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SYNTHETIC AMOEBICIDES: PART II. THE ANTI-AMOEBIC ACTION OF QUINOXALINE-1:4-DIOXIDE AND SOME DERIVATIVES

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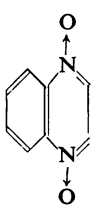
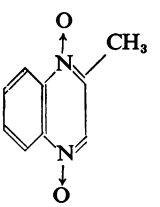
McIlwain (1943) prepared quinoxaline-1:4-dioxide and homologues as possible antagonists of vitamin K. He showed that these compounds possessed antibacterial properties against *Streptococcus haemolyticus* and *Corynebacterium diphtheriae*; further antibacterial studies with compounds of this class were made by Wiedling (1945), Frisk (1946), and Iland (1948).

In the course of routine screening tests of compounds as possible amoebicides, we selected (*a*) compounds which might function as antagonists of vitamins, and (*b*) those which might interfere with the oxidation-reduction potential of the parasites' environment. Whereas we had no evidence that *Entamoeba histolytica* was vitally dependent upon vitamin K, the work of Chang (1946) and Bradin and Hansen (1950) indicated the sensitivity of this parasite to changes in oxidation-reduction potential of the medium in which it was cultivated. Among the compounds examined was 2-methylquinoxaline-1:4-dioxide (ref. No. 7331), which was found to be highly active against an experimental amoebic infection of rats. Other quinoxaline dioxides behaved similarly.



Quinoxaline-1:4-dioxide (ref. No. 8173)



2-Methylquinoxaline-1:4-dioxide (ref. No. 7331)

We have found, however, that the quinoxaline oxides do not behave as oxidizing agents towards typical oxidation-reduction indicators, and that their biological activity is not antagonized by 1:4-naphthoquinone or vitamin K. Their mode of action, therefore, remains unexplained.

METHODS AND MATERIALS

Relative Therapeutic Potency.—The experimental amoebic infection of young rats (Jones, 1946) was used to assay the anti-amoebic effect of the compounds tested. Young rats (weighing 20–35 g.) were anaesthetized with ether and then injected intracaecally with 0.2 ml. of a suspension of *E. histolytica* harvested from several two-day-old cultures. In the morning and afternoon of each of the two days after this operation, a suspension or solution of the compound was administered orally. On the fifth day after the operation, the rats were killed and a careful assessment was made of the average degree of amoebic infection present in the caecum. By comparison of this figure with that obtained from a group of untreated controls a measure of the therapeutic effect could be made (Jones, 1946). Each of the compounds of this series was examined repeatedly, and that dose which would consistently produce a significant therapeutic effect was determined. This dose was used to characterize the relative potencies of the compounds.

Materials.—Quinoxaline-1:4-dioxide (8173) is a yellow crystalline substance, m.p. (when heated rapidly) 241–243° C. (decomp.). It is soluble in cold water to the extent of about 1.5%. Solutions of the drug are relatively unstable to visible or ultra-violet light, and in all the investigations described below precautions were taken to prevent photochemical-decomposition.

2:3-Dimethylquinoxaline-1:4-dioxide (7218) is a pale yellow crystalline substance, m.p. 193–194° C. It is soluble in cold water to the extent of 10%. In solution the substance is somewhat photo-labile, and, as with 8173, precautions were taken to prevent photochemical-decomposition. 7218 could also be estimated spectrophotometrically in blood or plasma.

6-Chloro-2:3-dimethylquinoxaline-1 : 4-dioxide (8218) is a pale yellow crystalline substance, m.p.

175–176° C. It is only sparingly soluble in water. As with 8173 and 7218, suspensions of the drug in water are unstable to light, and precautions were taken to prevent photochemical decomposition. 8218 was more slowly and less completely absorbed than 8173, as determined by spectrophotometrical studies of blood from mice dosed with the drug.

RESULTS

From the results shown in Table I it was apparent that quinoxaline-1 : 4-dioxide (8173), its lower homologues, and the related 8218 (6-chloro-2 : 3-dimethylquinoxaline-1 : 4-dioxide) were the most highly active of the series. With all of these compounds *E. histolytica* persisted in a proportion of the treated rats after the lesions were healed. For example, 8173, although having a very striking effect on the lesions at 2.5 mg./kg., eradicated *E. histolytica* from only 56% of the rats. Emetine hydrochloride produced a comparable effect at 0.6 mg./kg.

TABLE I
RELATIVE THERAPEUTIC POTENCY OF MEMBERS OF A SERIES OF QUINOXALINE-1 : 4-DIOXIDES (AGAINST EXPERIMENTAL AMOEBIASIS OF RATS)

Code Number	Substituents	Minimal Consistently Effective Therapeutic Dose mg./kg. b.i.d.×2
8173	None	2·5
7331	2-methyl	5
7218	2 : 3-dimethyl	5
9804	2-methyl-3-ethyl	1
10,323	2-methyl-3-*n*-propyl	5
10,376	2-methyl-3-*iso*propyl	10
9630	2-methyl-3-*n*-amyl	10
11,315	2-methyl-3-phenyl	25
8218	6-chloro-2 : 3-dimethyl	5
10,856	2-ethyl	5
9915	2 : 3-diethyl	1
10,931	2-ethyl-3-*n*-propyl	10
9833	2 : 3-di-*n*-propyl	25
8175	2 : 3-diphenyl	10

To determine, roughly, the relative oral toxicities of the more active compounds, groups of six male albino mice of uniform weight (18–20 g.) were dosed orally twice daily (once daily on the sixth and seventh day) for 12 days. The LD50 for each compound was estimated and the results are given in Table II.

It was necessary, at an early stage in these investigations, to select members of this series of compounds for further therapeutic and toxicological investigation, with a view to their subsequent trial in man. From the evidence of their therapeutic action in rats, their toxicity in mice, and their mode of absorption and excretion, 8173 (unsubstituted), 7218 (2 : 3-dimethyl-), and 8218 (6-chloro-2 : 3-dimethylquinoxaline-1 : 4-dioxide) were selected for further study. After oral dosage, 8173 was found to be well absorbed into the blood stream, and rapidly excreted; 7218 was rather more slowly absorbed, but was excreted much more slowly; 8218 was rather poorly absorbed. The minimal concentrations of these three substances causing inhibition of growth of *E. histolytica* in a simple liquid medium (Jones, 1946) were respectively 1 : 640,000, 1 : 80,000, and 1 : 640,000. The inhibitory concentration of emetine hydrochloride under these conditions was 1 : 3,000,000.

TABLE II
RELATIVE CHRONIC ORAL TOXICITIES, IN MICE, OF QUINOXALINE-1 : 4-DIOXIDE AND SEVERAL RELATED COMPOUNDS
Dosing: twice daily (once daily on the 6th and 7th days) for 12 days

Code No.	Substituents	Estimated LD50 in mg./kg.	95% Limits (mg./kg.)
8173	None	135	70–250
7331	2-methyl	80	65–100
7218	2 : 3-dimethyl	360	155–850
9804	2-methyl-3-ethyl	130	115–150
9630	2-methyl-3-*n*-amyl	1,085	745–1,575
9915	2 : 3-diethyl	175	150–195
8218	6-chloro-2 : 3-dimethyl	500	425–575

8173 (Quinoxaline-1 : 4-dioxide)

When administered orally to rats or monkeys in doses greater than 10 mg./kg., 8173 could be estimated spectrophotometrically in the blood or plasma, and was apparently present in unaltered form. Maximal blood concentrations were obtained 1–2 hours after dosing. Thereafter, a small proportion of the drug was excreted, but the greater part was rapidly metabolized. There was apparently no accumulation of drug in the blood of rats even after the daily oral administration of 100 mg./kg. for three weeks. The compound was retained to some extent in the metabolically inert tissues (fat), but was rapidly destroyed in the more active tissues such as liver and kidney. Mice, rats, and rabbits survived repeated daily oral doses of 100 mg./kg. up to three weeks; the LD50 in rats was 150 mg./kg. under these circumstances. In one monkey, daily oral doses of 250 mg./kg. caused slight anaemia and granulocytopenia; three daily oral doses of 500 mg./kg. caused the death of one monkey, which at necropsy showed fatty degeneration of the liver and tubular damage in the kidney; 100 mg./kg. did not affect the blood picture in the dog. No action was observed on blood pressure, respiration, or plain muscle. The toxic action of the drug when given to rats in doses exceeding 100 mg./kg. was directed mainly against the kidney, which showed glomerular haemorrhages and tubular desquamation, and the liver, which suffered fatty degeneration.

Daily oral doses of 250 mg./kg. given to one monkey caused cysts of *E. histolytica* to disappear from the stools ; five daily oral doses of 50 mg./kg. also caused the parasites to disappear, but they reappeared four weeks afterwards. Five daily oral doses of 20 mg./kg. had no therapeutic effect.

One kitten and one puppy were cured of experimentally induced amoebic dysentery by the administration of four daily doses of 5 mg./kg. and 20 mg./kg. of drug respectively.

Clinical Trial in Man.—Quinoxaline-1 : 4-dioxide (8173) was tried in 14 patients suffering from amoebiasis. Dr. A. R. D. Adams, of the Liverpool School of Tropical Medicine, carried out the trial ; the results can be summarized as follows.

Eight patients presenting minimal clinical symptoms, and consistently passing cysts of *E. histolytica* in the faeces, received 8.5 to 19 mg./kg./day in divided daily doses (3–5 for 21 days). Two of the eight ceased to pass cysts on the fourth and ninth days respectively of a 21-day course of thrice-daily treatment with 200 mg. (8.5–9.5 mg./kg.). The remaining six patients continued to pass cysts as before treatment.

Four patients suffering from acute amoebic dysentery were treated with 5.3–20 mg./kg. of the drug daily for 7–22 days. Two patients (Indian) responded well ; in one the acute attack had subsided by the third day and the parasites vanished on the fifth day. These two patients had 150 and 200 mg. 4-hourly five times daily for 17 and 21 days respectively (16.7 and 20 mg./kg.). The other two patients (both Europeans) showed no symptomatic response and parasites remained present. In one of these patients, treatment was abandoned after seven days because of toxic effects.

Two patients suffering from acute amoebic hepatitis responded to treatment within 2–3 days. Symptoms subsided and white cell counts diminished. Treatment was 200 mg. thrice and five times daily respectively for 12 days (10 and 19 mg./kg./day).

The drug was well tolerated by 9 of the 14 patients. The remaining five complained of nausea, vomiting, and cramps in the legs. In one patient the symptoms were sufficiently serious for treatment to be discontinued.

7218 (2 : 3-Dimethylquinoxaline-1 : 4-dioxide)

7218 was less toxic than 8173 ; repeated oral doses of 250 mg./kg. could be given to mice twice daily for 12 days. A similar dose was tolerated by rats. In mice poisoned by repeated large doses of 7218 (500 mg./kg. twice daily for 16–24 days) the most prominent symptom was tonic spasm of the abdominal muscles, hind limbs, and tail, and to a less extent of the back and neck ; the fore-limbs and facial muscles were not involved. At first these spasms appeared only when the mice were disturbed. Later, recurrent tonic spasms alternating with coarse general tremor and splaying and weakness of the hind-limbs followed. These toxic signs were of interest in view of the complaints of cramps in the legs made by some patients treated with 8173. The main pathological changes in mice and rats dosed repeatedly with large amounts of 7218 occurred in the middle and deeper cortex of the adrenal ; no characteristic damage could be detected consistently in other organs. One monkey (*M. mulatta*) was dosed orally with the drug ; three doses of 200 mg./kg. were given at 3-hourly intervals on the first day ; three doses of 100 mg./kg. at similar intervals on the second day, and two doses of 100 mg./kg. on the third day. A gradual build-up in blood concentrations of drug occurred and a maximum level of 43.4 mg./100 ml. was attained one hour after the last dose. At 20 hours after the last dose the blood level was 5.6 mg./100 ml. Toxic manifestations were excessive salivation, torpidity, and clamminess to the touch. An increased tendency for the blood to clot was apparent when samples were withdrawn for analysis. The monkey finally died five days after the last dose. Two monkeys dosed thrice daily for five consecutive days with 20 mg./kg. of 7218 caused *E. histolytica* cysts to disappear from the stools. One relapsed 26 days after treatment had ceased, but the other remained clear of parasites for three months. One kitten was cured of experimentally induced amoebic dysentery, after treatment with twice-daily doses of 1, 2, 2, 5, 10, 10, and 10 mg./kg.

7218 was submitted for clinical trial against amoebiasis, but was withdrawn after reports of serious toxic effect in a trial against lymphogranuloma venereum (Alergant, to be published).

8218 (6-Chloro-2 : 3-dimethylquinoxaline-1 : 4-dioxide)

Mice survived 100 mg./kg. of the drug given orally daily for 35 days. Rats survived 16 daily oral doses of 500 mg./kg. One dog survived 26 daily oral doses of 100 mg./kg. with no ill effects and no influence on the blood picture. Another dog vomited when dosed for two days with 200 mg./kg. One monkey survived 15 daily oral doses of 500 mg./kg. It was killed after the last dose, but showed only early fatty degenerative changes in the liver. Two monkeys dosed with 200 mg./kg. (one dose and three doses respectively)

showed no ill effects, and two monkeys given ten daily doses of 20 and 40 mg./kg. respectively were likewise unaffected. The smaller doses (20 and 40 mg./kg.) were without effect on the natural amoebic infection carried by the monkeys. The monkey receiving repeated daily doses of 500 mg./kg. became free of amoebic infection after the first dose of drug.

Clinical Trial in Man.—6-Chloro-2 : 3-dimethylquinoxaline-1 : 4-dioxide was tried in five patients suffering from amoebiasis. Dr. Adams again carried out the trial, and his results may be summarized as follows:

Four patients with chronic symptomless infection were treated over periods of 6 to 14 days with doses ranging from 100–300 mg. given thrice daily. The fifth patient, who was suffering from acute amoebic dysentery, received 200 mg. of drug thrice daily for 11 days. No therapeutic effect was evident in any of the five patients, and toxic signs included cramps in the hands and feet (2), palpitation and vertigo (1), vomiting (1), sore throat (1), enlarged neck glands (1), and an urticarial papular eruption in both hands, which were blue and cold (1).

Discussion

In addition to its action against amoebiasis, quinoxaline-1 : 4-dioxide (and certain related compounds) showed activity against certain of the larger viruses (Hurst *et al.*, 1953) and certain Gram-negative bacteria (Francis, personal communication). The selection of representatives of the series for clinical trial against amoebiasis was thus governed not merely by the action of any particular substance against this particular infection, but rather by the likely overall therapeutic value as judged from laboratory results with the three types of infection—protozoal, viral, and bacterial. In addition, the toxicity and mode of absorption and excretion were taken into account. Thus the three representatives chosen for trial, 8173, 7218, and 8218, had LD50s (in mice) of 135, 360, and 500 mg./kg. respectively; whereas after oral administration (to rats) of 100 mg./kg. of drug maximum blood levels of 3.1 mg./100 ml. after 20 minutes, 4.1 mg./100 ml. after 2½ hours, and 2.0 mg./100 ml. after 1½ hours, respectively, resulted.

Experiments in rats and monkeys showed that two other substances, 2-methyl-3-ethylquinoxaline-1 : 4-dioxide (9804) and 2 : 3-diethylquinoxaline-1 : 4-dioxide (9915) had superior therapeutic properties (against experimental amoebiasis) to the three compounds actually selected for trial in man. However, as a result of the alarming toxic reactions obtained with the latter compounds, the clinical trial of further representatives of this series of compounds was considered unwarrantably hazardous.

In view of the potent anti-amoebic action of the quinoxaline oxides as measured in rats, it was disappointing to find that these compounds were comparatively ineffective against the infection in man. Although there was some indication that 8173 had a therapeutic effect on the acute phases of amoebiasis, as indicated by a favourable response in two of four cases of acute amoebic dysentery, and both cases of amoebic hepatitis, the doses used were far in excess of the dosage required to produce a therapeutic effect on rats. Thus in the successfully treated cases the daily dosages were equivalent to 16.7, 20, 19, and 10 mg./kg. respectively. In rats a total daily dosage of 5 mg./kg. was adequate. If these dosages were compared on the basis of the metabolic rate formula, the differences would be more pronounced. The toxic symptoms produced in man, e.g., nausea, vomiting, cramps, and dermatitis, could not be foreseen from the laboratory toxicological studies. Further experiments were carried out in attempts to produce compounds of greater therapeutic activity and lesser toxicity. These will be described in a later publication.

Summary

Quinoxaline-1 : 4-dioxide was found to exert a therapeutic effect against experimental amoebiasis of rats, cats, dogs, and monkeys. Certain related compounds were also active against one or more of these laboratory infections.

Quinoxaline-1 : 4-dioxide showed only weak therapeutic action when tried in man, and also gave rise to serious toxic effects. The related compound, 6-chloro-2 : 3-dimethylquinoxaline-1 : 4-dioxide, had no therapeutic effect on the disease in man, and was also toxic.

We are considerably indebted to Dr. A. R. D. Adams from whose unpublished protocols we have summarized the results of the clinical trials of 8173 and 8218.

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