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U.S.-Japan Cooperative Medical Science Program Workshop on Leprosy Chemotherapy[1]

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A Workshop on Leprosy Chemotherapy was convened under the auspices of the U.S. Leprosy Panel of the U.S.-Japan Cooperative Medical Science Program of the National Institute of Allergy and Infectious Disease on 26 October 1975 in Bethesda, Maryland. The workshop provided a unique opportunity to bring together participants from other countries as well to discuss three of the most pressing issues of leprosy chemotherapy: 1) the persistence of drug-susceptible *M. leprae* in lepromatous leprosy despite apparently adequate long-term chemotherapy; 2) drug-resistant relapse in lepromatous leprosy; 3) chemotherapy trials in nonlepromatous leprosy. Discussion included elucidation of the magnitude and significance of the problems, newer work in leprosy pharmacology that might prove useful in considering future approaches to chemotherapy, and lastly, recommendations to deal with these problems and to plan new chemotherapy trials.

Dr. C. C. Shepard, Atlanta, who chaired the first session on bacterial persistence in leprosy, stated that the first evidence that this might be a problem in the treatment of leprosy came from a study in the Karimui of Papua New Guinea in which 5 of about 30 lepromatous patients were found to have solid bacilli in their smears despite regular treatment with acedapsone (DADDS) for three to five years. The treatment had produced the expected blood sulfone levels. Dr. M. F. R. Waters, Sungei Buloh, reported on a biopsy study with mouse inoculation of specimens from 12 Malaysian lepromatous patients who had completed a minimum of ten years of supervised dapsone (DDS) therapy in full dosage. All 12 patients showed full clinical response to the treatment although 3 patients were still smear-positive at one or two sites. Tissues studied included skin, skeletal muscle, peripheral nerve and dartos muscle. Ten of the 37 specimens and 7 of the 12 patients still harbored viable *M. leprae*. Three strains which were passaged were all shown to be fully sensitive to DDS (0.0001% DDS in mouse chow). Dr. R. J. W. Rees, London, reported a similar study of 21 Malaysian patients treated with 600 mg rifampicin (RMP) daily and thiambutosine (DPT), 1 gm daily by mouth or 1 gm twice weekly by injection, and 7 treated only with RMP for periods of one-half to five years. Of the 28 patients, *M. leprae* were isolated from one or more of the four tissues in 20. All 11 of those patients treated from two to five years with daily RMP yielded *M. leprae* that multiplied in mice. Three of these strains were passaged and all were found sensitive to RMP. Dr. S. R. Pattyn, Antwerp, attempted to infect mice with *M. leprae* isolated from skin, muscle and nerve of lepromatous leprosy patients after three months of treatment with daily DDS, daily RMP and weekly RMP. *M. leprae* were isolated from 2 of the 10 patients receiving DDS, none from 13 receiving daily RMP, and 1 from 8 treated with weekly RMP.

Dr. Rees chaired the second session on bacterial resistance. Dr. Waters initiated the discussion by describing the experience in Malaysia with the first 100 proven cases of sulfone resistance. Clinical evidence of relapse resulting from the development of resistance occurred between 5 and 24 years after the start of treatment with sulfones. All patients had responded to sulfone treatment for many years before relapsing. More than two-thirds of the patients whose records were complete had become smear-negative, some for many years, before they relapsed. The striking clinical feature was the simultaneous presence of new active leprosy lesions, which were often histoid and usually asym-

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metric, together with old healed disease. Bacteriologically, the patients' smears often showed marked variation among different sites. Drug-susceptibility testing in mice of *M. leprae* isolated from these patients showed that the strains of 85 of 98 patients were resistant to the largest concentration of DDS used. Low dosage favored resistance: initial treatment with solapsone resulted in a 7.8% incidence, whereas initial therapy with a full dose of DDS resulted in a 2.5% incidence. Furthermore, sulfone resistance (11%) has been encountered in patients treated with 50 mg DDS twice weekly, a relatively small dose. Dr. Waters also stated that almost all of the patients who relapsed with DDS-resistant organisms within ten years of starting treatment had either been irregular in their treatment or had started on 50 mg twice weekly or less. Dr. J. M. H. Pearson, Addis Ababa, described an even more serious incidence of sulfone resistance in Ethiopia, where approximately 15% of the patients observed initially treated with DDS developed sulfone resistance; extrapolating these data to 1984, 40% may be expected to develop DDS resistance. In Ethiopia over the past ten years the practice has been to initiate DDS therapy at 10 mg weekly and gradually increase over the next six months to a maintenance of 200 mg weekly. The proportion of high- to low-resistance isolates in Ethiopia is the reverse of that found in Sungei Buloh. About 70% of the resistant strains of *M. leprae* isolated in Ethiopia are resistant only to 0.0001% DDS in mouse chow but not to higher concentrations. Dr. Shepard described a situation in Costa Rica quite different from that in Ethiopia. Of the patients who started therapy during the period 1941 to 1955, principally with sodium glucosulfone (Promin®), 6.8% of those still alive had relapsed with DDS-resistant organisms. Dr. Pattyn has four patients with ethionamide-resistant *M. leprae* confirmed by mouse foot pad inoculation among a group of 102 patients who had been treated for at least five years with daily 500 mg ethionamide alone. Dr. Waters described 14 patients of fewer than 100 patients treated with either DPT or thiacetazone alone who became clinically resistant to these agents. Twelve patient-strains were proven resistant by mouse foot pad inoculation. Two strains were found sensitive to DPT on mouse foot pad inoculation; one of these two patients died before further studies could be carried out, but the other was shown to be a very poor absorber of DPT from the gastro-intestinal tract. Relapse occurred earlier in these patients than in those with DDS resistance, in some as early as two years. One of these patients was BB by the Ridley-Jopling scale initially, whereas DDS resistance has occurred only in more lepromatous patients.

In order to provide information that might be useful in approaching the problems of resistance and persistance, newer developments in the pharmacology of antileprosy drugs were presented. Dr. L. Levy, San Francisco, reviewed two published papers as well as newer work on the nature of the antimicrobial action of DDS against *M. leprae* in the mouse foot pad model. The results from different experiments are conflicting, with activity appearing at times bacteriopausal and at times bactericidal. In one experiment, treatment of mice with 0.1% DDS for one week during early plateau phase killed 99.4% of the *M. leprae*. Mr. G. R. Gordon, Menlo Park, California, presented work on tissue levels of DDS in mice and rats which showed that DDS penetrated all tissues well, including peripheral nerve. He also presented plasma sulfone levels determined in patients during treatment with DADDS. After an intramuscular injection of 225 mg DADDS, peak DDS levels were found 21 to 35 days later; the levels averaged 25 ng/ml immediately prior to the next injection, at least five times the plasma MIC of DDS for *M. leprae*. Dr. J. H. Peters, Menlo Park, and Dr. R. H. Gelber, San Francisco, presented work showing that significant individual differences in acetylation and plasma clearance of DDS do not predispose certain patients to the development of DDS resistance. Dr. Gelber also described a new drug interaction between RMP and DDS; concurrent RMP administration approximately doubled the rate of DDS clearance from the plasma. This probably occurred as a result of microsomal enzyme induction leading to an enhancement of the elimination of DDS by N-hydroxylation, rather than because of displacement of DDS from plasma protein binding sites or increased acetylation.

There was considerable discussion on how to utilize the costly but potent drug RMP most effectively. Dr. Rees reported that a low dose of 150 mg RMP daily showed significant activity and 60 mg daily some activi-

ty in a small clinical trial. In a small series of patients, Dr. Levy found that single doses of 1500 mg and 1200 mg and daily doses of 600 mg RMP always rendered organisms recovered from skin biopsy specimens noninfectious for mice as early as three to five days following initiation of treatment. With single doses of 900 or 600 mg or with daily 300 mg doses, there were some patients whose specimens were not rendered noninfectious in that time. By a few weeks, *M. leprae* recovered from the skin biopsy specimens of all of the patients treated with the lower single or daily dose were noninfectious for mice. Dr. E. J. Saerens, Brussels, presented data from tuberculosis chemotherapy trials with RMP that suggest that a critical ratio of anti-RMP antibodies to antigen causes a variety of systemic reactions to intermittently-administered RMP. The reactions increase in frequency with increased dosage, and the longest interval between doses utilized, one week, is associated with the highest frequency of drug toxicity. Dr. Shepard reported on a trial of 1500 mg RMP together with DADDS every 77 days. Of 26 patients who received between three and five doses, 8 had reactions. In four patients who had had reactions, none experienced a reaction when a subsequent dose was spread out over two days.

Another major theme of the workshop was the discussion of other antimicrobial agents. Because of the recent success of bactericidal agents in short-course chemotherapy of pulmonary tuberculosis, bacterial killing was emphasized. Mr. M. J. Colston, London, described the "proportional bacteriocidal" test, a new technic for quantitating the degree of lethal activity of an antimicrobial against *M. leprae* in mice. Thiocarlide, DPT, and thiacetazone showed no bactericidal activity. Clofazimine (B663) showed 2% survival, DDS 17% survival, and RMP less than 0.1% survival in this test. Dr. Shepard had previously reported that ethionamide is bactericidal for *M. leprae* in mice; and Dr. G. R. F. Hilson, London, argued from plasma levels in mice that serum bactericidal levels in man could be obtained using well tolerated doses of this drug. Dr. Shepard reviewed already published data from mice utilizing the kinetic technic that showed streptomycin was purely bacteriostatic. Dr. Waters presented the results of a small pilot study using one gram streptomycin daily in previously untreated lepromatous patients. Clinically all patients improved, and the MI fell at a rate not significantly different from that seen during treatment with DDS. Unfortunately the final skin biopsy was taken after six weeks of therapy and showed only a marginal loss of infectivity. After six weeks of daily RMP treatment, *M. leprae* recovered from skin biopsy specimens are almost uniformly noninfectious, whereas six weeks of daily DDS treatment usually produces little loss of infectivity. Mr. Colston found that the minimal inhibitory concentration (MIC) of thiacetazone in mouse plasma for *M. leprae* was 0.1 to 0.3 μg/ml; it is known that daily administration to patients of 150 mg thiacetazone produces peak levels of 2 μg/ml and trough levels of 0.6 μg/ml. He found that the MIC of DPT in mouse plasma for *M. leprae* was 0.3 to 1.0 μg/ml; therapy with DPT 1.5 gm daily produces peak plasma levels of 0.8 μg/ml and trough levels of 0.1 μg/ml. Thus, the MIC for *M. leprae* of the purely bacteriostatic drug thiacetazone is exceeded throughout the entire 24-hour cycle of administration, whereas the MIC of the similarly bacteriostatic DPT for *M. leprae* is clearly not exceeded for some considerable fraction of the 24-hour cycle.

Dr. N. E. Morrison, Baltimore, reported that a number of 2,4-diaminoquinazolines inhibited dihydrofolate reductase derived from *Mycobacterium* sp. 607 and also inhibited multiplication of *M.* sp. 607. DDS and active 2,4-diaminoquinazolines are truly synergistic against *M.* sp. 607 by virtue of sequential blockade of folate biosynthesis. Dr. Gelber reported the screening by the kinetic method of a number of dihydrofolate reductase inhibitors, including 2,4-diaminoquinazolines, against *M. leprae* in the mouse foot pad infection, and found that they were active. In combination with DDS there was suggestive evidence of synergism and killing of *M. leprae* not found with either drug alone. These dihydrofolate reductase inhibitors appear to have potential for new antileprosy drug development.

At first glance it appears inconsistent that viable *M. leprae* cannot be detected after a few months of DDS or even a few days of RMP but are regularly found after some years of therapy. A number of the participants offered the explanation that early in therapy the total number of organisms is so much larger than the number of viable *M. leprae* that viables cannot be detected by

mouse inoculation; they are diluted by the dead organisms, and their proportion is smaller than the minimum detectable in normal mice. Later in therapy, the BI decreases, as a result of the clearing of dead *M. leprae,* and the proportion of viable organisms increases so that detection of organisms by mouse foot pad inoculation again becomes possible. A number of participants described the experience of patients treated for many years who relapse after discontinuing therapy, some having become smear-negative for several years. Undoubtedly then, these persisters are clinically important. However, at present there is no information on what proportion of patients treated for 15 to 20 years will relapse if treatment is stopped. Dr. Bhojwani, the superintendent at Sungei Buloh, is conducting just such a study which should soon provide definitive data on the clinical significance of drug-susceptible *M. leprae* that survive treatment.

Dr. Shepard suggested the importance of developing laboratory models of persistence. Thymectomized, irradiated and bone-marrow-reconstituted mice, neonatally thymectomized rats, armadillos and nude mice were proposed as candidates. A number of chemotherapeutic approaches was suggested. First Dr. Rees emphasized that persisters were as common when RMP was used alone as when the purely bacteriostatic agent DPT was added. Dr. Saerens drew a parallel to the remarkable results of short-course therapy in tuberculosis and the importance of regimens including two or, better yet, three bactericidal drugs to success. Many participants suggested that the ability of combinations of drugs to prevent persisters ought to be fully explored in long-term clinical trials. Dr. Gelber underscored the need for the continued search for new agents that, like RMP, are primarily bactericidal. Only those drugs found to be bactericidal were considered for proposed treatment regimens. Besides RMP, these include DDS, B663 and ethionamide. Dr. Gelber and Dr. R. R. Jacobson, Carville, suggested that the "lepromatous" armadillo might be utilized for chemotherapy trials directed at the eradication of persisters that would yield early and important information concerning promising regimens for clinical trial.

The significance of the resistance problem was also vigorously discussed. Dr. Pearson suggested that treatment with low-dosage DDS appears to increase the risk, and that an incidence of 2.5% of patients relapsing over a period of about 25 years when full dosage of DDS is employed might be acceptable. Others felt the problem of DDS resistance was of sufficient magnitude that combination therapy of lepromatous leprosy must be recommended now, and that long-term clinical trials utilizing combinations of agents must be initiated. Most of the participants agreed that an initial period of RMP plus a sulfone would be best, but opinion was divided on which sulfone and how much RMP would be necessary. Various other drugs including streptomycin, DPT, ethionamide, thiacetazone, and B663 were suggested as third drugs or to replace sulfones or RMP in such trials. Dr. Waters and Dr. H. Sansarricq, (WHO) Geneva, suggested that the definition of ideal chemotherapy regimens should be accomplished first; afterwards, the issues of cost and methods of delivery for individual countries should be considered.

In treating patients with relapse in countries like Ethiopia where sulfone resistance is very common, Dr. Rees proposed that adding RMP and increasing the dose of DDS might be sufficient because of the low degree of DDS resistance found there. Dr. Shepard felt that this was risky and that two new drugs should be added.

The final session, chaired by Dr. Pearson, considered the treatment of nonlepromatous leprosy. Dr. Pearson began by discussing the design of clinical trials in tuberculoid leprosy. He concluded and later Dr. Shepard agreed that the only way to learn whether a patient has been cured is to stop the drug to see if relapse occurs. Dr. Pearson proposed that trials in tuberculoid leprosy may be carried out over a limited time, because relapse generally occurs 18 to 24 months following termination of therapy. Dr. Pearson is currently attempting to define criteria for relapse in tuberculoid disease and has found that the search for bacilli in skin and the study of histopathological changes are not useful, whereas the search for organisms in nerve biopsy specimens may be helpful. He suggested that trials in nonlepromatous disease might provide clues for treatment of lepromatous leprosy, because regimens that fail to prevent relapse in the former clearly would be insufficient to treat the latter.

Dr. D. A. Russell, Port Moresby, reported that 365 patients with paucibacillary leprosy

(tuberculoid, borderline-tuberculoid and indeterminate) in the Karimui were begun on DADDS therapy in 1967; all have responded satisfactorily, and in 1974 95% of these were completely healed. Dr. Shepard reported on a trial in progress in Cebu with the objective of determining the least amount of treatment necessary to prevent relapse of paucibacillary leprosy. Patients are to be followed for three years after they have reached inactivity by clinical and laboratory criteria. The treatment regimens have been set according to the Ridley-Jopling classification, and compare a course of DADDS only with a short course of RMP and DDS followed by DADDS. For example, one group of TT patients is receiving 225 mg DADDS every 12 weeks for 48 weeks and the other 2 weeks of 600 mg RMP plus 50 mg DDS daily followed by 225 mg DADDS every 12 weeks for 24 weeks. Those receiving the combined regimens appear to be showing somewhat more rapid clinical improvement. Also, there has been enhancement of Mitsuda reactivity in treated patients. Information on relapse rates after discontinuing therapy are not yet available.