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The First Joint THELEP-Sasakawa Memorial Health Foundation Workshop on Experimental Chemotherapy of Leprosy

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When the Study Group on Chemotherapy of Leprosy for Control Programmes ([15]), convened by the World Health Organization in November, 1981, designed the combined drug regimens to be recommended for the chemotherapy of leprosy for the purpose of leprosy control, it was guided by the increasing prevalence of dapsone resistance, and the antimicrobial potencies of the available drugs active against *Mycobacterium leprae.* The drug regimens were designed to be active despite the presence of dapsone-resistant organisms, to prevent the emergence of *M. leprae* resistant to dapsone or other drugs in patients whose organisms were susceptible, and to reduce the size of patients' bacterial populations so as to minimize the risk of relapse after stopping chemotherapy. In the course of its deliberations, the Study Group considered carefully the results achieved by combined chemotherapy of tuberculosis. Because these results appear, by analogy, to give direction to the combined chemotherapy of leprosy, it may be useful to approach a discussion of the chemotherapy of leprosy by reviewing the basis of chemotherapy of tuberculosis.

Basis of the chemotherapy of tuberculosis. Tuberculosis may be viewed as a localized disease characterized by pulmonary lesions. The most important of these lesions is the cavity, which may contain as many as 10^8 viable units** of *M. tuberculosis.* Within this large population of drug-susceptible *M. tuberculosis* are spontaneously occurring drug-resistant mutants, which occur in an average proportion of 10^{-6} (see Table 1). The organisms are primarily located extracellularly in the thin, liquid caseous layer that lines the cavity wall. In addition, there are smaller bacterial populations ($\leq 10^5$) in the macrophages and solid caseous areas of the lesion ([4]).

When, in 1945, streptomycin (SM) was introduced into the chemotherapy of tuberculosis, it was administered alone, with the result that, within three months, 80% of the patients so treated had relapsed as a consequence of the emergence of SM-resistant organisms. The explanation of this phenomenon was found to be simple: even before treatment, the population of 10^8 *M. tuberculosis* in a cavity includes approximately 100 spontaneous mutant organisms resistant to SM; these mutants were selected by SM administered alone.

As the result of this experience, it became clear that tuberculosis could not be cured by treatment with any antimicrobial agent, as long as it was not possible to prevent selection of drug-resistant mutants. After the introduction of *p*-aminosalicylic acid (PAS), in 1949, and isoniazid (INH), in 1952, it was possible to prevent selection of drug-resistant *M. tuberculosis,* and it became the practice to treat patients with a combination of active drugs. Because each drug was active against the mutants resistant to the other drugs in the combination, selection of mutants could be prevented in the majority of cases, as shown in Table 2.

The prevention of drug resistance is a necessary but not a sufficient condition for the cure of tuberculosis. It is also necessary to kill the drug-susceptible organisms. The next question to be addressed was that of the duration of treatment required to kill enough of the susceptible organisms to prevent relapse after stopping treatment. As shown in Table 3, the optimal duration of chemo-

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** A "viable unit" or "colony-forming unit" is understood to represent one or a few organisms.

TABLE 1. *Comparison of the characteristics of tuberculosis and leprosy.*

	Cavitary tuberculosis	Lepromatous leprosy
Disease		
Type	Localized	Disseminated
Main lesion	Cavity	Infiltrate
Extracellular organisms		
Present	Yes	No
Site; number	Inner layer of cavity wall; 10^8 Solid caseous material; $\leq 10^5$	
Intracellular organisms		
Present	Yes	Yes
Number	$\leq 10^5$	10^{11}
Doubling time	15–20 hours	10–14 days
Proportion of drug-resistant mutants	10^{-6}	?

therapy is a function of the drugs employed. To obtain a relapse rate no larger than 5% requires treatment for 18 months with the standard combination of INH, SM and PAS or thiacetazone (Tb1), for nine months with the combination INH-SM-pyrazinamide (PZA), and for six months with the combination INH-SM-rifampicin (RMP). When the four most potent drugs—INH, SM, RMP, and PZA—are administered in combination for six months, the relapse rate is 0–2% ([3]).

Considering all of the currently available information regarding the chemotherapy of tuberculosis, several conclusions are possible: a) some drugs, especially RMP and PZA, and some combinations of drugs, especially INH-RMP-PZA, are much more active (or more "sterilizing") than others, in the sense that treatment by these drugs and combinations is followed by a lower relapse rate; b) the rates at which *M. tuberculosis* are killed during chemotherapy with different combinations are quite similar during the initial (two-month) phase of treatment, but strikingly different during the secondary phase of chemotherapy; c) the differences among rates of bacterial killing are thought to reflect the special bactericidal activity of RMP and PZA on organisms located within macrophages and solid caseous areas that are not actively metabolizing (i.e., persisters), and the lack of such activity of the other drugs.

In summary, to be successful the chemotherapy of tuberculosis should first be capable of preventing selection of drug-resistant mutants; this is readily accomplished by the use of multidrug therapy. Second, the chemotherapy of tuberculosis should be capable of killing the drug-susceptible organisms, especially the persisters; this is readily accomplished by the use of RMP and PZA, administered in a course of treatment no shorter than six months.

Basis of WHO Study Group regimens for chemotherapy of leprosy. The basis of the WHO Study Group regimens for leprosy, which has been elegantly described by Ellard ([2]), is similar to that for the chemotherapy of tuberculosis. The objectives of the chemotherapy of leprosy are the same as those for the chemotherapy of tuberculosis—to prevent the selection of drug-resistant mutant *M. leprae,* and to kill the drug-susceptible organisms.

Although both tuberculosis and leprosy are infectious diseases caused by a species of *Mycobacterium,* the diseases differ in at least three respects. As shown in Table 1, the more serious, infectious form of tuberculosis is cavitary pulmonary tuberculosis, a localized disease process, with a bacterial population of 10^7–10^9 organisms, mostly extracellular. By contrast, the serious, infectious form of leprosy is lepromatous leprosy, a disseminated process, with about 10^7–10^9 organisms per gram of tissue, and a total bacterial population of 10^{10}–10^{12} organisms, most of them located within macrophages and other cells ([12]). Because of the size of the population of *M. leprae,* the risk

TABLE 2. *Capability of different drug regimens to prevent the selection of drug-resistant* M. tuberculosis.

Regimen	% Failures with acquired drug resistance
SM alone*	80
INH alone**	50
INH + PAS**	15
INH + PAS + SM***	2–3

* Adapted from reference no. 9a.
** Adapted from reference no. 2b.
*** Adapted from reference no. 2a.

TABLE 3. *Duration of chemotherapy of tuberculosis, and the mean percentage of relapse after stopping treatment.**

Regimen	Duration (mo)	Relapses (%)
INH + SM + PAS or Tbl	6	20–30
	12	10–15
	18	5
INH + SM + PZA	6	10–15
	9	5
INH + SM + RMP	6	5
	9	0–2
	12	0–2
INH + SM + RMP + PZA	6	0–2

* Adapted from references no. 3 and 4.

of selecting drug-resistant mutants is great. Moreover, because of the intracellular location of the organisms, only those drugs capable of penetrating macrophages are active; in addition, the activity of these drugs may be limited by the slow metabolism of the organisms within macrophages.

It is clear that drug-resistant *M. leprae* are indeed selected by monotherapy. In the case of leprosy, the drug employed in monotherapy was dapsone (DDS). For almost 20 years, beginning in the early 1950s, leprosy was treated only by DDS and sulfonamides, which possess the same mechanism of action as DDS, but are much less potent ([15]). The result was an increasing prevalence of DDS-resistant strains of *M. leprae.* In 1964, the prevalence was estimated to be only about 1 per 1000 ([11]); however, during the next decade, the prevalence increased to 25 per 1000 ([10]), and is now about 100 per 1000 ([2]).

Soon after RMP became available in 1967, it became clear that the drug was strongly bactericidal against *M. leprae.* Despite its high cost, RMP was used, often as monotherapy, to treat both patients who had relapsed during DDS monotherapy and newly diagnosed patients. The response to treatment with this drug was uniformly good, with rapid clinical improvement and loss of the infectivity of the patients' organisms for the mouse. However, it became apparent ([5,6]) that a proportion of patients relapsed with the emergence of RMP-resistant *M. leprae* within 4–10 years after beginning treatment (see Tables 4 and 5).

Experience gained in the chemotherapy of tuberculosis had demonstrated that the co-administration of at least two potent drugs effectively prevented the selection of drug-resistant mutants. Both drugs are active against the drug-susceptible organisms, and each drug prevents multiplication of the mutants resistant to the other drug. Because the population of viable *M. leprae* in the lepromatous patient is not greater than 10^{12} ([12]), and the proportion of mutant *M. leprae* resistant to each drug is believed, by analogy with *M. tuberculosis,* to be about 10^{-6}, the proportion of mutants resistant to both drugs should be no greater than $(10^{-6})^2 = 10^{-12}$. Therefore, doubly resistant mutants are unlikely to be encountered in the lepromatous patient with fully susceptible organisms.

Because of the increasing prevalence of DDS resistance, both primary and acquired (Table 5), it is not now possible to rely entirely upon DDS to prevent the multiplication of the organisms resistant to the second drug. On the other hand, because of its low cost and potential for toxicity, DDS remains an obligatory component of drug regimens for chemotherapy of leprosy. For these reasons, the WHO Study Group recommended that two additional drugs be combined with DDS: RMP; and clofazimine (CLO), rather than a thioamide, because of the potential for hepatotoxicity, which limits the usefulness of the thioamides in the field.

Having chosen the members of the drug combination to be used to prevent the selection of drug-resistant mutant *M. leprae,* there remained the task of selecting dosages and rhythm of administration. The best in-

TABLE 4. *History of development of RMP resistance.*

Patients with RMP resistance		Duration of leprosy (yr)	Duration of RMP treatment (mo)	Interval* (yr)
Site	No.			
Carville	1	30	43	3.5
	1	19	45	3.7
Paris	12	24.5 ± 7.1**	28.9 ± 21.7	7.4 ± 2.6

* Interval between beginning treatment with RMP and the diagnosis of RMP resistance.
** Mean ± standard deviation.

formation on dosages and rhythms of administration has come from studies of the rate of decrease of infectivity for the mouse of organisms recovered from the biopsy specimens of patients obtained during monotherapy with each of the drugs. The available data show that DDS must be administered for four to six months in a daily dosage of 100 mg, in order to render the patient's *M. leprae* non-infective for the mouse ([8]). And CLO must be administered for six months in a dosage of 100 mg at least three times weekly to be maximally effective; the drug was less effective when administered in a dosage of 600 mg on two consecutive days once monthly ([1]). Therefore, the Study Group recommended that DDS be administered in a daily dosage of 100 mg, and that CLO be administered in a daily dosage of 50 mg, supplemented by single, "topping-up" doses of 300 mg monthly, to protect the patient if he has not been fully compliant. Because a single 600-mg dose of RMP has been shown to render a patient's organisms incapable of multiplication in the mouse ([9]), it has not been possible to determine whether daily administration of the drug would be more effective than the administration of RMP at intervals of one week or one month. However, because a single 600-mg dose of RMP had been shown to be at least as effective as daily treatment with DDS or CLO for six months, the Study Group recommended that RMP be administered in supervised monthly doses of 600 mg. It was believed that a large proportion of the RMP-susceptible *M. leprae* are killed by the first dose of RMP ([13]), and that the role of subsequent doses is to kill the remaining RMP-susceptible organisms or, at least, to prevent their multiplication. The role of DDS and CLO is to kill the RMP-resistant mutants. However, because both drugs are less rapidly bactericidal than is RMP, it was difficult to estimate the duration of treatment required to eliminate the RMP-resistant *M. leprae*. Therefore, it was determined that both drugs should be administered, in combination with RMP, for the entire duration of chemotherapy.

To cure a patient, it is necessary not only to prevent the selection of drug-resistant mutants, but also to kill enough of the drug-susceptible organisms to minimize the risk of relapse after stopping treatment. For this purpose, the Study Group could recommend only the administration of the same three drugs—RMP, DDS and CLO—in the same dosage and rhythm of treatment as those recommended for preventing the selection of drug-resistant mutants.

However, there remained a most difficult question: For what period should treatment be administered? Except for measurement of the relapse rate after stopping treatment, no tools were available with which one could measure the number of viable *M. leprae* surviving after some long duration of treatment. It was decided to recommend that treatment should be continued for at least two years, and optimally until the patient's smears had become negative. The minimum of two years was selected on practical grounds: a shorter duration of treatment was likely to be insufficient, when this duration is compared to the duration of treatment for tuberculosis; and it was believed that a treatment duration of two years was probably the minimum that could be readily accepted by patients and a treatment infrastructure that had long been accustomed to life-long treatment of lepromatous patients. The recommendation with respect to smear negativity was based on tradition; during the era of DDS monotherapy, it had been recommended that only those patients who

TABLE 5. *Drug resistance of* M. leprae *measured in Guadeloupe and Martinique during the period 1980–1984.*

Source of *M. leprae*	No.	Resistance to DDS				Resistance to RMP	
		S*	0.0001**	0.001	0.01	S	R***
Previously treated patients	69	7	6	21	35	56	13
Untreated patients	70	23	34	7	6	70	0

Adapted from reference no. 5.
* Susceptible.
** Concentration of DDS in the diet, in g per 100 g diet.
*** Resistant.

remained smear negative for at least 10 years be considered to be candidates for stopping treatment. In fact, the optimal duration of chemotherapy by the Study Group regimens will only be determined in the future, when relapse rates will have been measured.

Outstanding questions regarding chemotherapy of tuberculosis and leprosy. There remain a number of unanswered questions with respect to the chemotherapy of leprosy and tuberculosis. Annually in the World, there are more than 3 million deaths from tuberculosis, and more than 10 million new cases are discovered ([7]). And more than 10 million patients suffer from leprosy, of whom half are believed to be unregistered and, therefore, untreated ([14]).

The present chemotherapy of tuberculosis is extremely effective. But cure of the patient requires that costly drugs be administered daily for six months. This requirement is easily met in industrialized countries, but this is not the case in developing countries. Therefore, the first question to be answered in the area of chemotherapy of tuberculosis is how to apply in developing countries regimens that are fully effective in industrialized countries but operationally difficult in developing countries. Extensive research has already been carried out in the area of the more effective use of existing drugs; without new drugs as active as RMP, INH and PZA, it appears unlikely that new regimens can be developed that would permit effective treatment in fewer than six months. New drugs would be also of interest for the increasing number of patients whose *M. tuberculosis* are resistant to the existing major drugs. Finally, it should be stressed that chemotherapy would benefit greatly from advances in immunological research; the use of non-specific immunoadjuvants and specific immunotherapy could increase the effectiveness of chemotherapy by activation of macrophages.

A number of important questions with respect to the chemotherapy of leprosy also remain to be answered. Concerning the Study Group regimen for multibacillary leprosy, three questions deserve special attention. The first question is that of the duration of treatment by DDS and CLO required to eliminate RMP-resistant mutant *M. leprae.* A short-term trial to measure the activity of the combination DDS-CLO is planned by THELEP. It is also important to know how long chemotherapy must be continued in order to reduce the size of the patient's bacterial population to a level that produces an acceptable relapse rate, once treatment has been stopped. The information now available leads to great optimism; no confirmed relapses have yet been encountered during the first five years following treatment by the Study Group regimen for multibacillary leprosy. And the numbers of persisting organisms, measured at several intervals during treatment by combined drug regimens not very different from the Study Group regimen, were reassuringly small ([13]). Finally, it would be extremely useful to have available some tool for measuring the activity of a chemotherapeutic regimen, other than that of stopping treatment and awaiting relapses.

In addition to these more general questions with respect to the chemotherapy of leprosy, there remain more specific questions related to the use of antileprosy drugs: a) What is the mechanism of the initial rapid killing of *M. leprae* by RMP? Does the enzyme, DNA-dependent RNA polymerase, differ among mycobacterial species, and is the enzyme of *M. leprae* more sensitive than

that of *M. tuberculosis* to the action of the drug? Or does the small proportion of *M. leprae* viable at the beginning of treatment somehow bring about the rapid loss of viability of *M. leprae* during treatment with RMP? b) Why do persisting *M. leprae* not respond to treatment by RMP, whereas persisting *M. tuberculosis* respond? In other words, why do *M. leprae* behave like susceptible organisms at the beginning of treatment, and like resistant organisms later in the course of treatment, whereas *M. tuberculosis* respond slowly throughout treatment with RMP? c) Are persisting *M. leprae* that have survived long-term chemotherapy with RMP likely to cause relapse, or are they likely to remain in the persister state? What is the risk of relapse among multibacillary patients who have been treated by a combined drug regimen? d) Is it possible to increase the activity against *M. leprae* of the existing drugs by changing the rhythm of administration, or by changing their formulations—e.g., incorporating them in liposomes? e) Can *M. leprae* be expected to respond differently to new drugs—i.e., is the response of the organism to treatment by RMP determined by the drug, the organism, or some interaction between organism and host-macrophage? f) Can immunotherapy enhance the response of the multibacillary patient to chemotherapy—i.e., will activation of macrophages by the administration of *M. leprae*-specific antigens together with effective antimicrobial chemotherapy increase the rate at which persisters are killed, and decrease thereby the risk of relapse after stopping treatment?

It is evident that a number of studies are needed to answer these questions. Some studies will obviously be difficult, because *M. leprae* cannot be cultivated *in vitro,* and because of the cost, scarcity, and limited precision of some of the available tools. On the other hand, it is clear that the chemotherapy of leprosy for the purpose of leprosy control will be made more effective by the availability of new compounds that exert bactericidal activity against *M. leprae.* Among the compounds found recently to possess activity against *M. leprae,* the new fluoroquinolones—e.g., pefloxacin and ofloxacin—appear most promising; the initial results of studies of their activity in the mouse and in man are favorable. In addition, fundamental research, certainly including research in the area of molecular biology, should assist us to gain an understanding of the mechanisms of action of antileprosy drugs, and to use them more effectively; in addition, research in this area may be expected to uncover leads to new drugs.

The Workshop. In recognition of the unmet needs, in terms of the outstanding questions just listed, on the one hand, and the unexploited potential for new and needed research efforts, represented by many laboratories currently employing the mouse foot-pad technique, and particularly those in which immunosuppressed rodents are employed, on the other, the Steering Committee of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and the Sasakawa Memorial Health Foundation planned a Workshop on Experimental Chemotherapy. The objectives of the Workshop were to: a) review and summarize the present situation with respect to chemotherapy for leprosy control; b) review the contributions of experimental chemotherapy to leprosy control; c) exchange experiences and information in the area of experimental chemotherapy of leprosy, to obtain a better understanding of the methods appropriate for research in animals; d) standardize techniques and criteria for experimental work in animals; e) identify the important outstanding questions with respect to the chemotherapy of leprosy which could be answered by experimental work in animals, and formulate the questions as precisely as possible; and f) prepare protocols for the experimental studies.

The Workshop occupied eight working days, and consisted primarily of copiously illustrated lectures and detailed, prolonged discussions of the topics encompassed in the lectures. For the preparation of protocols, the Workshop participants and faculty met in small groups, the results of which meetings were presented to the entire Workshop for further discussion.

The Workshop was very much a successor of the two earlier workshops, conducted by THELEP in Chingleput in November

1979, and in Shanghai in March 1984. All of these workshops were designed to promote application of the mouse foot-pad technique for cultivation of *M. leprae,* with the final aim of contributing to the control of leprosy.

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