COMMITTEE 3: ADVANCES IN EXPERIMENTAL CHEMOTHERAPY

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Progress in the last decade now allows the same topics to be studied in leprosy as in other bacterial diseases. They are:

1. Screening of new drugs, and determination of minimal effective dosage (MED).
2. Characterization of antileprosy drug activity: bactericidal, bacteriostatic, or bacteriopausal (prolonged bacteriostasis).
3. Methods of measurement of drug in blood and tissue, determination of minimal inhibitory concentration (MIC), and pharmacokinetics including repository effect.
4. Drug toxicity in relation to MIC.
5. Metabolism of the drug.
6. Short-term clinical trials to determine whether a given drug is also active in man.
7. Long-term clinical trials to determine whether a drug's activity continues to the point of smear-negativity.
8. Very long-term follow-up to see if smear-negativity is maintained or if drug-resistant *M. leprae* eventually emerge.

These steps should be followed in the development of antileprosy drugs. Patients should not be deprived of standard dapsone (DDS) therapy in order to test compounds that have not been tested against *M. leprae* in animals or compounds that appear on the basis of results in animals to be clearly less efficacious than standard therapy.

DRUG SCREENING AND CHARACTERIZATION OF ANTILEPROSY ACTION

Experimental model. In the absence of significant growth of *M. leprae in vitro*, all work must be done in animals. Most research has been done in the mouse model. This infection is very consistent. Genetically uniform mice are readily available and easily maintained in standard conditions. Hence, the mouse continues to be the animal of choice. Other animals may be useful when particular findings must be checked in another species. For studies requiring larger populations of *M. leprae*, the thymectomized-irradiated mouse, the neonatally-thymectomized rat and the armadillo may provide suitable animal models.

Methods of study. The **continuous method** of drug administration (from the day of infection to the end of the experiment) reveals whether a drug is active against *M. leprae*. The **kinetic method** (administration of drug during a limited period, beginning early in the logarithmic phase of growth of bacilli), determines whether a drug produces bactericidal, bacteriostatic or bacteriopausal effects. Administration of drugs in graded dosages allows the MED and MIC to be determined.

Results of drug screening and characterization. With these methods, more than 200 drugs have been tested. Only a few have exhibited bactericidal (or bacteriopausal)

activity and these few appear to include most of the drugs of real promise in leprosy. These drugs include:

1. DDS (4,4'-diaminodiphenylsulfone) and other sulfones giving rise to DDS in the gut or in tissues.
2. Rifampicin. The related antibiotic streptovaricin is distinctly less active.
3. Clofazimine (B663) and another phenazine dye, B1912.
4. Long-acting sulfonamides. These compounds appear to have MIC's close to toxic blood levels in man.
5. Ethionamide. In the dosage apparently required for man, gastrointestinal distress is frequent.

MIC of DDS. This was found to be 0.01 to 0.001 μg/ml (microgram/ml) in the mouse, corresponding approximately to an oral dosage of 1 mg a day in man. This finding in mice led to the introduction of treatment with acedapsone (DADDS), which releases DDS at a steady rate of 2.5 mg daily, following injections of 225 mg every 75 days. The therapeutic efficacy of this regimen, and that of 1 mg oral DDS given daily, confirmed these predictions. It needs to be emphasized that this study of 1 mg DDS daily was carried out to compare the MIC of DDS in mouse and man, and not to evaluate, or encourage very low dose DDS therapy as a practical therapeutic regimen.

Bactericidal effect of rifampicin. The curves of blood concentration in the mouse and in man are very similar, and studies of the anti-*M. leprae* effect of per kilo dosages in mice have been predictive of the results in man. With the kinetic method in mice, rifampicin was found to produce as much bactericidal effect in a few days as DDS in a few months. Bactericidal rates for these two drugs in man appear to be the same as in the mouse.

Clofazimine. Studies of the MIC of clofazimine have not been practical because the drug is very unevenly distributed in the tissues; thus, blood and tissue levels may not accurately reflect the concentration of drug in the immediate environment of the organism.

Demonstration of drug-resistant M. leprae. The mouse provides the only method of proving drug resistance. DDS-resistant *M. leprae* have been demonstrated in mice from some patients who have relapsed on prolonged DDS therapy. By contrast, DDS-sensitive organisms have been isolated from relapsed patients who have, in fact, stopped taking the drug. Studies have shown that 1% to 10% of sulfone-treated patients eventually undergo relapse caused by DDS-resistant *M. leprae*. Combinations of antileprosy drugs appear to offer the most promise for the prevention of these relapses. Thiambutosine- and thiacetazone-resistant *M. leprae* have been isolated from patients who have relapsed after therapy with these drugs.

PHARMACOKINETICS AND METABOLISM OF DRUGS

Comparative studies of the pharmacology of drugs in the mouse and man are necessary for understanding their antileprosy actions.

DDS. In man there are great individual differences in the rate of DDS elimination. The half-life (T½) of the drug varies from 5 to 50 hours. Among patients with relapses caused by DDS-resistant *M. leprae*, there is a significant excess of persons with very short drug half-lives. Since it is not possible to determine the T½ of all patients, regimens need to be designed so that they will take care of patients with shorter T½. Fifty milligrams DDS daily would ensure continuous blood levels well in excess of the MIC, whereas 350 mg once weekly (the same total dosage) would not. For this reason, it is insufficient to describe dosage merely in terms of the total weekly intake.

The only metabolite of DDS found in human blood is monoacetylated DDS (MADDS). Humans have been found to be genetically polymorphic in their acetylation capacities, resulting in rapid and slow acetylators. Rapid acetylators have higher ratios of MADDS/DDS in their plasma, but do not eliminate DDS more rapidly, so *a priori* one would not expect the acetylator status to affect the response of leprosy patients to DDS. Nevertheless, some studies, but not others, have suggested an excess of rapid acetylators among patients with relapses caused by DDS-resistant *M. leprae*.

Rifampicin. Pharmacokinetic studies of rifampicin are complicated because the half-life of rifampicin varies with drug concentration and because blood levels tend to

decrease after the patient has been receiving the drug for several weeks.

Clofazimine. Similar studies with clofazimine have not been possible because the drug is accumulated in the tissues.

TRIALS IN MAN

Background. Application of experimental chemotherapeutic findings to man has been inadequately understood. For a better understanding, the bacterial populations in human leprosy have to be considered (Table 1). A lepromatous patient with a Bacterial Index (BI) of 4+ (Ridley) and a Morphological Index (MI) of 10%, may be estimated to have 10^{11} *M. leprae* in his body, of which 10^{10} are viable. For example in line 3 of the table, after one to three months of DDS treatment, the MI is less than 1%, so that the number of viable *M. leprae* is less than 10^9. If mouse inoculation is negative, the number of viable organisms is less than a hundredth of its original value and may be estimated to be less than 10^8. With each decrease of the BI by one unit, the corresponding numbers of bacilli fall to a tenth of the preceeding value (decrease by one exponent). When the BI is less than 2+, measurement of the MI or inoculation of mice with standard numbers of bacilli is not possible, so that measurement of the proportion of viable bacilli is impossible. Consequently it is not technically possible with present procedures to estimate the number of viable *M. leprae* present in the body at any number less than 10^7. These considerations allow one to understand how there can be many viable bacilli present in the body if treatment is stopped after the MI has reached baseline values and infectivity for mice can no longer be demonstrated. To explain relapse in a patient, it is clearly not necessary to assume that non-solid bacilli have become viable. Similarly, in a patient with negative smears, it is not necessary to assume that non-acid-fast viable forms of *M. leprae* exist, since there could be as many as 10^6 typical, viable but undetected *M. leprae* present in the body. The survival of living *M. leprae* during treatment appears to occur by two mechanisms. One, which is not unusual with other drug-bacteria combinations, is the survival of a small fraction of drug-sensitive bacilli in the continued presence of the drug. Such bacilli do not multiply,

and since they are dormant or metabolically inactive, they remain relatively insensitive to the drug, until they resume normal metabolism. Moreover, the location of the bacilli in the tissues may be important, and some believe the location of *M. leprae* in nerve or muscle favors their survival. Because of the large numbers of bacilli present, factors affecting even a very small fraction of the population of *M. leprae* become important.

The second, unrelated, mechanism of survival of *M. leprae* is drug-resistance. A small fraction of bacilli is genetically insensitive to the drug and can multiply in its presence. Again, because large numbers of bacilli are present in lepromatous leprosy, a small resistant fraction may constitute a large absolute number of bacilli.

Clinical trials. In the treatment of leprosy, distinct differences exist between (a) the rate of loss of viability and (b) the rate of disappearance of acid-fast bacilli. Nearly all drugs that have been tried in leprosy were selected on the basis of their ability to carry out process (a), and such drugs do not affect process (b). During early treatment, (a) is much faster than (b).

Except for special studies in relapsed patients, all trials should be carried out in previously untreated lepromatous patients.

Short-term trials. These are carried out to confirm in man laboratory results of the anti-*M. leprae* effect of a drug. Two criteria may be applied:

1. Measurements of the MI. These provide immediate results, but are difficult to standardize between laboratories, and are technically demanding.
2. Mouse inoculations. They provide firm evidence of bacterial viability, are more sensitive than measurements of the MI, but they require greater investment of personnel and facilities, and results are available much later. Mouse inoculations may show more rapid bactericidal effect than do MI measurements, probably because changes in bacterial morphology may lag by perhaps two weeks behind loss of ability to multiply, a difference particularly evident with rifampicin.

Short-term trials may now be limited to a period of six months, or even much less, depending upon the regimen. The BI

changes little in this period and is therefore of no value in such trials.

Long-term trials ("five-year trials"). These are carried out to determine whether a drug's activity continues until smear negativity and clinical and histological quiescence are reached. Not many patients on standard DDS treatment reach this stage within five years. For practical reasons, these trials usually need to involve commitments by appropriate organizations to ensure long-term continuity. Mouse inoculations are particularly helpful when treatment failure is suspected, in which case tests of drug-sensitivity provide crucial information. Measurements of the MI, if they can be performed reliably, may provide the first indication of treatment failure.

Very-long-term studies. Because of the very long generation time of *M. leprae,* a complete picture of the therapeutic efficacy of a drug cannot be obtained unless patients are followed for very long periods, perhaps for the rest of their lives. Therefore leprosy services that successfully practice very-long-term follow-up of lepromatous patients can provide invaluable information on the final efficacy of a regimen. As pointed out, smear-negativity does not sig-

nify that the patient is free of bacilli, but rather that the number of bacilli in the body is less than 10^7. The minimal number of viable *M. leprae* needed to cause a relapse in a lepromatous patient may be very small, since such a patient would not be expected to possess immunity against *M. leprae.* Therefore, treatment may need to be continued indefinitely. In these studies, it will be essential to determine whether relapse is caused by drug-sensitive or drug-resistant *M. leprae.* Experience with sulfone therapy has shown that such relapses may occur 5 to more than 20 years after the commencement of treatment.

CONCLUSION

The application of the mouse model has at last placed the chemotherapy of leprosy on an objective bacteriological and pharmacological basis. It has provided sensitive procedures for the assessment of new drugs, the response to treatment, and the detection of drug-resistance. It has also led to clarification of the theoretical basis of long-term and very-long-term clinical trials. These are difficult and expensive to carry out, but without them the final value of a regimen cannot be determined.

Table 1. *Estimated number of* M. leprae *in typical lepromatous patients at various times during response to regular DDS therapy.*

				FINDINGS		INTERPRETATION	
			BI	MI	Mouse inoc.	Total *M. leprae*	Viable *M. leprae*
1.	Untreated		4+	10%	Pos	10^{11}	10^{10}
2.	Dapsone	1-3 mo	4+	1%	N.D.[c]	10^{11}	10^9
3.		1-3 mo	4+	<1%	N.D.	10^{11}	$<10^{9\,d}$
4.		1-3 mo	4+	<1%	(Pos)[e]	10^{11}	10^8
5.		1-3 mo	4+	<1%	Neg	10^{11}	$<10^8$
6.		ca. 1 yr	3+	<1%	N.D.	10^{10}	$<10^8$
7.		ca. 1 yr	3+	<1%	Neg	10^{10}	$<10^7$
8.		ca. 2 yr	2+	<1%	Neg	10^9	$<10^7$
9.		ca. 2 yr	2+	<1%	Neg	10^9	$<10^6$
10.		ca. 3 yr	1+	N.P.	N.P.[f]	10^8	$<10^{7\,g}$
11.		ca. 5 yr	0	N.P.	N.P.	$\leq 10^7$	$<10^{6\,g}$
12.	Required for cure						0

[a] When carried out according to the specifications described for "solid ratios" so that the proportion of "solid" bacilli is the same as the proportion of bacilli infective for mice.

[b] When 1×10^3 to 1×10^4 bacilli are inoculated.

[c] Not done.

[d] For example, $<10^9$ means that the number may lie between 0 and 10^8 (inclusive).

[e] Weakly positive (long incubation period and irregular results in mice), indicating that the number of viable *M. leprae* is near the limit of detectability.

[f] Not possible.

[g] Since it is not possible to determine the MI with a BI of less than 2+, the estimate of viable *M. leprae* is based on the supposition that not more than 10% of the total are viable. This does not imply an increased number of viable *M. leprae.*