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THE AMOEBICIDAL ACTIVITY OF 2-DIETHANOLAMINO-5-NITROPYRIDINE

BY

R. A. NEAL AND PATRICIA VINCENT

From the Wellcome Laboratories of Tropical Medicine, London

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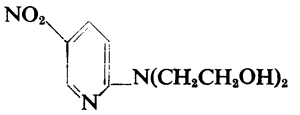
During the examination of a miscellaneous collection of chemicals for amoebicidal activity, 2-diethanolamino-5-nitropyridine (263C49) showed marked activity associated with low toxicity. Activity against *Entamoeba histolytica* by this type of chemical structure has not previously been described (cf. Copp, to be published).



2-diethanolamino-5-nitropyridine

MATERIAL AND METHODS

Caecal Infection in the Rat.—Young rats weighing less than 35 g. were infected by injecting cultured amoebae directly into the caecum, exposed at laparotomy. The rats were then given six daily doses of drug by mouth, the first being administered on the day following inoculation, and were finally killed and examined on the seventh day. Drugs were given in solution or suspended with compound powder of tragacanth in distilled water. The extent of caecal ulceration was given a score, the condition of the wall (normal 0, extensive ulceration 4) being scored separately from the contents (normal 0, mucus only 4), thus making a maximum score of 8 for each animal (cf. Neal, 1951). The caecal contents were examined microscopically for *E. histolytica*; if none was seen the contents were cultured in a medium consisting of an inspissated horse-serum slope covered by egg-white diluted with Ringer solution plus rice starch. The cultures were examined after 24, 48, and sometimes 72 hours' incubation at 37° C. Three strains of *E. histolytica* were used, one invasive (*M*) and two (*STC* and *EA*) which rarely invaded the caecal wall.

Liver Abscess in the Hamster.—The technique for determining the effect of the drug on liver abscesses was similar to that described by Thompson and Reinertson (1951). About 0.1 to 0.15 ml. of a suspension of amoebae from culture containing at least 20,000 amoebae was injected directly into the livers of hamsters exposed under hexobarbitone anaesthesia. The hamsters were given an oral dose of drug on each of the following 4 days and killed 7 days after inoculation. At autopsy the lesion was removed and weighed, the presence of amoebae being determined by microscopical and cultural examination. The strain used for producing liver abscess was a derivative of *M*, which had been serially passaged in the livers of hamsters on five occasions.

In vitro *Experiments.*—Investigation of the activity *in vitro* was made with two pure strains of *E. histolytica*, $P + A^1$ and $D + R^4$ (amoebae plus *Escherichia coli*). The drug and bacteria were added to the medium and incubated overnight; the amoebae were added the following day (Dobell, 1947). The tubes were examined microscopically for the presence of amoebae after 3 days' incubation at 37° C. The media used for the *in vitro* tests were dilute horse serum with 10% liver extract for $P + A^1$, and dilute horse serum with 0.1% dextrose for $D + R^4$. Rice starch was added to each medium.

RESULTS AND DISCUSSION

A comparison of the amoebicidal activity of 263C49 and chiniofon upon the caecal infection of *Entamoeba histolytica* in rats is shown in Table I.

TABLE I

EFFECT OF 263C49 AND CHINIOFON ON *E. HISTOLYTICA* IN RATS

Strain	Drug	Dose (mg./kg. ×6)	Proportion of Rats Cleared of Amoebae	Mean Caecal Score
STC (non-invasive)	263C49	250	4/11	0
		125	11/23	0·1
		62·5	1/11	0·3
	Controls	—	3/21	1·0
EA (non-invasive)	263C49	500	5/7	0·3
		250	7/17	0
		125	3/10	0·6
	Chiniofon	250	7/7	0
		125	6/7	0
		62·5	6/10	0·4
		31·25	4/10	0
	Controls	—	10/36	0·6
M (invasive)	263C49	250	5/6	0·3
		125	10/15	0·3
		62·5	1/7	2·4
	Chiniofon	250	6/6	0·3
		125	9/16	1·3
		62·5	0/7	5·6
	Controls	—	2/13	5·4

Against the invasive strain *M*, 263C49 has the same activity as chiniofon. The caecal scores show that the lowest dose of 263C49 (62.5 mg./kg.) decreased the degree of ulceration, although it did not eliminate the amoebae from the lumen. Chiniofon at this dose level had no effect on the infection rate or the extent of ulceration.

Against the non-invasive strains, 263C49 proved less effective than against the invasive strains. Chiniofon, on the other hand, was more effective on non-invasive strains than invasive strains. Esters of 263C49, which had low solubility in water, were just as ineffective upon the non-invasive strains as the parent compound. The simultaneous administration of 10,000 units per day of polymyxin B sulphate did not enhance the amoebicidal activity of 263C49.

Table II shows the effect on hepatic lesions in hamsters. Chloroquine was effective at 200 mg./kg., but 263C49 had no effect at 250 mg./kg.

TABLE II

EFFECT OF 263C49 ON AMOEBIC LIVER ABSCESS IN HAMSTERS

Expt.	Drug	Dose (mg./kg. ×4)	Proportion with Abscess	Average Lesion Wt. (g.)
1	Chloroquine	200	1/7	0·33
	263C49	250	5/8	2·36
	Controls	—	4/8	1·22
2	Chloroquine	150	5/6	1·61
		75	2/3	1·61
	263C49	150	5/6	2·51
		75	6/6	2·49
	Controls	—	3/4	1·56

In vitro, the minimum amoebicidal concentration of 263C49 was 10^{-5}, while chiniofon was effective at a concentration of 10^{-5} (strain $P+A^1$) and 10^{-4} (strain $D+R^4$). As the nitropyridine derivative showed no inhibition of the growth of *Escherichia coli* at a concentration of 10^{-4}, the drug action is probably directly upon the protozoa.

Toxicity experiments did not reveal any adverse effects on the haemoglobin, red cell, or white cell counts of rabbits. Microscopical examination of liver biopsies from these animals showed no abnormality. A group of mice received 53 daily doses of 250 mg./kg. without showing any inhibition of growth. The drug was rapidly excreted in the urine.

A small clinical trial was carried out by Dr. R. Elsdon-Dew at Durban, South Africa. He gave a very high dose, 51 g. orally over ten days, to a series of fourteen cases of amoebic dysentery and found that, though all cases were rapidly cleared of their symptoms, a proportion of them showed parasitological relapse after treatment.

The nitropyridine was tested for therapeutic effects upon laboratory infections of the following protozoa, but no activity was revealed—*Trypanosoma rhodesiense, T. congolense, T. cruzi, Leishmania donovani, Plasmodium gallinaceum,* and *Histomonas meleagridis.*

SUMMARY

1. The amoebicidal activity of 2-diethanolamino-5-nitropyridine (263C49) was equal to that of chiniofon on rats experimentally infected with an invasive strain of *E. histolytica.* 263C49 was less effective on non-invasive strains of *E. histolytica.* It had no activity on experimentally induced liver abscesses in hamsters.

2. *In vitro,* 263C49 was found to have a direct action on the amoebae at the same minimum concentration as chiniofon. On one strain, the new drug was ten times more active than chiniofon.

3. A small clinical trial showed that a very high dose cleared the clinical symptoms, but parasitological relapse occurred later.

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