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Prothionamide and Prothionamide-S-Oxide in Experimental Leprosy[1]

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Ethionamide (ETH) and prothionamide (PTH) are now well established as highly bactericidal drugs against *Mycobacterium leprae* in animals and man ([3,9]). Combinations of ETH with clofazimine, dapsone (DDS), or rifampin (RFM) have been found to act additively in mice infected with *M. leprae* ([13]), and ETH was effective in treating mice infected with *M. leprae* resistant to DDS and RFM ([5]). Thus, the thionamides are promising adjunctive drugs for combination chemotherapy with DDS and RFM.

Earlier studies ([1,4,6]) performed shortly after ETH was introduced in the late 1950s as a drug for tuberculosis showed that, in animals and man, the first biotransformation of ETH was oxidation to ETH-S-oxide (ETHSO). It was subsequently found that this metabolite was reduced back to ETH *in vivo*, but it is not known whether the oxidation reaction is reversible or whether two separate enzymatic steps operating cyclically are involved. ETHSO is also oxidized metabolically to an amide and subsequently hydrolyzed to an isonicotinic acid derivative. In man, metabolism of ETH is very extensive as shown from studies wherein only 1% of the administered ETH was found in urine ([12]). The S-oxide metabolite is particularly interesting because, of all known metabolites of ETH, it alone exhibited antimycobacterial activity ([2,8]). We subsequently found that the principal circulatory metabolite of PTH in rats and armadillos was the corresponding S-oxide, PTHSO ([10]). These results suggest that the therapeutic activity of thioamides after their administration may result from the action of both the parent drugs and their S-oxides.

In this report, we summarize results of tests of the comparative activities of PTH, PTHSO, and the compound, 2-propylisonicotinamide (PINA) *in vitro* against *M. tuberculosis* H37Rv and the comparative activities of PTH and PTHSO against *M. leprae* in mice.

MATERIALS AND METHODS

The PTH used in these studies was a gift from May and Baker Ltd., Dagenham, England. It was converted to PTHSO by adaptation of a procedure for the synthesis of ETHSO from ETH ([7]). PTH in pyridine solution was oxidized at 20–25°C by the dropwise addition of 32% hydrogen peroxide. The PTHSO was isolated from the reaction mixture by chromatography on a column of silica. Yellow crystals melting at 120–122°C (literature value 117°C ([11])) were obtained by crystallization from a 1:1 benzene : hexane mixture (v/v). Thin-layer chromatography on silica gel 60 of the PTHSO and PTH, using development with ethyl acetate, yielded single compact ultraviolet-absorbing spots at R_f values of 0.13 and 0.65, respectively. Ultraviolet absorption maxima for PTH and PTHSO in chloroform were at 295 nm ($\epsilon = 8 \times 10^3$) and 360 nm ($\epsilon = 7 \times 10^3$), respectively. Mass spectrometric analysis of PTH and PTHSO yielded parent ions at molecular weights 180 and 196, respectively, the latter corresponding to the addition of one oxygen to PTH. Purity of the synthesized PTHSO was estimated to be ≥95%. PINA was a gift of Dr. H. Franz, Saarstickstoff-Fatol, Pohl-

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[3] This material was presented in part by Dr. Peters at the 24th Joint Leprosy Research Conference, New Orleans, Louisiana, 26 September 1979.

TABLE 1. *Comparative activities*[a] *of PTH, PTHSO, and PINA on the growth of* M. tuberculosis *H37Rv.*

Level of compound in medium (μg/ml)	PTH		PTHSO	PINA
	Test 1	Test 2	Test 1	Test 2
10	−	−	−	++
5	−	−	−	++
2.5	−	−	−	++
1.25	−	−	−	++
0.625	−	−	−	++
0.312	−	−	−	++
0.156	+	−	−	++
0.078	+	++	−	++
0.039	++	++	+	++
0	++	++	++	++

[a] Tests were performed by Dr. T. Welch, USPHS Hospital, San Francisco, CA, during January (Test 1) and March (Test 2) 1978. Symbols used are: − no growth; + some growth; ++ growth comparable to controls.

heim, Germany. Our mass spectrometric analysis of this compound yielded results that testified to the authenticity of the assigned structure.

Tests of the activities of PTH, PTHSO, and PINA *in vitro* were performed using *M. tuberculosis* H37Rv in Dubos broth culture (7H9 without malachite green). Duplicate samples containing 0 to 10 μg of the compounds per ml of medium were incubated with assessments of growth made at 8 and 14 days.

The antileprosy activities of PTH and PTHSO were determined by the kinetic method ([14]). Strain CF1 mice were inoculated in the foot pads with 5 to 10 × 10^3 *M. leprae* obtained from the infected tissues of untreated patients or from nude mice with developed lepromatoid lesions. PTH and PTHSO, dissolved in 50% aqueous propylene glycol (Wako Pure Chemical Industries Ltd., Japan), were given by gavage in mg per kg of body weight or fed in the diet during the late lag or early log phase of bacterial multiplication at the doses shown in the tables.

RESULTS AND DISCUSSION

The results of tests of the activities of PTH, PTHSO, and PINA against *M. tuberculosis* H37Rv are shown in Table 1. PTH exhibited an *in vitro* minimal inhibitory activity (MIC) of 0.156 and 0.312 μg/ml in the two tests, not a significant difference. PTHSO exhibited an *in vitro* MIC of 0.078 μg/ml. Clearly, PTHSO was at least equal in activity to PTH and may be more active. These observations for PTH and PTHSO agree, in principle, with earlier findings that ETH and ETHSO exhibited approximately equal antimycobacterial activity ([2, 8]). PINA was found to be inactive in this test.

Tests of the antileprosy activity of PTH at doses of 5, 10, or 20 mg/kg 6 days per week for 30 days, starting on day 51 post-inoculation, are shown in Table 2 (Experiment 1). A repeat test using 2 and 20 mg/kg

TABLE 2. *Effects of graded doses of PTH on the growth of* M. leprae *in mouse foot pads.*[a]

Experiment	Treatment	No. of AFB (×10^5) in pooled foot pads on day					Growth delay[b] (days)
		105	140	175	210	245	
1[c]	None	0.24	0.66	3.1	8.2	14.2	
	PTH—20 mg/kg	—[e]	0.16	0.56	3.8	8.2	30
	PTH—10 mg/kg	—[e]	0.25	1.4	5.3	8.8	16
	PTH—5 mg/kg	0.13	0.27	1.9	6.4	11.6	11
2[d]	None	0.30	0.78	1.4	8.0	11.3	
	PTH—20 mg/kg	—[e]	0.32	0.45	1.1	4.6	45
	PTH—2 mg/kg	—[e]	0.46	0.66	9.8	18.6	3

[a] Treatment was 6 days per week.
[b] Estimated by graphical comparison with the control group.
[c] Drug administration was started 51 days post-inoculation and continued for 30 days.
[d] Drug administration was started 71 days post-inoculation and continued for 35 days.
[e] No AFB detected.

TABLE 3. *Effects of PTH and PTHSO on the growth of* M. leprae *in mouse foot pads.*

Experiment	Treatment	No. of AFB (×10⁵) in pooled foot pads on day													Growth delay[a] (days)
		120	133	135	168	170	180	198	210	240	245	254	273	289	
3[b]	None	1.3					4.1			8.7					
	PTH—10 mg/kg	—[c]					0.74			2.9					86
	PTHSO—10 mg/kg	—[c]					0.81			3.2					78
	PTHSO—5 mg/kg	—[c]					1.6			6.8					26
	PTHSO—2.5 mg/kg	—[c]					2.2			9.3					14
4[d]	None		0.30		2.1				3.8		9.1		11.5		
	PTH—0.01%		—[c]		0.14				0.28		1.4		3.3		68
	PTH—0.001%		0.05		0.73				1.4		4.9		9.4		24
	PTHSO-1—0.01%		—[c]		0.25				0.85		1.1		4.5		56
	PTHSO-1—0.001%		—[c]		1.0				0.91		5.7		12.3		20
	PTHSO-2—0.01%		0.09		0.20				0.75		2.2		3.9		61
	PTHSO-2—0.001%		0.15		0.49				1.1		6.5		11.4		18
5[e]	None			2.5		4.3		5.1				6.1		6.5	
	PTH—0.1%			—[c]		0.11		0.10				0.16		0.21	218

[a] Estimated by graphical comparison with the control group.
[b] Treatment was 6 days per week starting 91 days post-inoculation and continuing for 60 days.
[c] No AFB detected.
[d] Drugs were given in the diet, starting 66 days post-inoculation and continuing for 12 weeks.
[e] Drugs were given in the diet, starting 61 days post-inoculation and continuing for 12 weeks.

6 days per week for 35 days was also carried out (Table 2, Experiment 2). In these experiments, PTH caused growth delays of *M. leprae* that were less than the periods of drug administration in most cases. In only one treatment schedule (20 mg/kg in Experiment 2) was the growth delay slightly more than the period of drug administration.

In mice receiving 10 mg PTH or PTHSO per kg for 60 days, we found that the drug treatment caused approximately equal growth delays of 86 and 78 days, respectively (Table 3, Experiment 3). In this experiment, lower doses of PTHSO were relatively ineffective. Experiment 4 of Table 3 also compares the activities of dietary PTH and PTHSO. Again, the same doses of dietary PTH and PTHSO yielded nearly identical growth delays. It is apparent from these experiments that the antileprosy activities of PTH and PTHSO are equivalent whether given by gavage or in the diet.

Increasing the dietary level of PTH to 0.1% yielded a growth delay of 218 days, which was 134 days greater than the period of drug administration (Table 3, Experiment 5). This observation of a bactericidal effect at this dose of PTH agreed with conclusions of others [3, 9] on the bactericidal effects of PTH in mice infected with *M. leprae*. These authors also reported that PTH was only bacteriostatic at lower doses, as we have found.

The equivalence of activities of PTH and PTHSO both against *M. tuberculosis* and against *M. leprae* suggests that therapeutic activities of PTH in infected animals or man result from a summation of action of both PTH and PTHSO.

SUMMARY

PTHSO was at least equal in activity to PTH, the parent drug, against *M. tuberculosis in vitro*, but the isonicotinamide derivative of PTH was completely inactive. Using the kinetic method with the mouse foot pad model, PTH and PTHSO given orally at the same doses were found to have approximately equal activity against *M. leprae*.

RESUMEN

Se encontró que la droga PTHSO resultó cuando menos igualmente activa que la PTH, la droga progenitora, contra el *M. tuberculosis, in vitro*. El derivado isonicotinamídico de la droga fue completamente inactivo. Usando el método cinético en el modelo del cojinete plantar del ratón, se encontró que la PTH y la PTHSO, administradas oralmente y a las mismas dosis, mostraron aproximadamente la misma actividad contra el *M. leprae*.

RÉSUMÉ

Le PTHSO s'est révélé au moins aussi actif que le PTH, le composé médicamenteux dont il est tiré, contre *M. tuberculosis in vitro*, alors que le dérivé isonicotinamidique du PTH s'est révélé complètement inactif. En utilisant une méthode cynétique basée sur le modèle d'inoculation au coussinet plantaire de la souris, on a observé que le PTH et le PTHSO, par voie orale, à des doses identiques, avaient approximativement une activité équivalente contre *M. leprae*.

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