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CLINICAL TRIALS WITH ENTAMIDE FUROATE AND RELATED COMPOUNDS

I. IN A NON-TROPICAL ENVIRONMENT

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Much confusion in the treatment of intestinal amoebiasis has been caused by lack of controlled trials. In the majority of studies published at the present time, the efficacy of drugs in intestinal amoebiasis is judged by the disappearance of the parasites at the conclusion of treatment and follow-up examinations are seldom carried out. Even when they are, the possibility of re-infection is usually present and assessment of a true relapse rate is therefore difficult. For this reason studies of the efficacy of amoebicides is desirable in an area where locally acquired infection is known to be rare. MURGATROYD (1947) stated that there was remarkably little precise knowledge on problems relating to the treatment of amoebiasis, and that there were no generally agreed criteria for diagnosis and for test of cure. In published papers the method used for assessing cure, the number of examinations and the period of observation, were seldom stated, so that in general it was impossible to compare the results of different courses of treatment. This situation led to work being undertaken in the Medical Unit of the Department of Clinical Tropical Medicine at the London School of Hygiene and Tropical Medicine, and the Hospital for Tropical Diseases, London, to assess the results from well-tried remedies under standard conditions, and then to compare these with results from new remedies tried under the same conditions. The methods and results of some of these studies were first reported by WOODRUFF et al. (1956).

METHODS

Only those patients having trophozoites or cysts of *Entamoeba histolytica* in their faeces were treated and for this purpose they were admitted to the Hospital for Tropical Diseases, London. Samples from unformed stools containing blood and mucus were examined microscopically directly, and all faecal specimens were also examined after concentration by the formalin-ether method (RIDLEY and HAWGOOD, 1956). This method was also used for daily examination of faecal specimens during treatment to determine when parasites disappeared, and the same technique was used for follow-up stool tests. The intention was to examine faecal specimens from each patient monthly for 3 months and to continue follow-up examinations as long as possible. In practice a few patients returned overseas soon after treatment, and a few did not attend for follow-up examinations in spite of repeated written requests. *E. histolytica* cysts were measured with an eyepiece micrometer and those less than 10μ in diameter were designated *E. histolytica hartmanni* as recommended by HOARE (1952). Most patients had probably acquired their infection in India or tropical Africa, had suffered from alimentary symptoms or diarrhoea in the past and were either complaining at the time of examination or were found infected on routine examination of the faeces.

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DRUGS ASSESSED

Trials with dichloroacet-4-hydroxy-N-methylanilide, diloxanide or Entamide (Boots) showed WOODRUFF et al., 1956) that in doses of 12 mg. or more per kg. of body weight daily for 10 days, 11.8 per cent. of those excreting cysts relapsed, but each of three patients with acute amoebic dysentery relapsed. Entamide is absorbed from the gastro-intestinal tract relatively easily although it is comparatively insoluble in water. It was thought possible that its ineffectiveness in acute amoebic dysentery might be due to low concentrations of the drug in the colonic contents following absorption of most of the drug from the stomach or small intestine. The Research Department of Boots Pure Drug Co. therefore prepared derivatives of Entamide which were either more or less easily absorbed than the parent substance. Information provided by the Biology Division of the Company indicates that while Entamide piperazine sulphate is approximately equal to Entamide in toxicity for laboratory animals, the derivatives : Entamide benzoate and Entamide furoate are less toxic than the parent substance both on a weight for weight basis and in terms of Entamide content. Entamide itself has been used in man in doses of up to 55 mg. per kg. body weight daily for 10 days without serious toxic effects (FOLL, 1957).

Investigations on the absorbability of these compounds have been made by Boots Pure Drug Co. using C^{14} labelled Entamide, and these show that, following a dose of 50 mg. Entamide per kg. body weight in rats, the blood levels were at 2 hours 0.0048 mg. per g. blood, 0.0030 mg. at 6 hours, and 0.0014 mg. at 24 hours. Using 50 mg. Entamide benzoate, the blood levels in rats were at 2 hours 0.0043 mg. per g. blood, and at 6 hours, 24 hours and 48 hours less than 0.0015 mg. per g. blood. Entamide piperazine sulphate is more readily absorbed, and Entamide furoate is less readily absorbed than the parent substance, Entamide. Excretion of these drugs is both in the faeces and in the urine.

In vitro tests of the activity of these compounds (WOOLFE, 1957) show considerable variation, but by this method of testing, Entamide furoate is active at 10^{-8} and inactive at 10^{-9} ; whereas Entamide itself is active at 10^{-7}. Entamide benzoate and Entamide piperazine sulphate are both active at 10^{-6}. With regard to *in vivo* testing, Woolfe (pers. commun.) has found that using the " limit of activity test " in newly weaned rats, Entamide is usually active at about 12.5 mg. per kg. when tested for 5 days, the piperazine sulphate at about 25 mg. per kg., the benzoate at 6 to 12 mg. per kg. and the furoate at 3 to 6 mg. per kg. There is, however, considerable variation, using this technique.

Knowing these experimental data about the compounds, and having already carried out a trial with the parent substance, Entamide, it was thought possible that the trials, in addition to giving information on the relative efficacy of the drugs, might provide further suggestive evidence on their site of action and on the relative importance of high concentration in the blood or intestinal contents.

RESULTS

(i) *Entamide benzoate*

Entamide benzoate was supplied in tablets each of 0.5 gramme, and the dose was calculated on the basis of 40 mg. per kg. body weight daily for 10 days. No side effects or toxic effects of this drug were noted. In each case blood examination—including haemoglobin estimation, total erythrocyte and leucocyte count and a differential count—was made before and after treatment, but there were no significant changes in these values. Similarly the urine was examined for protein and the centrifuged deposit from it was examined microscopically but no evidence of nephrotoxic effects was found.

This drug was used for the treatment of 18 patients who had amoebic cysts in their faeces. In 11 whose faeces, examined daily during treatment, and which originally contained cysts, the faeces became free from them in an average of 3.6 days. Three patients in this group did not attend for follow-up examinations : two of them having returned overseas almost immediately after treatment. The 15 patients followed-up were examined on an average of 3.6 times each, the range being from one to eight examinations over a period of more than 12 months following treatment, and two were found to have relapsed. The results are shown in Table I.

TABLE I. Results of treatment of amoebic cyst-passers with Entamide benzoate 40 mg./kg./day for 10 days.

Parasite	No. treated	Not followed	Number followed-up and results: 1 month		2 months		3 months		6 months		12 months	
			Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
E. histolytica cysts	11	2	7	1	6	0	5	0	3	0	3	0
E. h. hartmanni cysts	4	1	2	0	1	0	3	0	2	1	1	0
Cysts of both races	3	0	2	0	2	0	3	0	1	0	3	0
Totals	18	3	11	1	9	0	11	0	6	1	7	0

TABLE II. Follow-up examination results in patients having intestinal amoebiasis treated with Entamide piperazine sulphate in a dose of 30 mg./kg./day for 10 days.

Parasite	No. treated	Not followed	Number followed-up and results: 1 month		2 months		3 months		6 months		12 months	
			Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
E. histolytica trophozoites	2	0	1	0	1	1						
E. histolytica cysts	10	1	6	0	4	0	3	1	3	0	1	0
E.h. hartmanni cysts	2	0	2	0	0	0	0	0	0	0	1	0
Cysts of both races	5	2	2	0	1	0	1	0	0	0	1	0
Totals	19	3	11	0	6	1	4	1	3	0	3	0

TABLE III. Follow-up examination results in patients with intestinal amoebiasis treated with Entamide furoate at approximately 20 mg./kg./day for 10 days.

Parasite	No. treated	Number followed-up and results										
		Not followed	1 month		2 months		3 months		6 months		12 months	
			Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
E. histolytica trophozoites	3	1	2	0	1	0	1	0	1	0	1	0
E. histolytica cysts	30	2	22	1	9	0	3	0	5	0	0	0
E. histolytica and *E. h. hartmanni* cysts	2	0	2	0	0	0	1	0	0	0	0	0
Totals	35	3	26	1	10	0	5	0	6	0	1	0

(ii) *Entamide piperazine sulphate*

Entamide piperazine sulphate was supplied in 0.5 gramme tablets and was administered in a dose of approximately 30 mg. per kg. body weight daily for 10 days. The patients tolerated this well and no side or toxic effects were observed. As with Entamide benzoate, examinations of blood and urine were made before and after treatment, but no evidence was found of any toxic effects. The faeces of eight patients examined daily during treatment and which on the first day of treatment contained amoebic cysts or trophozoites, became free from them after an average of 4 days. Nineteen patients were treated with Entamide piperazine sulphate, of whom two had *E. histolytica* trophozoites in their faeces with dysenteric symptoms. Of these two patients, one re-examined 1 month after treatment was free from parasites, and did not re-attend, the other examined 2 months after treatment was found to have *E. histolytica* cysts in the faeces and was re-admitted for further treatment. Of 17 patients who were excreting amoebic cysts before treatment, three did not respond to requests that they should attend for follow-up examination. The remaining 14 patients were examined on an average of twice each, the range being from one to six times over a period of more than 12 months. One patient was found to have relapsed. The results are given in detail in Table II.

(iii) *Entamide furoate*

This drug was supplied in 0.5 gramme tablets, and has been used in a dose of approximately 20 mg./kg. body weight daily for 10 days. Apart from increased flatulence noticed by some patients no side or toxic effects have been found. In 11 patients whose stools were examined daily during treatment, *E. histolytica* cysts disappeared in an average of 3.4 days from the start of treatment.

Of 35 patients treated with Entamide furoate, three had *E. histolytica* trophozoites in the stools. One of these did not attend for follow-up tests but of the other two, one was free from parasites 1 month after treatment and stools from the second patient were examined on five occasions in the next 12 months with negative results. Of 32 patients passing amoebic cysts in the faeces, 30 have attended for follow-up examinations, the mean number of such examinations being 1.5 per patient and the range extending from one to four over a period of 9 months following treatment. The smaller average number of examinations per patient is due to the fact that this drug has been used recently and many patients have not yet had time to attend for further tests. One patient has relapsed. The results are detailed in Table III.

The results of experimental studies on these three derivatives of Entamide, and their effectiveness in chronic infections with *E. histolytica* in this clinical trial are summarized in Table IV.

Discussion

1) *Efficacy of the drugs used*

Under the conditions prevailing in this study it is clear that Entamide furoate is outstandingly successful where the patient's stool contains the cystic forms of the parasite. The relapse rate following its use is approximately one-quarter of that following the use of Entamide (Table IV) in equivalent dosage and under similar conditions. No toxic effects have developed in the 35 patients treated with it and it would appear to be suitable for use for out-patients. It may well be that regular treatment of food handlers with it in large catering establishments would materially reduce the incidence of amoebic infection in those eating in such establishments.

TABLE IV. Absorbability and activity of Entamide compounds and their effectiveness in curing patients passing *E. histolytica* cysts.

Compounds	*Entamide*	*E. benzoate*	*E. piperazine sulphate*	*E. furoate*
Absorbability	Standard	Less than standard	More than standard	Less than standard
In vitro activity	10^{-7}	10^{-6}	10^{-6}	10^{-8}
Activity in weanling rats	12.5 mg./k. x 5 days	6-12 mg./k. x 5 days	25 mg./k. x 5 days	3-6 mg./k. x 5 days
Dose given to patients	12 mg./k. daily x 10 days	40 mg./k. daily x 10 days	30 mg./k. daily x 10 days	20 mg./k. daily x 10 days
No. of patients treated and followed-up	17	15	14	30
No. of patients relapsed	2	2	1	1
Relapse rate %	11.8	13.3	7.1	3.3

2) *Principal site of action of the drugs*

Entamide benzoate which is less absorbable than Entamide appears to be only about as effective as is Entamide when given in dosages double those of Entamide. By contrast, the more absorbable Entamide piperazine sulphate appears to be slightly more effective than Entamide benzoate, even when given in smaller doses than those of Entamide benzoate. No more than a suggestion of the most important site of action of these drugs can be gained from these trials, but that suggestion would be that when drugs of equal amoebicidal activity are used in equal doses the most effective is that which is most absorbable. Entamide furoate has an *in vitro* amoebicidal activity 100 times as great as both Entamide benzoate and Entamide piperazine sulphate, and in view of this it is probably unjustifiable to make deductions on its site of action based on comparisons with the other two drugs.

SUMMARY

1) In a non-tropical environment where reinfection can be discounted, three derivatives of dichloroacet-4-hydroxy-N-methylanilide (Entamide), the benzoate, piperazine sulphate and the furoate have been tried in human infections with *Entamoeba histolytica*.

2) Entamide benzoate, 40 mg./kg. body weight daily for 10 days resulted in disappearance of parasites from the stools in an average of 3.6 days, and of 15 patients passing cysts who were followed-up after treatment there were two relapses.

3) Entamide piperazine sulphate, 30 mg./kg. body weight daily for 10 days resulted in disappearance of parasites from the stools in an average of 4 days, and of 14 chronically

infected, treated and followed-up, one relapsed. Two patients having acute amoebic dysentery were treated of whom one relapsed.

4) Entamide furoate, 20 mg./kg. body weight daily for 10 days resulted in disappearance of parasites from the stools in an average of 3.4 days, and of 30 in whose faeces cysts were present and who were followed-up after treatment one relapsed. Three patients with acute amoebic dysentery were treated of whom two followed-up did not relapse.

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