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THE ANTITUBERCULOUS ACTIVITY OF ETHYL THIOLESTERS WITH PARTICULAR REFERENCE TO DIETHYL DITHIOL*ISO*PHTHALATE

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Diethyl dithiol*iso*phthalate exerts an antituberculous effect in mice comparable to that of isoniazid and streptomycin. It is effective when given in single doses, and against isoniazid-resistant strains. It is most active when given subcutaneously either by injection or by absorption through the skin. Drug resistance has been demonstrated in mice, and isoniazid is active against such strains. The effects of this and other ethyl thiolesters are antagonized by their methyl analogues.

In a previous paper (Davies, Driver, Hoggarth, Martin, Paige, Rose, and Wilson, 1956) it was suggested that the thiol esters were the most promising of the antituberculous derivatives of ethyl mercaptan. Since the completion of this work Solotorovsky, Winsten, Ironson, and Brown (1956) have described the antituberculous activity of ethyl mercaptan derivatives including thiol esters.

We describe here experiments in mice with diethyl dithiol*iso*phthalate (ETIP). This compound was selected for study in mice because it appeared to be the most suitable of the series for clinical trial in man.

METHODS

Male albino mice weighing 18 to 22 g. were infected intravenously with 0.5 mg. wet weight of *Mycobacterium tuberculosis*, human type, strain 905, suspended in 0.1 ml. of distilled water. The suspension was made by ball-milling bacilli harvested from a 14-day culture on Löwenstein's medium. Ethyl thiolesters were given subcutaneously to mice as solutions in arachis oil.

RESULTS

Acute Infections.—Table I shows the results of the first experiment in which ETIP was used in mice. ETIP showed high activity, and subcutaneous injections were more effective than doses given by mouth. Animals which received 500 mg./kg. of ETIP subcutaneously each day for 10 days were all alive 133 days after infection, while the untreated control mice had a mean survival time of 16.9 days.

The most active derivative of ethyl mercaptan which we had tested in mice before the discovery of ETIP was diethyl dithiolcarbonate. In the direct comparison shown in Table II, ETIP was as active as diethyl dithiolcarbonate at high doses, but less active at low doses. ETIP is virtually odourless, whereas the very unpleasant odour of diethyl dithiolcarbonate makes it unsuitable as a substance for use in man.

TABLE I

THE EFFECT OF DIETHYL DITHIOL*ISO*PHTHALATE (ETIP) AGAINST A TUBERCULOUS INFECTION IN MICE

Mice treated for 2 weeks, each of 5 days, starting from the day of of infection. 10 mice/group.

Dose of ETIP (mg./kg.)	Mean Survival Time (Days)
1,000 (oral)	63·7
500 ,,	25·8
500 (subcut.)	133 (no mice dead on the 133rd day)
None	16·9

TABLE II

COMPARISON OF ETIP WITH DIETHYL DITHIOLCARBONATE

Groups of 10 mice dosed for 2 weeks each of 5 days from day of infection.

Dose (mg./kg., subcut.)	Mean Survival Time (Days)	
	ETIP	Diethyl Dithiolcarbonate
250	80·0	69·7
125	50·2	59·2
50	36·0	80·7
25	23·2	42·2
12·5	21·0	35·3
5	21·4	28·4
None	21·6	

It was observed that application of ETIP to intact human skin resulted in an odour of ethyl mercaptan in the breath. This clearly indicated absorption of the drug through the skin and suggested that local application might give effects equivalent to subcutaneous administration. Groups of infected mice were treated by the daily application to the plucked skin for 10 days of a paste containing 75% ETIP and 25% magnesium stearate. The amount of paste applied contained in each dose the equivalent of 10 mg./kg. ETIP. To ensure that the effect was not due to mice licking the drug from one another, in a second experiment the mice were isolated in individual cages and watched for 1 hr. after application, when absorption appeared to be complete. The animals made no attempt to lick off the drug. In both these experiments a marked increase in the survival time (31 and 36 days respectively) was observed in the treated groups.

ETIP was also highly active against an intracorneal infection in mice (Table III). Naguib and Robson (1956) have shown it to be active in intracorneal infections with murine leprosy in mice.

TABLE III

THE EFFECT OF ETIP AND ISONIAZID ON AN INTRACORNEAL INFECTION IN MICE

Groups of 10 mice treated for 1 month from day of infection.

Treatment	Dose (mg./kg., by Mouth)	Number of Eyes Showing Lesions					
		Days after Infection					
		28	37	50	64	78	114
Isoniazid ..	100	1	1	4	5	5	6
ETIP ..	1,000	1	3	3	3	3	4
None ..	—	9	10	10	10	10	10

When single doses of 500 mg./kg. of ETIP were given subcutaneously to groups of 10 mice at various times before and after infection, activity was demonstrated with the compound given either 48 hr. before or 7 days after infection.

The Established Infection.—The compound was also markedly active against an established infection. The effect produced by daily subcutaneous doses of 500 mg./kg. of ETIP for 10 days was comparable to that produced by 50 mg./kg. of isoniazid given orally twice daily for 10 days. The 500 mg./kg. dose of ETIP, given twice daily orally, was as active as 100 mg./kg. of streptomycin, given subcutaneously once daily. The effect of ETIP appeared to fall off rapidly at doses below 250 mg./kg./day. The mean survival times in days of the groups in this experiment were: ETIP (500 mg./kg.) 78.7: ETIP (250 mg./kg.) 60.3: ETIP (125 mg./kg.) 19.3: streptomycin (100 mg./kg.) 35.5: isoniazid (50 mg./kg. twice daily) 76.3: controls 18.1.

Effect of Weekly Doses of ETIP.—A marked antituberculous effect was obtained with a single subcutaneous dose of 500 mg./kg. of ETIP given on the day of infection, and still larger effects were obtained by giving one or more further doses at weekly intervals (Table IV). One group of mice was dosed once weekly until death and the mean survival time of these animals was 105.3 days compared with the mean survival time of 19.3 days in the untreated group. Isoniazid was less active under these very stringent conditions.

TABLE IV

THE EFFECT OF ETIP AND ISONIAZID WHEN GIVEN AS SINGLE DOSES AT WEEKLY INTERVALS TO GROUPS OF 10 MICE

Treatment	Dose (mg./kg., subcut.)	Mean Survival Time (Days)
ETIP on day 0 only	500	28·1
,, ,, days 0 and 7 ..	500	55·5
,, ,, ,, 0, 7, and 14 ..	500	64·0
,, once weekly until death	500	105·3
Isoniazid on day 0 only ..	50	21·7
,, ,, days 0 and 7 ..	50	40·2
,, ,, ,, 0, 7, and 14	50	40·2
,, once weekly until death	50	94·2
None	—	19·3

Experiments with ETIP and Isoniazid.—As combined treatment is now well established in the therapy of tuberculosis, the effect of combinations of isoniazid and ETIP was studied. Table V shows that the combination of the two drugs was much better than either alone.

TABLE V

THE ADDITIVE EFFECT OF ETIP AND ISONIAZID

Isoniazid was incorporated in the food; the dose of ETIP was given subcutaneously. An asterisk indicates that these animals died from causes other than tuberculosis.

Treatment	Dose (mg./kg.)	Mean Survival Time (Days)
None ..	—	18·5
Isoniazid ..	8·5	49·3
ETIP ..	125	50·5
ETIP ..	50	24·7
		Days of Death of Individual Mice
Isoniazid + ETIP	7·5 / 125	*9, *29, 40, 69, 80, 100, 100, +3 survivors on 100th day
Isoniazid + ETIP	7·5 / 50	59, 69, 69, 73, +6 survivors on 100th day

A single dose of ETIP markedly prolonged the life of infected mice that had relapsed and were about to die after treatment with isoniazid. Treatment was effective even when the ETIP was given

only 5 days before mice in a group which had been treated with isoniazid alone started to die (Table VI).

TABLE VI

THE EFFECT OF SINGLE DOSES OF ETIP GIVEN TO INFECTED MICE AFTER A COURSE OF TREATMENT WITH ISONIAZID

All mice except the untreated controls were given isoniazid (7·5 mg./kg. in food) for the first 10 days after infection. S indicates a mouse which was alive 161 days and A 63 days after infection. K indicates that the mouse was killed.

Dose of ETIP (mg./kg.)	Days of Death of Individual Mice	Mean Survival Time (Days)
None ..	24, 30, 30, 36, 41, 41, 46, 84, K119, K119	57·0
500 (on day of infection)	36, 38, 50, 71, 90, 119, 119, 121, 126, 127	89·7
500 (20 days after) ..	71, 75, K119, K119, 133, S, S, S, S, S	132·2
500 (25 days after) ..	61, 83, 83, 97, K119, K119, 124, S, S, S	116·9
500 (30 days after) ..	27, 30, 31, 47, 77, 110, K119, K119, 146, 160	86·6
Untreated controls	18, 19, 20, 21, 21, 22, 22, 23, 23, 23	21·2
None ..	25, 26, 28, 28, 31, 32, 35, 49, A	34·8
50 (on day of infection)	23, 24, 25, 26, 28, 30, 33, 43, A, A	35·8
50 (20 days after) ..	26, 28, 59, A, A, A, A, A, A, A	55·4
50 (25 days after) ..	27, 28, 28, 28, 28, 29, 30, 30, 49, A	34·0
Untreated controls	18, 18, 18, 19, 20, 20, 20, 20, 21, 21	19·5

Drug Resistance.—One of the main needs in the present-day chemotherapy of tuberculosis is for a non-toxic substance, antituberculous in its own right, which will act upon tubercle bacilli that are resistant to isoniazid. Whatever the origin of such resistant strains, whether they are adaptive forms produced in response to the drug or spontaneous mutants, their emergence will probably be delayed in the presence of a second drug to which no cross-resistance is shown. We have demonstrated that there is no cross-resistance between isoniazid and ETIP. Mice infected with tubercle bacilli resistant to isoniazid respond to treatment with ETIP. The effect of ETIP on a streptomycin-resistant strain was not tested, since in an earlier experiment we had found that other ethyl mercaptan derivatives such as ethyl thiolbenzoate were effective against infections caused by such strains. An important factor governing the use of drugs in the treatment of tuberculosis is the ease with which tubercle bacilli become drug-resistant. All the commonly-used therapeutic agents (streptomycin, isoniazid, PAS) are active against tubercle bacilli *in vitro* and resistance to them can be demonstrated by *in vitro* tests. We have already shown (Davies *et al.*, 1956) that the slight *in vitro* activity of the compounds derived from ethyl mercaptan is probably irrelevant to their therapeutic action. Development of drug resistance, therefore, had to be studied *in vivo*. A group of 10 mice were infected and treated for 10 days with daily subcutaneous doses of ETIP at the rate of 250 mg./kg. On the 11th day after infection 5 mice were killed and the tubercle bacilli from their lungs cultured on Löwenstein's medium. After incubation for one month, these cultures were mixed and used to infect 2 further groups of mice, one of which was treated as before with ETIP, and the other left untreated. Resistance to ETIP was then complete, since a dose of 250 mg./kg. had no therapeutic effect and resistance once developed appeared to be fairly permanent, one culture being still resistant after seven monthly passages on Löwenstein's medium in the absence of drug. This strain was also resistant to other thiolesters. Isoniazid, however, was fully effective against the ETIP-resistant strain.

Antagonism.—A possible mode of action of ethyl mercaptan is that it interferes with a biological methylating or thiomethylating system. In support of this hypothesis it was found that the activity of sodium S-ethyl thiosulphate was antagonized by concurrent administration of sodium S-methyl thiosulphate. Similarly, ethyl thiolbenzoate, diethyldisulphide and diethyl dithiol*iso*phthalate were antagonized by methyl thiolbenzoate. The specificity of this action was demonstrated by the failure of methyl thiolbenzoate to antagonize the action of isoniazid and the failure of propyl- and *iso*propyl thiolbenzoates to antagonize ethyl thiolbenzoate. However, dimethyldisulphide did not antagonize either diethyldisulphide or ethyl thiolbenzoate, and ethyl benzoate slightly antagonized ethyl thiobenzoate and ETIP but not isoniazid (Table VII). Ethyl thiolbenzoate was not antagonized by cystine, cysteine, methionine or glutathione.

The experiments reported in this paper show that diethyl dithiol*iso*phthalate is a potent antituberculous agent in mice. Its high activity was apparent from the effect of single doses. Repeated subcutaneous administration resulted in a very marked increase in the survival time of infected mice (Table I), but tubercle bacilli were still recoverable from animals surviving for 133 days. This recovery could be accounted for in one of two ways: either the drug had failed to sterilize, or the mice had become reinfected. In unpublished experiments we have shown that this second possibility is real since normal mice become infected with tuberculosis when they are kept near diseased mice for several weeks.

TABLE VII

ANTAGONISM OF ANTITUBERCULOUS ACTION OF DERIVATIVES OF ETHYL MERCAPTAN

All treatments were started on the day of infection and continued for 10 days. Therapeutic effect is defined as the difference in days between the mean survival times of treated and control groups.

Active Agent	Daily Dose (mg./kg.)	Additive	Daily Dose (mg./kg.)	Therapeutic Effect (Days)		
				With Additive	Without Additive	Difference
Ethyl thiosulphate	500 oral	Methyl thiosulphate	500 oral	−0·6	5·6	6·2
Ethyl thiolbenzoate	250 s.c.	Methyl thiolbenzoate	250 s.c.	0·3	26·1	26·4
ETIP	250 ,,	,, ,,	250 ,,	10·2	33·2	23·0
Dimethyldisulphide	125 ,,	,, ,,	250 ,,	5·1	20·0	14·9
Isoniazid	25 oral	,, ,,	250 ,,	33·3	29·8	−0·5
Ethyl thiolbenzoate	250 s.c.	*n*-Propyl thiobenzoate	250 ,,	22 6	30·6	8·0
,, ,,	250 ,,	*iso*-Propyl thiolbenzoate	250 ,,	31·9	30·6	1·3
Diethyldisulphide	125 ,,	Dimethyldisulphide	125 ,,	23·9	28·2	4·3
Ethyl thiolbenzoate	250 ,,	,,	125 ,,	26·0	25	−1·0
Diethyldisulphide	125 ,,	Di-*n*-propyl disulphide	125 ,,	26·2	28·2	2·0
,,	125 ,,	Di-*iso*-propyl disulphide	125 ,,	32·0	28·2	−3·8
Ethyl thiolbenzoate	250 ,,	Ethyl benzoate	250 ,,	10·4	26·1	15·7
ETIP	250 ,,	,, ,,	25 ,,	18·8	33·2	14·4

The percutaneous activity of ETIP is a novel observation which suggests a method of administration suitable for use in man since pastes prepared from the drug appear to be stable and non-irritant.

Inclusion of isoniazid and ETIP in the same experiments showed that the two drugs were compatible and there was no evidence of cross-resistance. Of particular interest are the results shown in Table VI in which mice were allowed to relapse after treatment with isoniazid and then given single doses of ETIP; moribund mice recovered and lived for a further 100 days. The effect of weekly doses of isoniazid or of ETIP suggests that in mice it is not necessary to maintain constant high blood levels with these drugs. It should be pointed out, however, that these results may apply only to mice and the experiment is not regarded as providing sufficient evidence for the use of a similar dosage schedule in man.

The development of drug-resistance suggests that at least part of the action is exerted directly on the bacteria and is not mediated solely through the defence mechanisms of the host. Antagonism of ethyl thiol compounds by their methyl-analogues may indicate that ethyl mercaptan interferes with an essential methyl- or methylthiol-containing system. A number of such systems were investigated and reported in a previous paper (Davies *et al.*, 1956) and it may be that a hitherto unknown biological system is involved. However, dimethyldisulphide does not antagonize the action of ethyl thiol compounds. The metabolism of dimethyldisulphide may differ from that of diethyldisulphide, methyl mercaptan and the other derivatives of methyl mercaptan.

The partial antagonism of ethyl thiolbenzoate and ETIP by ethyl benzoate is probably explained by competition of the benzoate with the thiol esters for available esterase activity resulting in a reduced liberation of ethyl mercaptan. Isoniazid was not antagonized by ethyl benzoate.

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