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The Activity of Various Antituberculous Drugs in Suppressing Experimental *Mycobacterium leprae* Infection in Mice

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By means of the mouse footpad technique of Shepard, and using continuous administration of drugs in powdered diet, the following compounds have been tested for their ability to suppress experimental *Mycobacterium leprae* infection: (1) Trimethoprim; (2) Thiocarlide; (3) Compound AW 16′ 1989 (the isobutyl alcohol ester of N-carboxylated thiocarlide); (4) Compound 20541 RP, a polypeptide antibiotic; (5) Compound B.1912, a phenazine derivative related to clofazimine (B.663); and (6) rifampicin. Five of these compounds had been shown in the laboratories of the manufacturers, and in some cases by other workers, to have antituberculous activity. Trimethoprim (Compound 1) has no significant activity against *Mycobacterium tuberculosis:* however, in our laboratory we have found moderate activity of this drug against *Mycobacterium marinum in vitro.* The chemical structures of the compounds tested are illustrated in Figures 1, 2, 3, 4, and 5.

The methods used were essentially based on those of Shepard and Chang ([13]) and are briefly outlined as follows: Mice were inoculated in the left hind footpad with 10^4 *M. leprae* (counted as acid-fast bacilli) derived from homogenates of human lepromas or of footpads of previously infected mice. The mice were divided into control groups and groups receiving different concentrations of the drug under test, made up in powdered diet, and feeding of the drug was started from the day of inoculation, or 50 or 75 days later (periods which are within the "lag" phase of the infection). No drug concentration higher than 0.1 per cent was tested. Samples of three or four mice

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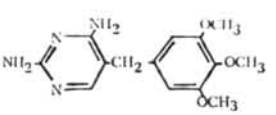


FIG. 1. *Trimethoprim* (Wellcome). (2, 4-diamino-5-(3, 4, 5-trimethoxybenzyl) pyrimidine). Action: Inhibitor of dihydrofolate reductase. Strong synergistic action with sulfonamides against fast-growing bacteria, with bactericidal effect.

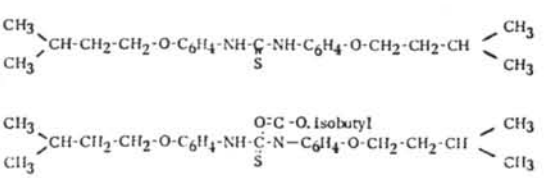


FIG. 2. Thiocarlide (upper) and AW 16′ 1989 (lower). *Thiocarlide* ("Isoxyl" Continental Pharma). 4-4′ diisoamyloxythiocarbanilide. *AW 16′ 1989* (Dr. A. Wander S.A., Berne). Isobutyl ester of N-carboxylated thiocarlide.

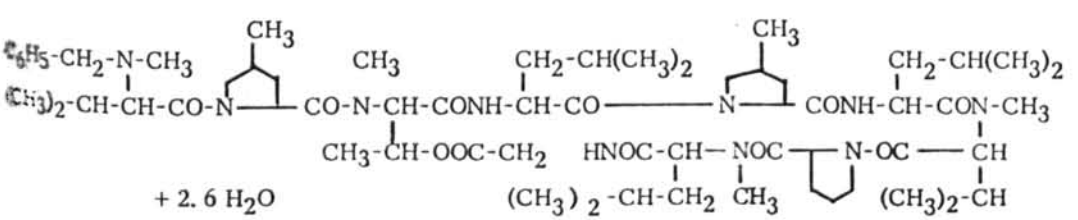
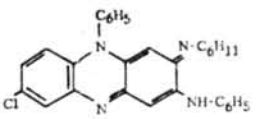
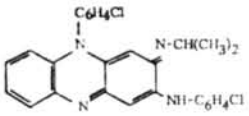


FIG. 3. *20541 R.P.* (May & Baker, Ltd.). A polypeptide antibiotic: $(C_{62}H_{100}N_{10}O_{11}) + H_2O$. (M.W. 1, 162).



B.1912



B.663

FIG. 4. *B.1912* (May & Baker, Ltd.). Related to clofazimine (B. 663): $C_{30}H_{27}N_4Cl$). (M.W. 479).

from the control groups were killed four months after inoculation, and the inoculated footpads homogenized and counts of their content of acid-fast bacilli (AFB) made by a standardized technique. Further samples were taken from the control groups at monthly intervals thereafter, and when these showed AFB counts approaching 10^6/footpad, samples of mice from the drug-treated groups were also taken and counts of AFB/footpad made. If AFB counts in the footpads of drug-treated mice were essentially equal to those in the controls, the drug was taken to be inactive at the concentration tested; if the counts were lower, "partial activity" was recorded; and if the counts were no higher than the original inoculum of 10^4/footpad, the drug was recorded as "active," i.e., completely suppressive of *M. leprae* growth.

The results obtained with drugs which were inactive, or active only at relatively high concentrations in the diet, are presented first, and are summarized in Table 1 (Compounds 1, 2, 3, and 4). *Trimethoprim* (TMP) (Compound I) adminis-

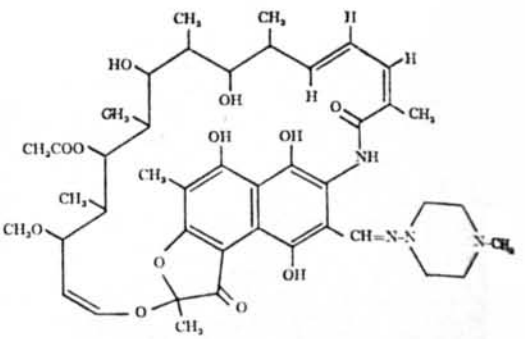


FIG. 5. *Rifampicin* (Lepetit). An antibiotic semi-synthetic derivative of rifamycin from *Streptomyces mediterranei*. Empirical formula: $C_{43}H_{58}N_4O_{12}$. Chemical Name: 3-(4-methyl-1-piperazinyl-iminomethyl)-rifamycin SV.

TABLE 1. *Data on drugs showing poor activity against* Mycobacterium leprae *in the mouse foot pad infection.* M. leprae *inocula* 10^4 *or* 5×10^3. *Viability (solid ratios): 12–33%. Counts of AFB at different times of harvest in control mice and mice receiving different drug treatments.*

Drug	Harvest at month	AFB in controls ($\times 10^4$)	AFB ($\times 10^4$) in mice on treatment with: 0.01% (25)	0.03% (75)	0.1% (250) in diet (mgm./kgm.)	Activity
Trimethoprim	4	88	140	—	20	Inactive
	5	190	11	—	100	
Thiocarlide	5	320	73	15	—	Active at 0.03% (75 mgm./kgm.)
	6	180	100	5.3	—	
	7	360	110	1.3	—	
AW 16' 1989 (Strain 1)	4	88	24	—	1.3	Active at 0.1% (treated from day 0)
	5	190	55	—	<1.0	
(Strain 2)	5	320	—	190	110	Inactive (treated from 75th day post-inoculum)
	6	180	—	140	130	
	7	360	—	430	200	
20541 R.P.	5	120	22	15	<1.0	Active at 0.1%

tered up to 0.1 per cent in the diet was inactive. It has also been previously found inactive (C. C. Shepard, 1969, personal communication) by the kinetic method [Shepard (11)], with no enhancement of dapsone (DDS) activity. We have found TMP with DDS is synergistic in inhibitory action *in vitro* (but with no bactericidal effect) on *M. marinum*, but in mouse footpad infections with this species TMP alone was ineffective, and it did not enhance the activity of DDS (unpublished). *Thiocarlide* (Compound 2) was active at 0.03 per cent in the diet, corresponding to a dosage of 75 mgm./kgm. This particular diphenyl thiourea has not apparently been tested previously in the mouse footpad; other related compounds have generally only been active experimentally at 0.1 per cent in the diet. There are favorable clinical reports of the activity of thiocarlide (Buu-Hoi (2, 3), Griffiths (6) and Sentilhe (10). The thiocarlide derivative (*AW 16′ 1989*, Compound 3), administered from the day of inoculation with one strain of *M. leprae*, showed definite activity only at 0.1 per cent in the diet, and against a second strain, the drug being given from 75 days post-inoculation, was inactive. Compound 4, the polypeptide antibiotic *20541 RP*, showed partial activity down to 0.01 per cent, but was only fully suppressive at 0.1 per cent dietary dosage. Neither this compound nor the previous one, AW 16′ 1989, has previously been tested experimentally or clinically.

B.1912 (Compound 5): the results are shown in Table 2, and suggest that a dietary concentration of 0.0001 per cent is active in the short term, but that 0.0003 per cent (0.75 mgm./kgm.) may be necessary for complete suppression. B.1912 has not previously been tested against *M. leprae;* against *Mycobacterium lepraemurium* the activity of B.1912 was found to be equal to that of B.663 (Y. T. Chang, 1970, personal communication); and Shepard (12) has shown activity of B. 663 against *M. leprae* at 0.0001 per cent dietary dosage. The activity of B.663 at higher dose levels is well established [Shepard and Chang (14), Rees (8) and Gaugas (5)] and the clinical value of the drug in leprosy is well recognized [Browne and Hogerzeil (1), Pettit and Rees (7)]. As in the case of B. 663, mice receiving B.1912 developed a reddish pigmentation. It is too soon to say whether B.1912 may be found to have any advantage in practice over B.663: in the case of both compounds the minimum clinically effective dose has yet to be found, and the relationship between blood levels in patients or in mice on treatment with the drugs is as yet unknown.

Rifampicin (Compound 6): the results of rifampicin treatment of infections in mice with two strains of *M. leprae* are shown in Table 3. Against both strains 0.0001 per cent of this drug in the diet showed partial activity of a similar degree, and 0.0003 per cent (0.75 mgm./kgm.) was completely suppressive in the case of one strain, the drug having been administered from the 75th day after inoculation. With the second strain, 0.01 per cent dietary dosage was effective: the intermediate dos-

TABLE 2. *B. 1912.* M. leprae *inoculum* 10^4 *AFB/footpad (solid ratio 22%). B. 1912 given in powdered diet from 50th day after inoculation.*

Harvest at month	AFB in controls ($\times 10^4$)	AFB ($\times 10^4$) in mice on treatment with: 0.0001 (0.25)	0.001 (2.5)	0.01 (25) % in diet (mgm./kgm.)
4	45	—	—	—
5	110	7.6 (mean of 2.2, 5.6 & 15)	<1.0	<1.0

TABLE 3. *Rifampicin.* M. leprae *inocula* 10^4 *AFB/footpad: 2 strains used (solid ratios 12 and 14%). Rifampicin given in powdered diet from 75th day after inoculation.*

Harvest at month	AFB in controls ($\times 10^4$)	AFB ($\times 10^4$) in mice on treatment with: 0.0001% (0.25)	0.0003% (0.75)	0.001 (2.5)	% in diet (mgm./kgm.)
5	120	13	1.0	1.0	(Strain 1)
6	190	25	1.0	<1.0	
		0.0001%	0.01%		
5	110	38	1.0		(Strain 2)

ages were not investigated in this case. *M. leprae* has previously been shown to be suppressed by 0.0025 per cent in mouse diet, which was the lowest dosage tested [Rees, Pearson and Waters (9)]. These authors reported the results of a pilot clinical trial, on the basis of which they concluded that this drug appears to show especially high bactericidal activity against *M. leprae*.

Assays were made by a microbiologic method of the concentrations of rifampicin in the serum of mice on continuous treatment with the drug at two levels of dietary dosage, and the method and results are shown in Table 4. The levels found appeared to be mutually consistent, since a tenfold difference in amount of drug administered corresponded to a tenfold difference in serum levels. If strains of *M. leprae* can be taken to be suppressed by a dietary dosage of 0.0003 per cent in mice, and the serum levels found at higher dosage may be extrapolated to the lower dose level, the minimum inhibitory concentration (MIC) of rifampicin *in vivo* for *M. leprae* appears to be of the order of 0.06-0.12 μg./ml. Serum levels of this drug in patients receiving 600 mgm. of rifampicin daily average about 2-4 μg/ml. [Furesz *et al.* (4)], which would apparently constitute a concentration at least tenfold higher than the MIC for *M. leprae* suggested on the basis of the experimental infection.

TABLE 4. *Rifampicin. Assay of serum levels in mice on continuous treatment at 2 dose levels*

Method	By tube titration with doubling dilutions of serum in nutrient broth + 50% horse serum. Test organism: *Sarcina* species with rifampicin sensitivity in the system of 0.01 μg/ml.
Results:	In mice on 0.1% Rmp, serum levels were 15–30 μg/ml; in mice on 0.01% Rmp, serum levels were 2–4 μg/ml.

CONCLUSIONS

Trimethoprim and the compound AW 1989 appear unlikely to be of practical value in the treatment of leprosy, and the potency of the latter appears to be inferior to that of thiocarlide, of which it is a derivative. Thiocarlide and the antibiotic 20541 cannot be completely dismissed, and much would depend on the determination of the MIC's of these compounds for *M. leprae* and their relationship to serum levels in patients on tolerated dosage. The potency of the compound B.1912 is clearly high, of the same order as that of the related riminophenazine B.663, and worthy of further investigation. The validity of the great interest being shown at present as to the value of rifampicin in leprosy is confirmed by the findings presented above.

SUMMARY

By means of the mouse footpad technique of Shepard, and using continuous ad-

ministration of drugs in powdered diet, the following compounds have been tested for their ability to suppress experimental *M. leprae* infection: (1) Trimethoprim; (2) Thiocarlide; (3) Compound AW 16′ 1989 (the isobutyl alcohol ester of N-carboxylated thiocarlide); (4) Compound 20541 RP, a polypeptide antibiotic; (5) Compound B.1912, a phenazine derivative related to clofazimine (B.663); and (6) Rifampicin.

Trimethoprim (1) at dosages up to 0.1 per cent in the diet (approximately 250 mgm./kgm.) was inactive; thiocarlide (2) was active in suppressing bacillary growth at 0.03 per cent (75 mgm./kgm.), but not at lower dosages; AW 16′ 1989 (3) was suppressive against one strain of *M. leprae* at 0.1 per cent but not lower dosage, and inactive at 0.1 per cent against another strain; and 20541 RP (4) was completely inhibitory at 0.1 per cent; at 0.03 and 0.01 per cent slight activity only was detected. Activity of a much higher order was found with B.1912 (5) and with rifampicin (6). Five months after inoculation B.1912 was completely suppressive at 0.01 and 0.001 per cent and partially active at 0.0001 per cent (0.25 mgm./kgm.) against one strain. In the case of rifampicin, 0.0003 per cent (0.75 mgm./kgm.) was completely suppressive, and 0.0001 per cent partially so, against one strain, these results being based on harvests at five and six months after inoculation; with a second strain 0.0001 per cent was also partially suppressive. By a microbiologic assay method rifampicin at 0.1 per cent in powdered diet was found to produce a blood level in mice of 15-30 μg/ml., and at 0.01 per cent a level of 2-4 μg/ml. These results suggest, therefore, by extrapolation, that the minimal inhibitory concentration in mice of rifampicin for *M. leprae* may be of the order of 0.06-0.12 μg/ml.

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