;[28] pefloxacin 150 mg/kg daily displayed bactericidal activity;[28] ofloxacin 50 mg/kg daily exerted the same effects as pefloxacin 150 mg/kg daily, and ofloxacin 150 mg/kg daily displayed profound killing activity.[30] These observations, confirmed by other investigators,[58,60,61] represented the first lead to an important new antileprosy drug in many years.

As a first clinical trial of a fluoroquinolone derivative in leprosy, 10 previously untreated lepromatous patients (two-fifths of them with primary dapsone resistance) were treated with pefloxacin 400 mg twice daily for 6 months.[29] Definite clinical improvement was observed in all 10 patients as early as 2 months after beginning treatment, and the morphological index (MI) has also drastically decreased to the baseline during the same period. The rapid bactericidal effects, as measured by serial mouse footpad inoculations with organisms recovered from biopsies taken before and at different intervals during treatment, were demonstrated to the extent that about 99% of the bacilli were killed during the first two months of treatment. However, the bacterial load (in terms of the bacterial index (BI) and the number of acid-fast bacilli per mg of tissue) of the patients was only moderately reduced. The side-effects were mild, and the patients tolerated the treatment well.

The second clinical trial was to compare the therapeutic effects and side-effects

between pefloxacin 800 mg and ofloxacin 400 mg once daily among 21 previously untreated lepromatous cases.[31] The trial consisted of two parts: monotherapy from Day 1 to Day 56 except stopping treatment from Day 2 to Day 6; and combined with WHO/MDT regimen for MB leprosy from Day 57 to Day 180. The clinical improvement, and the evolution of MI and BI during monotherapy with either compound were virtually the same as had been observed in the first trial. The most important observation was the demonstration of rapid bactericidal activities of both treatments by serial mouse footpad inoculations with immunologically intact (normal) and congenitally athymic (nude) mice. Although a single dose of pefloxacin or ofloxacin only displayed a modest degree of bactericidal effect, about 99·99%, or 4 'logs', of organisms viable on Day 0 were killed by 22 doses of either treatment, and no significant difference could be detected between the two regimens.[31] Except for rifampicin, no other drugs thus far tested in humans have demonstrated such a degree of bactericidal activity. The side-effects were rare and mild, except one patient who developed a psychic disorder after 21 doses of pefloxacin monotherapy. All patients tolerated extremely well the combination of fluoroquinolone plus WHO/MDT.

As it has been demonstrated that 4 'logs' of viable *M. leprae* (more or less the same amount of rifampicin-resistant mutants present in a lepromatous patient before treatment) have been killed after 22 daily doses of either pefloxacin or ofloxacin; and by definition, rifampicin-resistant mutants should be killed by fluoroquinolone at the same speed as rifampicin-susceptible organisms, thus, all the rifampicin-resistant mutants may be eliminated after 22 doses of either pefloxacin or ofloxacin. It is, therefore, possible that the combination of ofloxacin and rifampicin may considerably shorten the required duration of MDT. A multicentric field trial is being organized to test the hypothesis.

The fluoroquinolones are rapidly developing,[62] with many new compounds appearing that might be more active against *M. leprae* than pefloxacin/ofloxacin. It is important to be on the alert for new compounds with lower MICs against Gram-positive organisms, or those with favourable pharmacokinetic properties. Recently it was reported that 7 newer fluoroquinolones, i.e. AT-4140, OPC-17100, OPC-17066, PD-117596, PD-124816, PD-127391 and WIN 57273, were more active against *M. leprae in vitro* than ofloxacin.[58] Further *in vivo* evaluations of these agents are required to determine their potential for the treatment of leprosy.

MINOCYCLINE

Among the tetracyclines, minocycline is unique in being active against *M. leprae*,[32,63] probably because its lipophilic properties allow it to penetrate the cell wall of *M. leprae* more effectively than other tetracyclines. Two tetracyclines, doxycycline and minocycline, have been tested in the mouse footpad system because of their greater *in vitro* activities against certain microorganisms included slow-growing mycobacteria. Doxycycline was inactive. However, by kinetic method and proportional bactericidal method, 0·01% (wt/wt) dietary minocycline was bacteriostatic, and higher dietary concentrations of minocycline, i.e. 0·02% to 0·04%, were bactericidal; minocycline combined with dapsone, kanamycin and rifampicin resulted in 'additive' antimicrobial activity against *M. leprae*; and the minimal inhibitory concentration (MIC) of minocycline against *M. leprae* was estimated at about 0·2 μg/ml, which is considerably less than levels (2–4 μg/ml) easily obtained in plasma and tissues of patients treated with customary doses.[32] We have also

demonstrated strong bactericidal activity in mice treated with 20 daily doses of 25 mg/kg minocycline by a proportional bactericidal test (unpublished observation). Because minocycline is an established antimicrobial and it seems to be safe in the long-term therapy of acne,[32] clinical trials are being conducted, aiming to evaluate the bactericidal activity of minocycline 100 mg daily in previously untreated lepromatous patients.

MACROLIDES

It was reported that erythromycin at ≥ 2 μg/ml was active against *M. leprae* by several *in vitro* methods,[33,53,54,56] but failed to inhibit the multiplication of *M. leprae* in mouse footpads probably due to its poor pharmacokinetics in mice.[33] In any case, erythromycin has no future in the treatment of human leprosy because of the need for daily multiple dosing due to its short half-life. Recently several semisynthetic macrolides, included azithromycin, clarithromycin, roxithromycin, M-119-31 and M-119-49, have been tested by *in vitro* methods, radiorespirometric assay of palmitic oxidation[56] and ATP assay.[54] These newer macrolides have superior acid stability and serum half-life as compared with erythromycin. Azithromycin was less active than, and M-119-49 had similar activity to, erythromycin; M-119-31 and roxithromycin appeared somewhat more active; whereas clarithromycin was the most active compound, causing significant inhibition at 0·125 μg/ml. When the drugs were administered at 0·01% (wt/wt) in the diet by kinetic method, probably also due to poor pharmacokinetics, roxithromycin was unable to inhibit the multiplication of *M. leprae* in mouse footpad as erythromycin, whereas clarithromycin demonstrated strong bactericidal activity against *M. leprae*. We also demonstrated the bactericidal activity in mice treated with 20 daily doses of 12·5–50 mg/kg clarithromycin by proportional bactericidal test (unpublished observation). It was estimated that the dietary concentration of 0·01% clarithromycin in mice corresponds to 100 mg daily in humans,[33] which is far lower than the clinically tolerated doses, 250 to 500 mg twice daily. Because clarithromycin is well tolerated in phase II and III clinical trials for various clinical conditions, clinical trials with different doses of clarithromycin among previously untreated lepromatous cases are being conducted.

PHENAZINE DERIVATIVES

Clofazimine, a phenazine derivative, is one of the important components of MDT regimen for MB leprosy because of its bactericidal activity against *M. leprae* and its anti-inflammatory effects in preventing and controlling type 2 reaction (erythema nodosum leprosum). A problem is the skin coloration it caused. Recently, a series of phenazine derivatives have been synthesized that do not result in skin coloration. Structure-activity relationships of 12 phenazines against *M. leprae* have been investigated by using *in vitro* radiorespirometric assay.[55] Most of the chlorinated phenazines were considerably more active *in vitro* than clofazimine. Because the most active compounds, such as B4019 and B3786, contained a 2,2,6,6-tetramethylpiperidine (TMP) substitution at the imino nitrogen,[55] the effect of substitution at the para position of phenyl and anilino groups in TMP-substituted phenazines was further assessed.[59] All of the tested TMPs were clearly more active *in vitro* than clofazimine, and the most active compound was the bromine-substituted TMP (B4076) and a trichlorinated analog (B4090). The efficacies of the highly active derivatives require *in vivo* confirmation. A few compounds have already been tested

in mice and the preliminary results were not very encouraging.[59] Although B4019 has completely inhibited the multiplication of *M. leprae* when administered at 0·01% (wt/wt) in the diet, clofazimine remains the only phenazine to inhibit *M. leprae* at 0·001% by kinetic method. It was thought that the disappointing results were largely due to pharmacokinetic properties, in particular low lipophilicity, and the lipophilicity of phenazines was responsible for skin coloration. If the assumption has been confirmed, it seems difficult to develop a nonpigmented phenazine with superior *in vivo* activity against *M. leprae* which may substitute for clofazimine in the treatment of leprosy.

RIFAMYCIN DERIVATIVES

In the mouse footpad system, strong bactericidal activities have been demonstrated by several rifamycin derivatives included rifabutin (LM 427),[64–66] rifapentine (DL 473)[66–69] and R-76-1 (isobutylpiperazinylrifamycin SV).[69] All these derivatives were more effective on a weight for weight basis than rifampicin. The greater activity of rifapentine is apparently due to its much longer half-life; and the favourable results of rifabutin and R-76-1 are probably due to their greater intrinsic activities. A pilot clinical trial has demonstrated that R-76-1150 mg daily was very active among 20 lepromatous cases.[69] However, because no difference on bactericidal activity could be detected between rifampicin 600 mg daily or intermittent therapy,[14] the new derivatives could contribute significantly to the treatment of leprosy only if they are active against rifampicin-resistant strains of *M. leprae*. Although it was reported earlier that rifabutin was active against rifampicin-resistant *M. leprae*,[65] unfortunately, this has not been confirmed in further experiments (unpublished data). Therefore, it is unlikely that the available rifamycin derivatives may further improve the efficacy of the current MDT regimen.

OTHER ANTIBIOTICS

Among beta-lactam antibiotics, cephaloridine, cephaloglycin, 7-aminocephalo-sporanic acid, cefuroxime and cefoxitin displayed various degree of bactericidal-type activity against *M. leprae* by kinetic method in the mouse footpad system.[70,71] It was thought that these antibiotics interfere with the cell-wall synthesis of *M. leprae*. However, except cephaloglycin (a drug no longer produced), most of the active beta-lactams must be administered by injection, and the injections have to be repeated frequently because of their short half-lives. Therefore, the prospect of applying the beta-lactam antibiotics for the treatment of leprosy is bleak.

With respect to aminoglycosides, streptomycin was found to be purely bacteriostatic[35] or to have varying degrees of bactericidal activity[72,73] against *M. leprae* in mice. An intraperitoneally injection of kanamycin or amikacin 100 mg/kg daily also showed impressive killing, but a 20 mg/kg daily injection of gentamicin or tobramycin were much less active.[73] Recently, because the high doses of aminoglycosides might be toxic the efficacy of lower dosages and intermittent therapy was evaluated. It was reported that reducing the dosage to 12·5 mg/kg 5 times a week or reducing the frequency of administration to 100 mg/kg once a week, streptomycin exerted a decreased but still significant bactericidal activity, and kanamycin no longer displayed bactericidal activity. In addition, once-monthly rifampicin plus streptomycin was more active than either drug alone.[74] Nevertheless, because streptomycin has to be administered by injection, it is unlikely that it might be used as a component of an MDT regimen in the field.

Clinical trial in measuring the therapeutic effect of new antileprosy drugs and new MDT regimens

The major objectives of a clinical trial are to evaluate the efficacies and side-effects of the treatment. Here, we are focusing on the techniques related to the monitoring of the therapeutic effects.

Clinical assessment and evolution of BI during chemotherapy were the most important parameters in earlier trials. Although a definite clinical improvement was observed in previously untreated lepromatous patients during treatment with all established antileprosy drugs and other new compounds such as pefloxacin,[29,31] ofloxaxin[31] and R-76-1,[69] the assessment of clinical improvement is very much subjective and difficult to quantify for comparison. The BI reflects the total bacterial load including both dead and viable organisms. Because the great majority of bacilli were dead even before treatment,[29,31] and also because the dead organisms persisted in the tissue and were eliminated by a process unrelated to the antimicrobial activity of the treatment; the reduction of BI did not differ significantly between dapsone monotherapy and MDT or other rifampicin-containing regimens although it is well known that rifampicin is far more bactericidal than dapsone. The MI was a major development in measuring the proportion of viable *M. leprae* in the host, and clinical trials based on changes of MI permitted identification of effective drugs after treatment of small numbers of patients for only a few months. Nevertheless, the technique is difficult to standardize and to perform with accuracy; also because it is difficult to examine more than 50–100 organisms, it may monitor a decrease in proportion of viable organisms by no more than 90%, or one 'log'.[75,76] These parameters are still useful in clinical trials, but they are not sensitive enough to evaluate more accurately and precisely the bactericidal activities of treatments.[76]

Serial mouse footpad inoculations have been applied as one of the most efficient techniques, in the sense that it needs the fewest patients for monitoring the rate of the 'initial killing' of *M. leprae* during treatment with individual drugs or combinations of drugs in short-term trials among MB leprosy.[75] *M. leprae* are recovered from biopsies taken at different intervals before and during treatment from a skin lesion and are inoculated into normal mice for assessing their viability. Because the technique involves examination of a much larger number of organisms than does measurement of the MI, if the proportions of viable organisms in the bacterial population have been carefully titrated on different occasions by inoculating groups of mice with several 10-fold diluted inocula prepared from each biopsies, it allows to measure the bactericidal activity of the treatment up to 99% to 99·9%, two or three 'logs' depends upon the proportion of viables before treatment.[29,31] The sensitivity of measuring the killing cannot be further improved by using normal mice because of the limited inoculum size, i.e. 5×10^3 to 1×10^4 organisms per footpad. To improve the sensitivity, one has to inoculate more organisms into immunocompromized rodents: thymectomized-irradiated (TR) mice,[77] neonatally thymectomized (NT) rat[78] and nude mice.[79,80] Up to now, these immunocompromised rodents have been employed only in a limited number of trials,[14,31,81–84] but their superiority in detecting a small proportion of viable organisms have been clearly demonstrated. Because nude mice are extraordinarily susceptible to infection by *M. leprae*,[85] as many *M. leprae* as are available can be inoculated. In our recent clinical trial for testing pefloxacin and ofloxacin,[31] we inoculated both normal and nude mice with

different dilutions of bacterial suspensions prepared from biopsies taken on several occasions. It was possible to measure the killing rate up to four or even five 'logs', depending upon the proportion of viables in the pretreatment biopsies and the maximum available amounts of organisms for nude mice inoculation.

Nevertheless, the disadvantages in applying the serial footpad inoculations are evident. It is time-consuming, it takes at least 12 months to obtain the results; it requires many animals to monitor a single trial, and the application of nude mice enormously increases the costs because the purchase and maintenance of nude mice are very expensive. In addition, because no more than 10^6 organisms per mg of tissue can be recovered from biopsies of advanced lepromatous patients, and the small size of mouse footpads severely restricts the volume of inoculum, even with nude mice, at best one can measure the initial five 'logs' killing among lepromatous patients who may have 10^{10} viables organisms before treatment.[31] It has already been proved that TR mice[14] and NT rats[82] are not sensitive enough to evaluate precisely and to compare the extraordinarily rapid and strong bactericidal effects of various rifampicin-containing regimens; although nude mice have not been used for such comparison but, based on the available knowledge, they are unlikely to be able to detect any difference after a few doses of treatment with rifampicin. It seems none of the existing rodent systems are able to monitor the therapeutic effects of any regimen containing more than one strong bactericidal drug, such as the combination of rifampicin and ofloxacin. Apparently, more rapid, simple and sensitive systems should be developed for measuring the killing of *M.leprae* by the treatment. It is unclear whether or not any of the above mentioned *in vitro* systems can meet the requirements because, to our knowledge, none of the systems has been tested in a clinical trial for monitoring the bactericidal effects of the treatment. Nevertheless, in view of the rationales and the procedures of these methods, it is unlikely that the available *in vitro* methods may be as sensitive as mouse inoculation in detecting a tiny proportion of viables among dead organisms. Recently, it has been reported that small numbers of *M. leprae* ($\sim 10^2$) can be detected by a simple procedure based on polymerase chain reaction (PCR).[86] In comparison with serial mouse (normal and nude) footpad inoculations, studies are being carried out by us to determine whether or not the *M. leprae* detected by PCR are viable organisms.

The other method for monitoring the therapeutic effects of treatment is to follow-up the relapse rate after stopping treatment. In MB leprosy, the relapse rate is thought to be proportionally correlated with the number of viable organisms at the time when the treatment is stopped, and therefore the relapse rate may reflect the bactericidal activity of the treatment. Under present circumstances, this seems to be the most reasonable approach to evaluate new combined regimen(s) containing more than one strong bactericidal drug. Unlike the footpad inoculation which requires a certain amount of organisms and therefore can be applied only for the trials of MB leprosy, this approach may also be employed for monitoring the therapeutic effects of treatment in PB leprosy; although it is still unclear whether or not the relapse in PB leprosy does reflect the treatment failure and is caused by the remultiplication of viable organisms.

The trials should be conducted as a double blind. Because the relapse rate after completion of MDT is already low and the relapse in MB leprosy after treatment with rifampicin-containing regimens may occur late,[20] in order to prove that the new combined regimen(s) is as good as or even better than the current MDT regimens, the sample size must be sufficiently large and the follow-up should be long enough. Patients should be

allocated randomly, and each regimen should have at least 500 patients which will be followed-up after 7 years in MB trials and 5 years in PB trials after completion of the treatment. The MB patients should have active, skin-smear positive lesions, and must be either previously untreated or only treated with dapsone monotherapy for a limited period, say, less than 12 months; the PB patients should be previously untreated, and with active skin lesions. Because it is unlikely that such amounts of patients may be recruited by any single centre within a reasonable period, e.g. 24 months, the trial is bound to be multicentric. Besides monitoring the relapse rate, the tolerance, side-effects and feasibility of the regimens will also be evaluated during the trials.

Since the assessment of the therapeutic effects of the regimens heavily depends upon the relapse rate, the criteria of relapse must be well defined in advance. In MB leprosy, relapse refers to the evidence of remultiplication of the organisms. Whenever a BI increase of at least 2+ from any single site over the previous value is detected, with or without new lesions, relapse should be suspected and a biopsy should be taken from the site for mouse footpad inoculation. Relapse will be confirmed only after viable organisms have been demonstrated in the mouse. In PB leprosy, because of the difficulties in distinguishing relapse and reversal reaction,[8,87] and also because the rapid response of reversal reaction to treatment with corticosteroid whereas relapse does not; unless the PB patient has become skin-smear positive, all suspected relapsed cases should be confirmed by corticosteroid therapeutic trial. Patients who respond well by the end of 4 weeks of corticosteroid treatment are diagnosed as having suffered from reversal reaction, and those who do not respond properly are diagnosed as relapse.

Future research activities related to chemotherapy in leprosy

Within the next five years, a substantial increase of information about the long-term therapeutic effects of the current MDT regimens will be accumulated, screening and synthesizing of new drugs will be continued and expanded, clinical trials of various new antileprosy drugs and field trials of new combined regimens will be mounted. However, the development of more powerful regimens does not necessarily result in better disease control, the key factor is to apply the effective regimens properly under routine field conditions. There is still a large gap between the number of cases who have been or are being treated with MDT and the total number of registered cases, needless to mention the gap between the former figure and the total number of estimated cases, which is probably still between 10 and 12 million in the world.[8] The gap is particularly wide in Africa, only 19% of the registered cases are under MDT as compared to 50% of cases for the rest of the world,[10] although the problem of leprosy on the African continent is significant. To control leprosy eventually, the gap should be reduced to the minimum. The weakness in the operational aspects are always the reasons that MDT cannot be implemented successfully. It appears that operational research, including health systems research and social-economic research, may provide a better understanding of the reasons of and the possible approaches to cope with the weakness in operational aspects. Unfortunately, this has been a neglected area in leprosy research,[88] therefore, all efforts should be made to promote operational research. The other important research area is related to leprosy reactions and nerve damage, which may occur during or even after MDT. Within the last two decades, there has been almost no progress either in prevention or in treatment of

these two important clinical events. Basic research leading to a better understanding of the mechanisms are needed, and appropriate animal models should be established.

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