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COMBINATIONS INVOLVING DAPSONE, RIFAMPIN, CLOFAZIMINE, AND ETHIONAMIDE IN THE TREATMENT OF M. LEPRAE INFECTIONS IN MICE

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There are two substantial reasons for using combinations of anti-leprosy drugs. The first is to decrease the risk of developing drug-resistant M. leprae. Studies in Malaya and in Costa Rica have found that among lepromatous patients for whom sulfones were prescribed in standard dosages, less than 10% develop relapses due to DDS-resistant M. leprae. One would expect, therefore, that the addition of a second effective drug with independent mechanism of action would decrease the rate of relapse due to drug-resistant M. leprae to a very low level.

The second reason for using combinations is that the killing of the M. leprae may be more rapid than with single drugs. We know from therapeutic trials involving mouse inoculation methods that the initial phase of killing, the reduction in numbers of viable bacteria by a factor of 10^3 or so, is fairly rapid. The killing of the remaining viable M. leprae takes many years, however. We know this from the incidence of relapses when treatment is stopped, and from recent mouse inoculation stidies by Rees, Waters, and Pearson and by Pattyn. The reason for the survival of this small fraction of drug-sensitive organisms are not known. Some possible explanations are that the surviving bacilli are in a latent state and therefore not incorporating much drug into their metabolic products, or they are in a site where drug does not reach them, or they are in a cell that supplies them with a physical or nutritive factor that allows them to escape drug. Whatever the explanation, it seems possible that the surviving bacteria might not be identical for each drug. Moreover, a combination of drugs might produce a combination of different biochemical lesions, from which a bacillus might have increased difficulty in recovering.

Combinations of drugs can be antagonistic, however, and before combinations are tried in patients they ought to be tested in animals. Several years ago we studied combinations of clofazimine (B663), dapsone (DDS), and ethionamide (Eth) against M. leprae in mice in a kinetic experiment. We tested the drugs at high and at low levels, and at both levels we found no evidence of antagonism and instead found evidence of additive effects (Internat. J. Leprosy (1972) 40:33.

We have repeated the studies, in two experiments, now adding

rifampin (RMP) to the three drugs studied earlier. Experiments of this kind can get very large, and it is not possible to try more than one dosage of drug in one experiment. Moreover strains of M. leprae vary in sensitivity to drug, so what is needed for each drug given alone is a dosage that will have a level of effect that will allow either antagonistic or additive effects to be detected in the combinations. Experience helps in the selection of the dosage, but one also needs a degree of luck. Fortunately both experiments succeeded.

The results of one experiment are shown in Table 1. The single drugs are arranged in an order that allows one to look down the column to compare combinations to the most effective single member of the combination. There were 11 combinations and 7 had some increase over the most effective single drug in the combination.

The experiment, with some modifications in dosages in line with increased experience, was then repeated with the results shown in Table 2. The general results were similar. Additive effects were seen with 9 of the 11 combinations.

Comparisons of the various combinations are more easily made in Table 3, where the results with the drug combinations in all three experiments are summarized. In this table positive values indicate increases in growth delay (relative to the most effective single member of the combinations), and negative values represent decreases. Of the 30 entries in the table, 23 represent increases. The 99% confidence limits for the ratio 23/30 = 0.77 are 0.52 - 0.92, so the difference from the expected ratio if there were no additive effect (0.50) is highly significant. Thus we may conclude that more rapid bacterial killing usually resulted from the use of the drug combinations.

Are there any combinations that are especially effective? The only combinations of two drugs that did not have any negative entries are B663-RMP and DDS-Eth. The entries involving these two pairs with or without other drugs number 18, and 17 of these are positive. The entries not involving these two pairs number 12, and 5 of these are positive. Thus there is a suggestion that the excessive additive effects are caused by two combinations, B663-RMP and DDS-Eth.

The four drugs selected for testing in combination are the only acceptable drugs that have been found so far to have bactericidal-type effects in kinetic experiments in mice. There are a few other drugs that also give bactericidal-type effects but there are clear contraindications to their use. Fortunately among the four drugs tested there was no indication of antagonism.

Bactericidal-type effect can, however, be caused by one of three mechanisms--true bactericide, bacteriopause (that is, persisting bacteriostasis after the elimination of drug), or repository effect of drug. Moreover, increase in the antibacterial effect in a combination could also be caused by a drug interaction through the host, for example, by slowed

Table 1. Results of first experiment testing combinations of the four drugs.

	Growth delay[a] (days)			
	RMP[b]	B663[c]	Eth[d]	DDS[e]
RMP	(255)[f]			
B663	311[g]	(239)		
Eth	271	218	(118)	
DDS	222	153	206	(45)
B663-Eth	320			
B663+DDS	303			
Eth+DDS	275	211		
B663+Eth+DDS	343			

[a]Relative to the curve of the mean for four control groups, which passed $10^{5.0}$ M. leprae per mouse at 118 days after infection.
[b]RMP, 20 mg/kg, by gavage on the 90th and 91st day after infection.
[c]B663, 0.004% in the diet, from the 77th-91st day.
[d]Eth, 0.1%, from the 77th-91st day.
[e]DDS, 0.01%, from the 77th-91st day.
[f]Parentheses indicate results with single drugs.
[g]Underlined values indicate increases over the longest delay caused by a single member of the combination.

Table 2. Results of second experiment testing combinations of the four drugs.

	Growth delay[a] (days)			
	RMP[b]	B663[c]	Eth[d]	DDS[e]
RMP	(151)[f]			
B663	226[g]	(188)		
Eth	147	192	(74)	
DDS	173	154	147	(42)
B663+Eth	212			
B663+DDS	>357			
Eth+DDS	212	217		
B663+Eth+DDS	212			

[a]Relative to the curve of the mean for four control groups, which passed $10^{5.0}$ M. leprae per mouse at 118 days after infection.
[b]RMP, 30 mg/kg, by gavage, on the 84th day after infection.
[c]B663, 0.004% in the diet, from the 84th-111th day.
[d]Eth, 0.1%, from the 84th-111th day.
[e]DDS, 0.01%, from the 84th-111th day.
[f]Parentheses indicate results with single drugs.
[g]Underlined values indicate increases over the longest delay caused by a single member of the combination.

Table 3. Summary of experiments with drug combinations.

	Change in growth delay with combination[a] (days)											
	RMP				B663				Eth			
	IA[b]	IB[b]	II[c]	III[d]	IA	IB	II	III	IA	IB	II	III
B663			56	75								
Eth			16	-4	>63	59	-21	4				
DDS			-33	22	>65	-8	-86	-34	>173	55	88	73
B663+Eth			65	61								
B663+DDS			48	>206								
Eth+DDS			20	61	>66	91	28	29				
B663+Eth+DDS			88	61								

[a]Relative to growth delay for the single member (of the combination) producing the longest growth delay.

[b]Experiment I (Internat. J. Leprosy (1972) 40:33): Drugs were given from the 70th-130th day after infection. The dosages in IA were B663, 0.004%; Eth, 0.1%; DDS 0.01%. The dosages in IB were B663, 0.0001%; Eth, 0.01%; DDS, 0.0001%.

[c]Experiment II: That of Table 1, this paper.

[d]Experiment III: That of Table 2, this paper.

elimination of one drug as a result of the presence of another drug.

Our experience in studies of single antileprosy drugs would suggest that studies of these combinations should now be shifted to human trials. The differentiation between the various causes of the bactericidal-type effect will be automatically taken care of, and one will be able to focus on drug metabolism in man. The absence of antagonistic effect between these drugs removes the ethical objection to human trial. The human trials would need to include a) short-term clinical trials with mouse inoculations to monitor the initial rate of killing, b) intermediate-term clinical trials with the methods, now under study, that allow large numbers of M. leprae to be inoculated, c) long-term clinical and bacteriological follow-up, and d) studies of drug levels achieved with various combinations. These four types of studies could, of course, all be carried out in the same patients.

There is reason for haste. Although the problem of drug-resistance of M. leprae is manageable now, it may become increasingly difficult to handle if transmission from patients with drug-resistant infections continues. In the meantime some safe and acceptable combinations probably ought to be recommended for all multibacillary patients, perhaps rifampin and dapsone or rifampin and acedapsone. The recommendations can be modified or changed later, as the outcome of the trials suggested above become available.

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