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Further Studies of the Action of Antithyroid Drugs on *Mycobacterium leprae*[1] (40251)

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In an earlier study (1), the antimicrobial action of methimazole and several additional antithyroid agents was studied in mice infected with *Mycobacterium leprae*. The drugs, methimazole, propylthiouracil, thiambutosine and 5-*n*-heptyl-2-thioxo-4-thiazolidinone, were found to possess in common both antimicrobial and antithyroid properties. Methimazole was active against *M. leprae* even when it was co-administered with exogenous thyroid substance. In an earlier study (2), in which the effect of methimazole treatment had been compared with that of ^{131}I-iodide administration, additional evidence had been adduced that the antimicrobial properties of methimazole were independent of its antithyroid activity. In an effort further to clarify the relationship between the antimicrobial and antithyroid activities of methimazole, additional experiments have been carried out. The results of these experiments appear to confirm that the two properties are unrelated.

Materials and methods. Methimazole was supplied by the Lilly Research Laboratories, Indianapolis, Indiana, 5-*n*-heptyl-2-thioxo-4-thiazolidinone by the Sterling-Winthrop Research Institute, Rensselaer, New York, and thiambutosine by the CIBA-GEIGY Corporation, Summit, NJ; propylthiouracil was purchased from the Aldrich Chemical Company, Milwaukee, WI.

The *M. leprae* were of a strain originally isolated from a lesion of a patient with previously untreated lepromatous leprosy by C. C. Shepard, Center for Disease Control, Atlanta, Georgia, and subsequently carried in mouse passage. Inocula of *M. leprae* were prepared, and *M. leprae* were harvested from infected foot-pad tissues by published methods (3, 4). Locally-bred female BALB/c mice were employed. Drugs were incorporated into the mouse chow by means of a liquid–solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, PA).

The sources of all of the cultivable mycobacterial strains are given in an earlier publication (5), except for the following: *M. intracellulare*, our strain no. 74, obtained from the American Type Culture Collection (ATCC 15985); *M. kansasii*, our strain No. 33, obtained from A. Back, City-County Health Department, San Francisco; and *M. scrofulaceum*, our strain no. 66, from the Trudeau Institute (TMC 1321). Stock cultures of the cultivable *Mycobacteria* on Lowenstein-Jensen slants were stored at 4°. Tubes of Dubos 7H9 broth with oleic-albumin supplement (Difco Laboratories, Detroit, Michigan) were inoculated from stock cultures of the cultivable mycobacterial strains and incubated at 37° until the absorbance as measured at 580 nm in a Coleman Sr. spectrophotometer exceeded 0.1. At this time, the cultures were diluted to yield an absorbance of 0.1, and 0.1 ml aliquots were used to inoculate triplicate 10-ml vol of drug-free and methimazole-containing Dubos 7H9 broth with oleic-albumin supplement. Methimazole dissolved in 10% (v/v) ethanol had been added to some of the culture media to yield final concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} *M*. Equal volumes of 10% ethanol were added to the drug-free cultures. The absorbance of the drug-free and methimazole-containing cultures was measured at intervals during incubation at 37° until the absorbance of the drug-free cultures achieved a level of 0.5. At this time, bacterial growth in drug-containing media was scored as "fully inhibited" if there had been no increase in the absorbance of these cultures, and as "partially inhibited" if the absorbance of the cultures had increased to a level no greater than 70% that of the

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drug-free control cultures. For 21 of the 29 mycobacterial strains studied, aliquots of drug-containing cultures in which no increase of absorbance had been observed were plated at intervals on Dubos 7H9 agar to learn if bacterial killing had occurred.

Results. Studies in M. leprae*-infected mice.* Groups of 30 female BALB/c mice were inoculated in the right hind foot pad with $10^{3.7}$ *M. leprae.* On the same day, treatment of all but one of the groups was begun with 0.05 or 0.005 g methimazole per 100 g of mouse chow, 0.1 or 0.01 g propylthiouracil per 100 g, 0.1 or 0.01 g thiambutosine per 100 g, or 0.15 or 0.015 g of the thiazolidinone per 100 g mouse chow. Harvests of *M. leprae* were performed from the pooled foot pad tissues of four mice between 145 and 152 days after inoculation; at this same time, additional mice in each group were sacrificed by exsanguination, and pooled heparinized plasma specimens were used for measurement of the concentrations of protein-bound iodine (PBI) and thyroxine (T4).

The results of this experiment are shown in Fig. 1. Multiplication of *M. leprae* in untreated mice may be seen to have proceeded during logarithmic phase with a doubling time of 16 days, reaching the level of 10^6 AFB per foot pad on day 144. The results of harvests of *M. leprae* from the foot pad tissues of treated mice demonstrate that all four compounds inhibited multiplication of *M. leprae.* The relative efficacy of the compounds in the dosages administered was: thiambutosine > the thiazolidinone > methimazole > propylthiouracil. With the exception of the thiazolidinone, the two concentrations of each compound proved equally effective in terms of inhibition of multiplication of *M. leprae*; with the exception of propylthiouracil, administration of the lower concentration of each compound was not associated with decreases of the PBI and T4.

Thus, all four antithyroid compounds were again found to be effective inhibitors of the multiplication of *M. leprae.* The relative potency of the four compounds was much the same as that shown in the earlier study (1). In general, the antimicrobial potency of these compounds was not reduced when the dosages were reduced by 90%, whereas the antithyroid potency as measured by the PBI and T4 was markedly reduced.

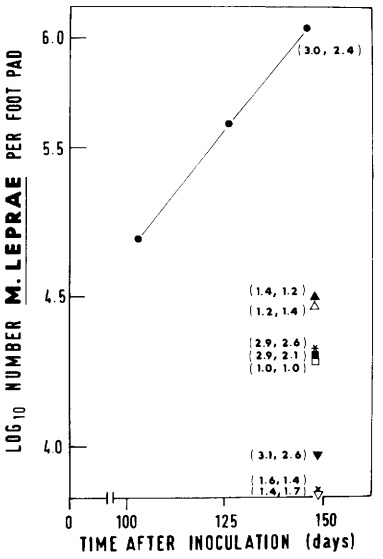

FIG. 1. Log_{10} number of *M. leprae* in mouse foot pads as a function of time after inoculation. ●—no treatment; △—0.1 g propylthiouracil per 100 g mouse chow; ▲—0.01 g propylthiouracil per 100 g; ×—0.15 g 5-*n*-heptyl-2-thioxo-4-thiazolidinone per 100 g; *—0.015 g 5-*n*-heptyl-2-thioxo-4-thiazolidinone per 100 g; □—0.05 g methimazole per 100 g chow; ■—0.005 g methimazole per 100 g; ▽—0.1 g thiambutosine per 100 g mouse chow; ▼—0.01 g thiambutosine per 100 g. All drugs were administered incorporated in the mouse chow continuously from the day of inoculation. The pairs of numbers enclosed in parentheses represent the PBI and T4 in μg per 100 ml mouse plasma measured in mice administered the corresponding drug and dosage.

Studies with cultivable Mycobacteria. The susceptibility to methimazole was studied of 29 strains representing six species of *Mycobacteria,* of each of which at least three strains were available in our collection. The results of this study, summarized in Table 1, demonstrate that all 29 strains were completely inhibited from multiplication by methimazole in a concentration of 10^{-1} *M*; almost all of the strains were partially inhibited by 10^{-2} *M* methimazole. The effect of 10^{-1} *M* methimazole was found to be bactericidal on virtually all of the strains in which the possibility of bactericidal effects was searched for. Two of the four strains of *M. gordonae* studied were found to be completely inhibited by methimazole in a concentration of 10^{-4} *M*, the smallest concentration studied, and 10^{-3} *M* methimazole was shown to exert bacteri-

TABLE 1. EFFECT OF METHIMAZOLE ON MULTIPLICATION OF CULTIVABLE *Mycobacteria in vitro*.

Species	Strain[a]	Concentration of methimazole (M)		
		Full inhibition	Partial inhibition	Killing[b]
M. avium	2	10^{-1}	10^{-2}	N.S.[c]
	10	10^{-1}	10^{-2}	10^{-1}
	29	10^{-1}	10^{-2}	N.S.
M. gordonae	1	10^{-2}	10^{-3}	10^{-2}
	24	10^{-4}	N.A.[d]	10^{-3}
	36	10^{-4}	N.A.	10^{-3}
	52	10^{-1}	10^{-2}	N.S.
M. intracellulare	5	10^{-1}	10^{-2}	10^{-1}
	14	10^{-1}	10^{-2}	N.S.
	74	10^{-1}	10^{-2}	N.S.
M. kansasii	16	10^{-1}	10^{-2}	10^{-1}
	17	10^{-1}	10^{-2}	N.S.
	33	10^{-1}	10^{-2}	10^{-1}
	46	10^{-1}	10^{-2}	10^{-1}
	47	10^{-1}	10^{-2}	10^{-1}
	48	10^{-1}	10^{-2}	10^{-1}
	51	10^{-1}	10^{-2}	N.S.
M. scrofulaceum	8	10^{-1}	None[e]	10^{-1}
	20	10^{-1}	10^{-2}	N.S.
	25	10^{-1}	10^{-2}	10^{-1}
	37	10^{-1}	10^{-2}	10^{-1}
	66	10^{-1}	10^{-2}	10^{-1}
M. smegmatis	9	10^{-1}	None	10^{-1}
	26	10^{-1}	None	None
	42	10^{-1}	None	10^{-1}
	44	10^{-1}	10^{-2}	10^{-1}
	45	10^{-1}	None	10^{-1}
	63	10^{-1}	10^{-2}	10^{-1}
	64	10^{-1}	None	10^{-1}

[a] Accession number.
[b] When a bactericidal effect was noted, the cultures were sterilized in virtually all instances.
[c] Not studied.
[d] Not applicable; the smallest concentration of methimazole studied produced full inhibition.
[e] Not produced by any concentration of methimazole studied.

cidal effects against these two strains. Thus, methimazole appears to exert an antibacterial effect against many cultivable *Mycobacteria*.

Discussion. Previous work in this laboratory has shown that the administration to mice of methimazole, a second antithyroid drug, propylthiouracil, and two antimicrobial compounds that exerted antithyroid effects—thiambutosine and 5-*n*-heptyl-2-oxo-4-thiazolidinone, was associated with the inhibition of multiplication of *M. leprae* in the foot pad (1, 2). Moreover, treatment of mice with ^{131}I-iodide was not accompanied by inhibition of multiplication (2). Finally, the administration of U.S.P. thyroid substance together with methimazole antagonized completely the antithyroid effects of methimazole without blocking the inhibitory effects on *M. leprae* (1). Yet, because the administration of thyroid substance alone was associated with inhibition of multiplication of *M. leprae* (1), one could not be completely confident that the antimicrobial effects of methimazole were independent of its effects on the thyroid function of the host.

In order further to evaluate the relationship between the antimicrobial and antithyroid properties of methimazole, two additional experiments have been carried out. The four antithyroid and antimicrobial compounds were administered to *M. leprae*-infected mice in the concentrations originally employed (1) and in concentrations one-tenth the original, in the hope that smaller dosages of the compounds might produce only one of the two effects. This hope was largely realized. The antimicrobial effects of three of the four compounds were as pronounced when the compounds were administered in smaller dosages as when the larger dosages were employed. And the lower dosages of two of these compounds did not produce decreases of the PBI and T4. This is certainly further evidence of dissociation of the antimicrobial and antithyroid properties.

In the second experiment, methimazole in a concentration of 10^{-1} *M* inhibited at least partially the multiplication *in vitro* of 29 cultivable mycobacterial strains, and two strains of *M. gordonae* were fully inhibited by 10^{-4} *M* methimazole. Moreover, evidence that methimazole was bactericidal was found for all 21 of the strains in which such evidence of bactericidal effects was sought. These results suggest that the antimicrobial effects of methimazole may be expressed without the intermediacy of an animal host. Thus, although the antithyroid and antimicrobial effects of the four compounds studied may depend on a common mechanism of action, it appears that the antimicrobial effects of methimazole and some related compounds on *M. leprae* in the mouse do not result from inhibition of

thyroid function of the mice by these compounds.

No reports have been found of the concentration of methimazole in plasma after administration of the compound. Sitar and Thornhill found (6) the half-time of disappearance ($T_{1/2}$) of methimazole from the plasma of the rat to be about 4 hr, very like the $T_{1/2}$ of dapsone from the plasma of the rat (7). Ozawa et al have reported (8) that administration to mice of dapsone in a concentration of 0.01 g per 100 g mouse chow produces a plasma dapsone concentration of about 1 μg/ml; the larger dosage of methimazole employed in this experiment might therefore be expected to produce a plasma methimazole concentration of about 5 μg per ml. A 10^{-4} *M* concentration of methimazole is equivalent to 8.7 μg/ml. Thus, the susceptibility of *M. leprae* to methimazole appears to be about the same order as that of two of the strains of *M. gordonae* studied here.

Summary. Methimazole, an antithyroid drug, and thiambutosine, an antimicrobial active against *M. leprae* in the mouse, were both found to inhibit multiplication of *M. leprae* in the mouse foot pad when administered in concentrations too small to inhibit thyroid function of the mice. Moreover, methimazole inhibited multiplication *in vitro* of a number of cultivable *Mycobacteria.* Although the antimicrobial and the antithyroid effects may be exerted through a common mechanism, it appears that methimazole nevertheless possesses direct antimicrobial properties.

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