ANTIAMOEBIC ACTION OF DRUGS AND SYNTHETIC COMPOUNDS AGAINST TROPHOZOITES OF *ENTAMOEBA HISTOLYTICA* UNDER AXENIC AND POLYXENIC CULTURE CONDITIONS AND IN THE INFECTED RAT CAECUM

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IN VITRO tests for determining the amoebicidal action of test agents against trophozoites of *Entamoeba histolytica* are routinely used in preliminary trials for developing drugs against human amoebiasis because the methods are cheap, simple and clinically effective drugs demonstrate varying degree of amoebicidal properties under these procedures. However, it is well recognized that test procedures impart variable results. This has been adequately discussed for compounds possessing antibacterial activity in tests employing amoeba-bacteria cultures[1].

The development of axenic cultivation of *E. histolytica*[2] has offered a suitable approach for determining the amoebicidal action of test agents without the complications arising from bacterial factors. Reproducible results were obtained when known numbers of trophozoites of *E. histolytica* were exposed to a drug dilution in modified TPS-1 medium keeping a constant volume of the medium and time of incubation[3].

The studies reported in this communication deals with the comparative amoebicidal action of test agents against trophozoites of *E. histolytica* growing under polyxenic and axenic culture conditions and in the infected rat caecum.

STA, B-1, IB-1 and NIH-200 strains of *E. histolytica* were used in this study. The first three strains were maintained in association with an undetermined bacterial flora through thrice weekly passage in modified Boeck and Drbohlav medium[4]. NIH-200 was maintained both under axenic condition in modified TP-S-1 medium[5] and also in the Boeck and Drbohlav medium in association with the bacterial flora from B-1 strain of *E. histolytica*.

The drugs used in this study included: emetine hydrochloride, dehydroemetine dihydrochloride, vioform, entobex, metronidazole, furazolidone and sulphaguanidine. The synthetic compounds consisted of aryloxy propanol piperazines, aryloxy propanol amines, alkyl and aroyl piperazino pyridines, arylketones and other miscellaneous heterocyclic compounds.

The stock solutions of the test agents were prepared in distilled water. The insoluble ones were dissolved in dimethyl formamide and the required volume made up with distilled water.

The amoebicidal action of the test agents against polyxenic *E. histolytica* cultures was determined by methods described earlier[6].

The therapeutic efficacy of drugs and synthetic compounds was determined in rats infected with *E. histolytica* by the following method (unpublished data) 21 days old, laboratory bred, weanling rats (Druckrey strain) weighing 18–20 g were fed on autoclaved rice diet for 7 days. They were then inoculated intracaecally with 0.3 ml of an amoebic inoculum (obtained by pooling 24 hr old cultures) containing about 100,000 trophozoites of *E. histolytica*, rice starch particles and associated bacteria. The animals were placed on the rice diet till the time of sacrifice. The rice diet alters the caecal pH of rats to mildly acidic range for favouring the development of *E. histolytica* infection.

The infected rats were administered the test agent in desired doses (maximum tolerated dose or 100 mg/kg) once daily for five consecutive days by the oral route after 48 hr of infection. They were sacrificed a day following the treatment schedule and the scrapings from caecal wall and caecal contents examined for viable amoebae microscopically and by culture method. Controls consisting of untreated rats and those treated with metronidazole (100 mg/kg dose) were included.

The results presented in table 1 shows that drugs like emetine hydrochloride, dehydroemetine dihydrochloride and metronidazole exert comparable amoebicidal action which is not affected by the culture conditions of the amoeba. In the case of vioform and entobex, the amoebae remained less sensitive to the action of drugs in tests conducted under axenic conditions as compared to those under polyxenic condition. The reverse was true in the case of furazolidone.

Some of the synthetic compounds also presented interesting differences in their action against *E. histolytica* growing under axenic and polyxenic culture conditions. These have been included in table 2. On the basis of these results, the compounds can be classified

Table 1 *Comparative amoebicidal action of known antiamoebic and antibacterial agents against polyxenic and axenic cultures of E. histolytica.*

Drug	M.I.C. μg/ml polyxenic cultures (after 48 hr)				Axenic culture (after 72 hr)
	STA	B-1	IB-1	NIH-200	NIH-200
Emetine hydrochloride	8	8	8	8	8
Dehydroemetine dihydrochloride	8	8	8	8	8
Metronidazole	8	8	8	4	4
Vioform	16	31	31	16	1000
Entobex	31	16	16	31	250
Furazolidone	—	1000	1000	1000	31
Sulphaguanidine	—	1000	1000	1000	1000

*Minimum inhibitory concentration

into four groups *viz.* (1.) Compounds possessing comparable amoebicidal action irrespective of the culture conditions. (2.) Compounds exerting greater amoebicidal action against *E. histolytica* growing in association with bacterial flora. (3.) Those exerting greater amoebicidal action on amoebae growing under axenic conditions and (4.) those that exert varying degree of amoebicidal activity against *E. histolytica* growing in association with bacterial flora, but totally ineffective under axenic conditions.

The results on the *in vivo* therapeutic efficacy of drugs, however, obliterated all the differences recorded from *in vitro* tests and except for metronidazole (100 mg/kg dose) which produced total cures, none of the drugs and synthetic compounds were effective. Treatment with emetine hydrochloride (4 mg/kg) and dehydroemetine dihydrochloride (10 mg/kg) resulted in healing of mucosal ulcers but failed to eradicate viable trophozoite of *E. histolytica* from the caecal contents of a majority of rats. Vioform and entobex each in doses of 200 and 100 mg/kg body wt respectively failed to cure caecal amoebiasis of rats. In the case of furazolidone (100 mg/kg) the mucosal ulcers were not healed though the consistency of the caecal contents were normal. None of the synthetic compounds were effective and motile trophozoites of *E. histolytica* were seen with ease both from caecal contents and scrapings from caecal wall.

To ascertain whether the *in vivo* failure of the drugs was due to false positive results through inherent defects of *in vitro* procedures, tests were conducted to determine the antibacterial action of a few synthetic compounds and the effect of furazolidone on the redox potential of the test medium.

Counted numbers of mixed bacteria (by a haemocytometer) from *E. histolytica* cultures were inoculated into diluted inactivated bovine serum in tubes and incubated for 18 hr at 37°C. After this, the test compounds in amoebicidal concentrations were added and the tubes incubated for a further period of 48 hr. The total count of bacteria and optical density by Bijau Colorimeter, using 625 μm filter, were then determined.

To determine the action of furazolidone on the redoxpotential of the test medium, tests were conducted in the following manner: To 9 ml of diluted inactivated bovine serum containing 0.2 percent L-cysteine hydrochloride and 0.1 percent indigocarmine in screw capped test tubes (15 × 125 mm), 1 ml of furazolidone in amoebicidal concentrations, dissolved in distilled water was added. Controls were kept with the addition of distilled water alone. The tubes were incubated at 37°C.

The results presented in table 3 shows that there was a marked inhibition of bacterial growth due to addition of synthetic compounds at amoebicidal concentrations. Similarly, indigocarmine failed to become decolourized in tubes containing furazolidone. In the control tubes it remained decolourized upto 72 hr. It thus becomes apparent that the addition of test agents renders the medium unsuitable for healthy growth of *E. histolytica*.

Furazolidone has been reported to be ineffective at concentrations as high as 200 μg/ml against several strains of *E. histolytica* growing in association with mixed bacteria[7]. In the present study, this drug produced false positive results in tests conducted under axenic conditions while it failed to be amoebicidal under polyxenic conditions and in the infected rat caecum.

The limitations of methods using cultures of *E. histolytica* growing in association with mixed bacterial

Table 2 *In vitro action of synthetic compounds against polyxenic and axenic cultures of E. histolytica*

Code No	Compound	MIC* µg/ml	
		NTH-200	STA
1	2	3	4
67–125	1-(3-(β-Naphthoxy)-2 hydroxy propyl)-4-(2-methylphenyl) piperazine HCl	31	62
68–52	1-(3-phenyl-2-hydroxy propyl)-4-(O-methyl phenyl) piperazine	125	62
71–179	1-(3-cyanophenoxy)-2-hydroxy-3 (N^4-phenyl piperazinyl) propane	125	8
69–229	1-(3-Trifluoromethyl phenoxy)-3-(3,4-dimethoxy phenyl ethyl amino) propanol-2-	NA*	125
67–153	4-(O-chlorophenyl)-1-(3-nitro-4-pyridyl) piperazine	NA	125
67–159	4-(1-Naphthyl)-1-(3-amino-4-pyridyl) piperazine	NA	125
67–190	1-(3-Nitro-4-pyridyl)-4-(α-phenylethyl) piperazine	62	62
64–14	4-β-Phenethylamino-3-amino-5-nitropyridine	125	125
64–22	2,3-Diamino-5-nitropyridine	31	125
64-311	5-Methyl-7, bromo-8-hydroxyquinoline	125	62
65–318	1-β-Diethylamino ethy-2,3-diohenyl indole	125	125
66–357	4-β-Phenyl ethyl amino-3-amino quinoline, 2HCl	125	31
67–84	3-β-Phenyl ethyl 1-2,3,4,4a,5b-hexahydro-(1H) pyrozino-(1,2-a) quinoline	125	125
67–90	4-Amino- 2,3-hexamethylene quinoline	62	125
67–148	1:3 diphenyl-1-(4-N-methyl piperazinyl)-propane, HCl	125	125
67–352	1-β-(4-methyl-1-piperazinyl)-ethyl-3-phenyl-2,3,4,5, tetra hydro-1-1-1-1 hexaazepine,2 HCl	125	125
67–371	4-(3-Amino-4-pyridyl) amino diphenyl	62	62
67–372	2-(β-hydroxy) phenethylaminomethyl-1,2,3,4 tetrahydroquinoline	NA	125
68–15	1-(3:4-dichlorophenyl)-1-(1-piperidyl)-3-phenyl propane HCl	NA	62
69–13	1-(β-hydroxy-V-(4-propiophenoxy)propyl) 23,4,4a,5,6 hexahydro piperazinoquinoline, HCl	125	125
69–14	1-(β-hydroxy-V-(p-chlorophenoxy)propyl)2,3,4,41,5,6 hexahydro piperazinoquinoline, HCl.	NA	125
70–331	Bis-(3-Nitro-4-morpholino)-phenyl sulphone	62	62
70–419	1-(3-Diethylaminoethyl)-2,3 cycloheptyl-5 methoxy indole oxalate	125	62
71–283	4-(n-Hexylamino)-2,3-tetramythylene quinoline HCl	31	4
71–284	4-(cyclohexylamino)-2,3-tetramethylene quinoline, HCl	31	2
71–354	10-Morpholino acridine	31	31
71–535	(2-(γ-p-Fluorobenzoyl propyl)-1,2,3,4,4a,5,11,111 octahydro-41, 11a-trans 6H pyridine (3,4–6) indol.	31	31
71–536	2-(3-(p-Fluorobenzoyl)propyl)-1,2,3,4,4a,5,11,11a octahydro-6H pyrido (4,3-b) carbazole	NA	62
65–290	4,5-Diphenyl-3-(4-hydroxyphenyl)imidazole	NA	62
70–468	β-(3-hydroxyphenyl)propiophenone	NA	125

* NA = Non amoebicidal at 1000 µg/ml.

Table 3 *Antibacterial effect of compounds against mixed bacterial flora of E. histolytica cultures (STA).*

Compounds	No. of bacteria (× 10 ml) after drug treatment	Optical density
65–290	880	0.367
67–153	72	0.284
67–372	104	0 450
68–15	28	0 149
69–229	40	0 184
70–468	1,000	0.382
Control	1,280	0.530

An inoculum of 128×10^7 bacteria per ml was used

flora or selected metabolic associates have been well established[1]. The results of our present study shows that use of axenic cultures in these tests also fail to eliminate such uncertainties. These tests apparently cannot be used as substitutes for *in vivo* procedures and the results needs to be interpreted with caution in studies dealing with structure activity relationship in developing drugs against human amoebiasis.

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ANNOUNCEMENT

NEW DIRECTOR FOR THE INDIAN INSTITUTE OF SCIENCE, BANGALORE PROF. C. N. R. RAO, F.R.S.

We are happy to report that Prof. C. N. R. Rao, F.R.S., one of India's very distinguished scientists (and the present President of the Current Science Association) has been appointed Director of the Indian Institute of Science, Bangalore—one of the most prestigious research institutes in India and which is now celebrating its Platinum Jubilee. Prof. Rao is well known for his outstanding contributions to spectroscopy, surface science, solid state chemistry and materials science. He is the recipient of numerous honours and awards (see *Current Science*, Vol. 51, page 351) and is the President-elect of the IUPAC, a distinction not achieved by any Indian so far. He is now in England as the first Jawaharlal Nehru Professor at the University of Cambridge. On his return in the first week of August 1984, he will be taking over as Director from Prof. S. Ramaseshan.

We wish Prof. C. N. R. Rao all success in this important assignment.