Brit. J. Pharmacol. (1954), **9**, 437.

THE ABSORPTION, TOXICITY AND EXPERIMENTAL ANTITUBERCULOUS ACTION OF 5-AMINO-7-METHYL-1:2:4:6-TETRA-AZAINDENE

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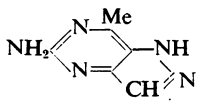
(RECEIVED JULY 7, 1954)

During the course of routine testing of compounds in the chick embryo, Francis and Hoggarth (1953) detected antituberculous activity in the purine analogue 7438, 5-amino-7-methyl-1:2:4:6-tetra-azaindene. The chemistry of this



substance, together with that of many related compounds which had little or no antituberculous action, has been described by Rose (1952). The tetra-azaindene was next tested in the mouse and found to be of the same order of activity as streptomycin when treatment commenced at the time of infection. This led to an investigation of its activity in guinea-pigs, dogs and monkeys, and to toxicological studies preparatory to a trial in clinical tuberculosis. Although such a trial was not subsequently undertaken, the observation of marked therapeutic activity in experimental infections of this novel chemical type is felt to justify publication of the results as they now stand.

METHODS

The drug exhibits a very strong fluorescence in ultraviolet light. It was estimated in blood by precipitating the proteins with trichloroacetic acid and reading the fluorescence of the filtrate in the Coleman fluorimeter. It may also be estimated spectrophotometrically.

The antituberculous action of the drug was assessed in mice by the methods previously described (Martin, 1946; Hoggarth and Martin, 1950). The method of assessing lesions in guinea-pigs was that described by Francis, Spinks, and Stewart (1950).

For the intracerebral inoculation of guinea-pigs a 20 gauge needle and 0.1 ml. of inoculum were used. A small hole was made in the skull just to one side of the midline, on a line joining the right eye and left ear.

RESULTS

Absorption and Toxicity in Various Species

Mice.—When a dose of 250 mg./kg. of 7438 was given by stomach tube, the drug was well absorbed, reaching a maximum concentration in the blood of 5–6 mg./100 ml. Its rate of clearance from the blood was similar to that of sulphanilamide, the concentration falling to 1–2 mg./100 ml. about 9 hr. after dosing.

The drug was of low toxicity to mice. Animals given doses of 300 mg./kg. twice daily *per os* for 6 weeks gained weight at the same rate as untreated controls, but similar doses of 400 mg./kg. caused the death of 6 out of 12 mice in one week. Doses of 500 mg./kg. killed 8 out of 12 mice in two days. Mice kept on powdered food containing 0.25% of 7438 (i.e. intake approximately 0.5 g./kg./day) for 6 months gained weight at exactly the same rate as control mice.

Guinea-pigs.—Guinea-pigs increased normally in weight when dosed twice daily with 50 mg. 7438/kg., but not when dosed with 75 or 100 mg./kg. They gained in weight when given 0.2% but not 0.4% 7438 in powdered food. One hr. after dosing with 100 mg./kg. three animals had the following blood concentrations: 1.75, 4.31 and 5.27 mg./100 ml.; 16 hr. after dosing the concentrations were 0.71, 1.6 and 3.3 mg./100 ml. Groups of four guinea-pigs which had received food containing various concentrations of 7438 for 19 days had the following mean concentrations of drug in the blood: from 0.1% of drug in the food, 0.2 mg./100 ml.; 0.2%, 0.7 mg./100 ml.; and 0.4%, 1.3 mg./100 ml.

Dogs.—Four dogs were given single oral doses of 7438. The concentrations of drug in the blood after the administration of 100 mg./kg. rose to

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maxima ranging between 2.5 and 5.5 mg./100 ml. after 2–3 hr., and fell to about half these values 7 hr. after dosing. The concentration of 7438 in the cerebrospinal fluids of the dogs was about two-thirds of the concentration in the blood, showing that the drug passed freely into this medium.

Three dogs were given two doses of 7438 daily. One dog was given 100 mg./kg. morning and evening for one week, a second dog 75 and 100 mg., and a third dog 50 and 75 mg. The animal receiving the largest doses vomited once, but showed no other signs of toxic effects, and it was therefore decided to give morning doses of 50 mg. and evening doses of 100 mg./kg. to tuberculous dogs. After four days this dose was increased to 100 mg./kg. twice daily, but it caused vomiting in two dogs, one of which died. The liver was yellow and there was extensive central necrosis of the lobules There was also some necrosis of the epithelium of the renal tubules. After 22 days' treatment the dose of 7438 was stabilized at 30 mg./kg. twice daily, which maintained blood concentrations between 1 and 4 mg./100 ml. The dogs were killed after about 15 weeks' treatment and, apart from the tuberculous lesions, the dogs treated with 7438 showed some cirrhosis of the liver and vacuolation of liver cells (sections were not stained for fat).

Monkeys.—Two monkeys, recently purchased, which had a positive reaction to tuberculin, were given a single dose of 7438. The first received 200 mg. and the second 100 mg./kg. The latter died suddenly whilst the third hourly blood sample was being taken; post-mortem examination revealed extensive tuberculous broncho-pneumonia. The concentrations of 7438 in the blood of the former 1, 2, 3, 5, and 24 hr. after dosing were 3.2, 3.4, 3.6, 5.6 and 2.5 mg./100 ml., respectively. Another monkey was dosed with 30 mg./kg. twice daily for 60 days. During this period the weight of the animal increased from 3.2 to 3.6 kg. Blood concentrations were determined on five occasions during the period of dosing and averaged approximately 5 mg./100 ml. The concentration of blood urea on the 29th, 42nd and 56th day was 71, 65 and 40 mg./100 ml., respectively. Blood counts and haemoglobin estimations, done at the same times as the 7438 estimations, revealed no significant changes.

Action on Tuberculosis

7438 had little action on tubercle bacilli *in vitro*. Some growth occurred in Dubos' medium containing 1:1,000 of the substance, and growth was normal in the presence of 1:27,000.

Mice.—When given by mouth to mice infected intravenously with "human" type tubercle bacilli, the tetra-azaindene produced a marked increase in mean survival time. For this reason it was used for a considerable period in these laboratories as a standard or reference substance, being given for the first 14 days after infection. It was also active when given subcutaneously from 1–5 or from 8–12 days after infection. The results of a number of experiments are shown in Table I. These effects listed are of the same order as those produced by the subcutaneous administration of streptomycin, 1 mg./20 g. twice daily for the same limited period.

TABLE I

INCREASE IN MEAN SURVIVAL TIME (M.S.T.), IN DAYS, OF GROUPS OF 10 MICE INFECTED I.V. WITH *M. TUBERCULOSIS* AND TREATED WITH 7438 FOR THE FIRST 14 DAYS

Drug Given *per os* Twice Daily. Each Dose, mg./kg.			Drug Given S.C. Once Daily in Oil. Dose, mg./kg.	Drug Given in Food. Total Daily Intake, mg./kg.		
50	150	250	500	200	500	1,000
0	4·7 5·7 5·7	3·8, 2·8 4·1, 6·0 4·7, 2·2	7·7	3·9	4·4, 3·2 5·1, 2·1 4·8, 5·0 5·7, 4·4 3·8, 4·3	7·3

M.S.T. of control groups 19·2–23·5 days. Increase of M.S.T. required for significance ($p = 0·05$), 1·2–2·3 days.

When administration of 7438 (500 mg./kg. per day in food) was continued from the time of infection (with 0.75 mg. of bacteria) up to 180 days, only one specific death occurred (on the 150th day) in a group of 15 mice. The remainder were alive and apparently in excellent condition (mean weight 43 g.) when the experiment was terminated on the 210th day, but on examination *post mortem* they were found to have some tuberculous lesions in the lungs. It appeared that the mice might have died from a tuberculous infection after a very long time. Streptomycin was not included in this experiment, but 80% of a group treated with *p*-ethylsulphonyl benzaldehyde thiosemicarbazone (8388) (500 mg./kg. per day in food) were dead at the end of the experiment.

Treatment with 7438 (500 mg./kg./day in food) of a group of mice infected intravenously with 0.02 mg. of organisms was included in Expt. No. 241 (Hoggarth and Martin, 1950), and gave results very similar to those given by 8388, i.e. results much less striking than those of streptomycin at a dose of 37.5 mg./kg. twice daily. The effect of 7438 was therefore much less when it was used

to treat an established infection than when it was given from the time of infection onwards.

Guinea-pigs.—Five groups of seven guinea-pigs were inoculated subcutaneously with 0.1 mg. of human tubercle bacilli (Weybridge C) and given medicated food or dosed as shown in Table II. Only one control guinea-pig had died from tuberculosis, after 42 days, when the rest were killed and the severity of the lesions assessed. It will be seen from Table II that 7438 had about the same effect when given in the food as by mouth and that it was considerably less effective than streptomycin but more effective than 4:4'diaminodiphenyl sulphone.

TABLE II

EFFECT OF VARIOUS DRUGS ON TUBERCULOSIS IN GUINEA-PIGS INJECTED SUBCUTANEOUSLY WITH 0.1 MG. "HUMAN" TUBERCLE BACILLI (WEYBRIDGE C); TREATMENT WAS BEGUN IMMEDIATELY

Treatment	No. of Animals per Group*	Average Score of Lesions	Average Weight of Spleen g.
50 mg./kg. streptomycin twice daily	7	0·23	0·96
50 mg./kg. 7438 orally twice daily	5	1·0	1·8
0·25% 7438 in food	6	1·0	1·2
0·3% 4:4'diaminodiphenyl sulphone in food	4	2·3	5·0†
Controls	4	3·8	4·0

* Some non-specific deaths occurred.
† Sulphone specifically increases the size of the spleen.

The next experiment in guinea-pigs was one already described (except for the result with 7438) by Francis, Spinks, and Stewart (1950). Briefly, animals were injected intramuscularly with 0.005 mg. of human tubercle bacilli (Weybridge C) and 38 days later they were weighed. Six guinea-pigs were killed and the extent of the lesions assessed; the others divided into groups and treatment commenced. The guinea-pigs were in rather poor condition when treatment began and the drugs caused only a slight prolongation of life, but 7438 and streptomycin were placed in the same order of activity on the basis of survival time and the assessment of lesions (Table III).

Action of 7438 and Streptomycin on Tuberculosis Produced by Intracerebral Injection.—Thirty guinea-pigs were divided into three equal groups. One group was given food containing 0.25% 7438 and a second group streptomycin 20 mg./kg. twice daily. The following day all guinea-pigs were injected intracerebrally with 0.01 mg. of human tubercle bacilli (Weybridge C) and treatment was continued. The control animals survived for an average period of 19 days and those receiving streptomycin for 30.5 days. The 7438 treated animals survived for an average period of only 16 days.

TABLE III

EFFECT OF VARIOUS DRUGS ON TUBERCULOSIS IN GUINEA-PIGS INJECTED INTRAMUSCULARLY WITH 0.005 MG. "HUMAN" BACILLI (WEYBRIDGE C); TREATMENT WAS BEGUN 38 DAYS LATER

Treatment	No. of Animals per Group	Average Time in Days from Commencement of Treatment to Death	Average Score of Lesions
0·25% 7438 in food	14	19·7	2·5
0·25% 8388 „ „	14	21·2	2·0
20 mg./kg. streptomycin twice daily	13	23·3	1·6
Controls	14	17·9	3·7
Animals killed 38 days after infection (when treatment of others began)	6	—	2·6

DISCUSSION

In guinea-pigs, dogs, and monkeys 7438 in single oral doses of 100 mg./kg. produced maximum blood concentration of about 4 mg./100 ml. after three to five hours. The drug was more persistent in monkeys than in dogs and produced higher concentrations on repeated dosing. Mice tolerated oral doses of 250 mg./kg. twice daily, but guinea-pigs, dogs, and monkeys tolerated only about one-tenth of this.

The results presented in this paper, together with those recorded by Hoggarth and Martin (1950), indicate that, although 7438 has a very good action if mice are treated immediately after infection, the drug is considerably less active than streptoymcin, and probably less active than *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (8388), against established tuberculosis in mice and guinea-pigs. Francis (unpublished) obtained essentially similar results in dogs and monkeys, and unless 7438 is considerably less toxic to man than to other species it is unlikely to be of value in the treatment of human tuberculosis.

SUMMARY

1. 5-Amino-7-methyl-1:2:4:6-tetra-azaindene (7438) was well absorbed when given orally to mice, guinea-pigs, dogs and monkeys; it was fairly persistent in the blood and passed freely into the cerebrospinal fluid of dogs.

2. Mice tolerated repeated oral doses of 250 mg./kg. of the drug, but guinea-pigs, dogs and monkeys tolerated repeated doses of only 25–50 mg./kg. Doses of 100 mg./kg. in dogs produced necrosis of the liver and death.

3. At its maximum tolerated doses, 7438 was of the same order of effectiveness as *p*-ethylsulphonylbenzaldehyde thiosemicarbazone against established tuberculosis in mice. The drug was considerably less effective than streptomycin against established tuberculosis in mice and guinea-pigs. Against cerebral tuberculosis in guinea-pigs 7438 had no effect, whereas streptomycin given systemically caused a 50% increase in the mean survival time.

4. It is concluded that 7438 is unlikely to be useful in the treatment of human tuberculosis.

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