Recent Advances in Protozoan Diseases, Proceedings of Symposium held at Hindustan CIBA-GEIGY Research Centre, Bombay, November 28-29,1983

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Development of new amoebicides

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Abstract

Amoebiasis is a wide-spread disease with manifold manifestations, the causative organism being *Entamoeba histolytica*. The sequelae can range from mild stomach upsets to fatal amoebic liver abscess. Development of amoebicides has been facilitated by the elaboration of various animal models for both the invasive and non-invasive varieties of the disease. In this communication drugs discovered thus are briefly mentioned, especially with respect to their mode of action. Reference is also made to the role of plant products in this area.

The advent of metronidazole can be considered to be a breakthrough in the treatment of amoebiasis. Tinidazole and ornidazole belong to the nitroimidazole group. Satranidazole, Go-10213, a drug under registration is the latest addition and is superior to these in animal models of amoebiasis, trichomoniasis and anaerobic infections.

The role of the nitro group in the antimicrobial activity of nitroimidazoles is highlighted. Other possible ways of specific interference with the metabolic processes of amoeba are mentioned as alternative approaches to the synthesis of new amoebicides.

It is a privilege to be called upon to participate in this symposium and present a paper. My approach to the topic 'Development of new amoebicides' will be essentially that of a medicinal chemist, but the treatment of the subject will have a broad perspective rather than be based on narrow structure-activity considerations.

I shall preface my talk with a quick look at the magnitude of the problem of amoebiasis, its multifarious manifestations and the tools available for screening candidate amoebicides. This will be followed by a brief consideration of the currently available amoebicides and their mechanisms of action wherever these are known. The talk will conclude by surveying recent additions to the information on the biochemistry of amoeba which may help a rational design of the amoebicides of the future.

The causative organism of amoebiasis, *Entamoeba histolytica* is estimated to affect 10% of the world population, with a low incidence of about 10-25% in temperate zones, but a higher prevalence rate, upto 100% in some tropical countries.

Contribution No. 717 from Research Centre

In India, 8·5-85% are reported to be affected in various regions, and more than 50% of the population is harbouring the parasite with or without symptoms. It would be a matter of considerable interest and should be a matter of some concern for the foreign delegates to this conference to know that 50-80% of Bombay's population is affected by *E. histolytica*[1]. Most of the well-known manifestations of amoebiasis may be summarised as (a) acute intestinal amoebiasis causing diarrhoea with blood, mucus and colicky pain; (b) chronic intestinal amoebiasis seen as chronic diarrhoea or constipation with or without blood or mucus and (c) extra-intestinal amoebiasis manifesting itself variously as hepatic amoebiasis—mild, diffuse hepatitis to multiple large abscesses and very rarely as amoebic brain abscess or cutaneous amoebiasis or even infestation of the genital organs.

An excellent review on experimental amoebiasis and development of antiamoebic compounds is available[2], while the clinical approaches to chemotherapy of amoebiasis have been reviewed elsewhere[3]. Comprehensive articles both on general[1] and on medicinal chemistry[4] are available. Experimentally, candidate antiamoebic drugs may be initially screened *in vitro* for their action on trophozoites of *E. histolytica* in polyxenic or axenic cultures. The former would represent both direct and indirect action while activity in the latter system would be evidence for a direct action. *In vivo* models of invasive amoebiasis consist of hamsters with induced liver abscesses (hepatic infection), in hamsters or young Wistar rats or mice[5] having caecal infections (intestinal amoebiasis) or the hamsters carrying dual infections in the liver and caecum[6]. Models for noninvasive or luminal amoebiasis consist of the hamsters carrying natural infection of *E. criceti* or the rats with *E. muris* infection[7]. Jirds, cats, guinea pigs and monkeys have also been used as *in vivo* models for screening for antiamoebic activity[2].

Turning to results with antiamoebic chemotherapeutics, *in vitro* activity data are given in Table 1 for representatives of several types of antiamoebic agents against axenic *E. histolytica*[2]. The nitroimidazoles(8,10,11) are seen to be the most potent ones in this test and are followed by emetine(20). Less active are the dichloracetamides like diloxanide(1) while phanquone(14), hydroxyquinolines(15,18) and chloroquine(19) are weaker, potency decreasing in the order given. Among the antibiotics, paromomycin is quite potent, but the tetracyclines are weaker, suggesting an indirect mode of action for the latter. Structures of some of the more important drugs are given in Figs. I-III. Table 2 reproduces data that have been obtained for representative clinically used antiamoebic agents in animal models of caecal and hepatic infections[2]. Besides emetine(20), the nitro-imidazoles, metronidazole(8), ornidazole(10) and tinidazole(11) are found to be active at both sites of infections, while the dichloracetamides, diloxanide(1), its furoate and etophamide(5) are highly active against caecal amoebiasis, but are inactive in the hepatic model. Diiodoquin(16) is only feebly active in the former. Interestingly, while nitroimidazoles(8,10,11) are quite active in curing hamsters of caecal amoebic infections, the dichloracetamides are inactive even at very high doses. On the other hand, preliminary studies indicate that the latter are considerably more active than nitroimidazoles against lumen-dwelling natural *E. muris* infection in rats. This is likely to be the case with natural *E. criceti* infections in hamsters.

Plant extracts and pure plant products have not been perhaps as exhaustively studied for antiamoebic activity as for example, antihypertensive and antitumour

TAB

Activity of
against axenic E

Drug
Phanquone (14)
Clioquinol (15)
Iodo hydroxyqui
sulphonic acid (1
Chloroquine (19)
Chlorbetamide (
Diloxanide (1)
Dehydroemetine
Emetine (20)
Metronidazole (8
Ornidazole (10)
Tinidazole (11)
Paromomycin
Tetracycline
Oxytetracycline

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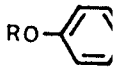

1 R = H
2 R = 2

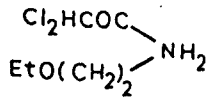

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TABLE 1

Activity of amoebicides against axenic E. histolytica (2)

Drug	ED_{50} (ug/ml)
Phanquone (14)	5.8
Clioquinol (15)	8.4
Iodo hydroxyquinoline sulphonic acid (18)	33
Chloroquine (19)	85
Chlorbetamide (3)	7.5
Diloxanide (1)	0.88
Dehydroemetine (21)	0.25
Emetine (20)	0.09
Metronidazole (8)	0.01
Ornidazole (10)	0.03
Tinidazole (11)	0.03
Paromomycin	0.73
Tetracycline	29
Oxytetracycline	83

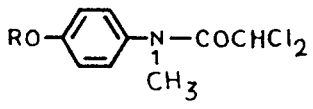
TABLE 2

Activity of antiamoebic compounds in hepatic infection in hamster and caecal infection in rat (2)

Drug	ED_{50} mg/kg p.o. × number of doses	
	Caecal infection in rat	Liver abscess in hamster
Diiodoquin (16)	600 × 5	—
Diloxanide (1)	10 × 6	inactive
Etophamide (5)	4.0 × 5	inactive
Emetine (20)	2.5 × 6	0.9 × 5 (s.c.)
Metronidazole (8)	30 × 3	7.3 × 5
	10 × 5	10 × 5
Ornidazole (10)	10 × 3	21 × 3
Tinidazole (11)	5.0 × 5	25 × 5

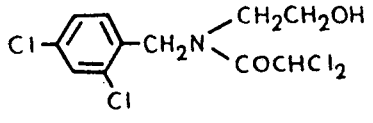
activity. The pride of place for a useful natural product in this field goes to emetine(20) which has been largely replaced with synthetic dehydroemetine(21) for the treatment of both liver abscess and amoebic dysentery. Conessine, the steroidal alkaloid of *Holarrhena antidysentrica* (Kurchi) was used in the humans for both extraintestinal

Figure I : Dichloracetamides

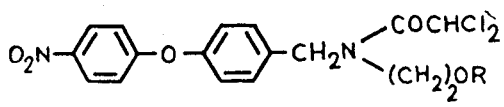


1 R = H; Diloxanide

2 R = 2-Furoyl; Diloxanide furoate

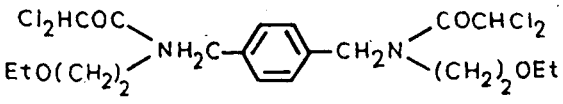


3 Chlorbetamide

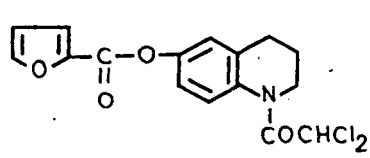


4 R = H; Clefamide

5 R = Et; Etophamide

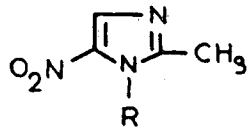


6 Teclozan



7 Quinfamide

Figure II : Nitroimidazoles

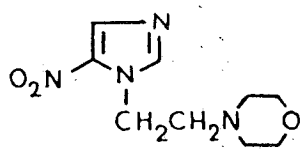


8 R = CH_2CH_2OH, metronidazole

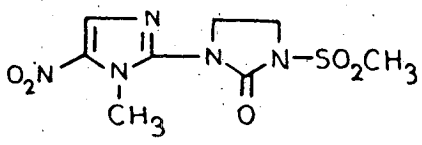
9 R = $CH_2CH(OH)CH_3$, secnidazole

10 R = $CH_2CH(OH)CH_2Cl$; ornidazole

11 R = $CH_2CH_2SO_2Et$; tinidazole

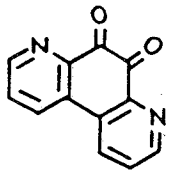


12 Nimorazole

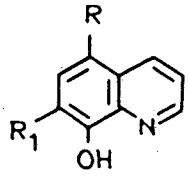


13 Satranidazole

Figure III : Miscellaneous antiamoebic compounds



14 Phanquone

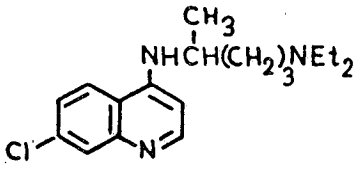


15 R = Cl, R_1 = I Clioquinol

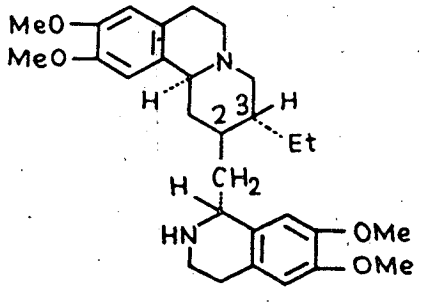
16 R = R_1 = I Diiodoquin

17 R = R_1 = Br Broxiquin

18 R = SO_3H, R = I Chiniofon



19 Chloroquine



20 (-) Emetine

21 (double bond at 2.3) Dehydroemetine

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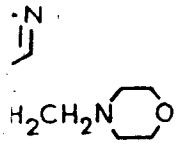

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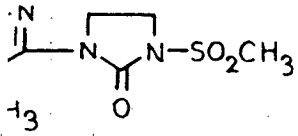

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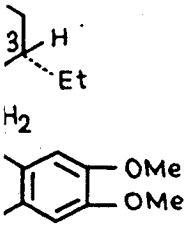

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and intestinal amoebiasis, but given up due to toxic CNS side effects[4]. In the hamster model, it was active in the liver at 75 Mg^x 2 p.o. and in caecal infections in hamster at 75 Mg^x 4 p.o. Semisynthetic analogues related to another steroidal alkaloid, paravellerine (from *Paravelleris microphylla*) have been claimed to have antiamoebic activity[8]. *Wrightia tomentosa*, an adulterant of Kurchi has been reported to have *in vitro* antiamoebic activity[9]. Berberine from *Berberis aristata* is reportedly active in low doses against amoebic infection in hamster liver and rat caecum[10], but this could not be confirmed in our laboratories. *In vitro* activity has been mentioned for extracts of *Euphorbia hirta*[11] which is claimed to have clinical utility in intestinal amoebiasis. Glaucarubin, the amaroid constituent of Simarouba and quassin, the bitter principle of *Quassia amara* have been found to be antiamoebic[4]. The former has been reported to be useful for chronic as well as acute intestinal amoebiasis but has not established itself well in clinical practice. Intestinal amoebiasis in man has been treated with powdered leaf of henna, *Lausonia alba*[12]. Another lead is ailanthone, the diterpene from *Ailanthus glandulosa*[13]. Anacardic acid from *Anacardium occindentalle* and *Semecarpus anacardium* is active against caecal infections in the rat at very high doses[14]. Routine screening of Indian medicinal plants has revealed several *in vitro* leads[15].

Among the antibiotics, the aminosugar polysaccharide, paromomycin has been used successfully for curing intestinal infections, while the tetracyclines are used in combination, sometimes with chloroquine. Anisamycin, a *Streptomyces* antibiotic, is a simple p-methoxybenzylpyrrolidine derivative. It is active both *in vitro* and *in vivo* but has not found clinical application[4].

Among the synthetic compounds, metronidazole(8) belonging to the nitroimidazole group may be considered to have introduced a new era in the treatment of amoebiasis. Apart from their activity against both intestinal and extra-intestinal amoebiasis, the nitroimidazoles have a wider range of useful biological activities[16] (Table 3), compared to the diloxanide(1) group of dichloracetamides which are singularly active only in luminal amoebiasis. Ten years ago, we embarked upon a programme of

TABLE 3

Spectrum of activity of nitroimidazoles

Amoebiasis, giardiasis, balantidiasis
Trichomoniasis
Dracunculiasis
Pinworms (Mouse)
Coccidiosis, histomoniasis
Aerobic bacteria
Anaerobic bacteria—Vincent's disease, post-operative surgical infections, particularly gynaecological
Radiosensitisation of hypoxic cell tumours
Hirsutism!

TABLE 4

Comparison of antiamoebic and antitrichomonal activities of satranidazole (13) with other nitroimidazoles

Drug	Hepatic amoebiasis (hamster) ED_{100} mg/kg p.o. × 2	Caecal amoebiasis (hamster) ED_{100} mg/kg p.o. × 4	*T. vaginalis* (mouse) ED_{100} mg/kg p.o. × 4
Satranidazole (13)	22	30	10
Metronidazole (8)	>45	>40	40
Ornidazole (10)	45	>40	15
Tinidazole (11)	>45	>40	25
Secnidazole (9)	>45	>40	25
Nimorazole (12)	>45	>40	>50

synthesis and evaluation of nitroimidazoles for antiparasitic activity. From the screening of more than 400 new derivatives, resulted satranidazole(13) Go 10213, a highly potent, well-tolerated and clinically useful antiamoebic-antitrichomonal agent, which is now under registration in India and in advanced clinical trials abroad[17]. Satranidazole has been found to be twice as active as metronidazole against axenic *E. histolytica*[18]. Table 4 gives a comparison of satranidazole with some clinically useful nitroimidazoles in experimental infections of amoebiasis and trichomoniasis and provides proof for its superior antiamoebic action[19,20,21]. Additionally, satranidazole has been found to have a wide spectrum of *in vitro* activity against a number of anaerobes, being 6, 20 and 10 times respectively more potent than metronidazole against *Bacteroides fragilis*, *Spherophorus necrophorum* and *Bacteroides oralis*. Against 50 clinical isolates of anaerobes studied, satranidazole at a dose of 0·12 ug/ml was active against 45, unlike clindamycin(22) or nitroimidazoles(8) and(10) (<10). Satranidazole was also superior to metronidazole in a nonfatal subcutaneous *Bacteroides fragilis* abscess model in the mouse and in a fatal murine model of *Fusobacterium necrophorum* infection[22]. Satranidazole is well-tolerated by animals[23,24] and has excellent CNS-CVS tolerability[25]. Studies with radiolabelled[26] and cold satranidazole show that it is orally well-absorbed, and distributed uniformly in all tissues and body fluids. Good tolerability and efficacy have been established in intestinal and hepatic amoebiasis as well as in trichomoniasis[27] and the preparation has been submitted for registration after extensive phase III trials[28].

The post metronidazole era in amoebiasis has seen the appearance of a large number of analogues, only a few of which are listed in this review. A more comprehensive account is provided elsewhere[16]. In the dichloracetamide group of luminal amoebicides, there have been fewer successors to diloxanide, like etophamide(5) or teclosan(6). Quinfamide(7)[29], a very recent addition, is superior to diloxanide(1) in *E. criceti* infections. This had been synthesised by us in 1972 (Go-9863) and was found to be inactive against both hepatic and caecal infections in the hamster. There have been a distressingly small number of other leads which are represented

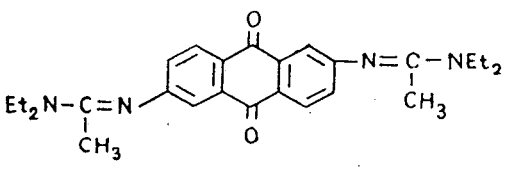
tivities of
s

T. vaginalis (mouse) ED_{100} mg/kg p.o. × 4
10
40
15
25
25
>50

itic activity. From the dazole(13) Go 10213, a -antitrichomonal agent, clinical trials abroad[17]. nidazole against axenic le with some clinically asis and trichomoniasis n[19,20,21]. Additionally, *n vitro* activity against vely more potent than *phorum* and *Bacteroides* anidazole at a dose of roimidazoles(8) and(10) nonfatal subcutaneous fatal murine model of olerated by animals[23,24] liolabelled[26] and cold uted uniformly in all ve been established in [27] and the preparation l trials[28].

appearance of a large review. A more com- mide group of luminal le, like etophamide(5) perior to diloxanide(1) 1972 (Go-9863) and ctions in the hamster. which are represented

Figure IV : New antiamoebic leads



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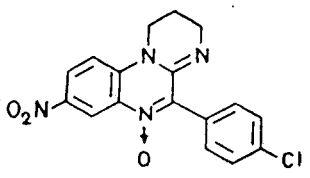

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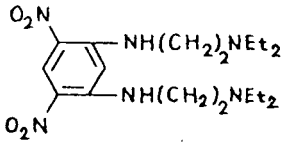

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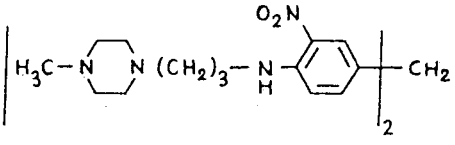

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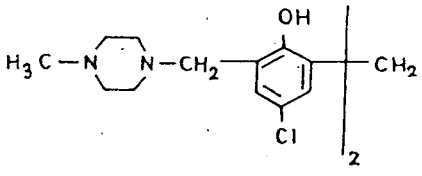

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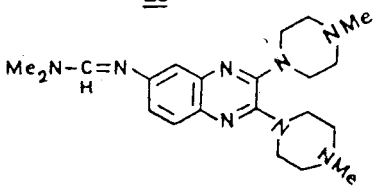

27

by structures(22,27) (Fig. IV). Their activities in experimental models of amoebiasis and trichomoniasis as determined in our laboratories are noted in Table 5. The amidine(22)[30-32] and pyrimidoquinoxaline(23) are easily comparable to metronidazole with respect to antiamoebic[33] although not to antitrichomonal activity while the dinitro derivatives(24,34,25) are weaker amoebicides and additionally non-trichomonacidal(35). The bis Mannich base(26) is similar in its spectrum and potency(36). None of the compounds had an acceptable level of tolerability in our hands for development for human trials. Bispiperazino quinoxaline(27) is claimed to have amoebicidal properties[37] *in vivo* but this could not be confirmed by us.

The question now arises whether there is a need for a new amoebicide and if so, what its profile and mode of action should be. This question will perhaps

TABLE 5

Activity of newer leads in hepatic and caecal amoebiasis (hamsters) and T. vaginalis infection (mouse). ED_{100} in mg/kg p.o. ×

Compound	Hepatic amoebiasis	Caecal amoebiasis	*T. vaginalis*
22	30 × 2	60 × 4	125 × 4
23	45 × 2	45 × 4	300 × 4
24	100 × 2	100 × 4	inactive
25	75 × 2	100 × 2	inactive
26	60 bd × 2	120 × 4	inactive

be raised in the panel discussion this afternoon and answered. The nitroimidazoles especially metronidazole(8), have been quite successful in the treatment of amoebiasis. Development of drug resistance by *E. histolytica* is not a problem, although this may be the case in trichomoniasis[16]. Incidentally preliminary studies show that metronidazole-resistant *T. vaginalis* is still sensitive to satranidazole(13),[38]. The clinical efficacy of metronidazole in luminal amoebiasis is the subject of recurrent debate, but luminal amoebicides of the diloxanide type have provided at least partial answers. Mutagenic and carcinogenic findings for metronidazole in experimental animals have been problem areas for nitroimidazoles in general but human experience gained so far has belied the fears[39]. Nevertheless, the toxicity of the newer drugs must be kept under scrutiny on a long-term basis. A safe, non-mutagenic and cheap chemotherapeutic agent with good activity against both invasive and luminal amoebiasis may still be a desirable objective in this field.

Modern approaches to drug design with such an objective would require that vulnerable sites of the parasite and steps in its metabolism should be identified and inhibited by agents which are non-lethal to the host. Before scrutinising recent additions to basic information on *E. histolytica* it would be of interest to survey briefly available knowledge concerning the mechanism of action of currently used anti-amoebic drugs. Hydroxyquinolines, e.g.(15) are likely to be amoebicidal because of their ability to chelate and remove Fe^{++} ions needed for growth[4]. Phanquone(14) may owe its activity either due to its redox property or by the ability of its reduction product, a hydroxyphenanthroline to chelate Fe^{++} ions. The antiamoebic activity of chloroquine(19) like its antimalarial property may arise from its propensity to intercalate with nucleosides. Emetine(20) [and probably dehydroemetine(21) also] has been shown to cause irreversible blocking of protein synthesis in all eucaryotes by inhibiting the movement of ribosomes along messenger RNA[3]. Not much is known about the mode of action of diloxanide(1), except for a passing statement that it is also an inhibitor of protein synthesis[3]. It is also interesting to speculate whether a reactive metabolite arising by dehalogenation of the dichloracetamido group(1) into an oxamyl chloride is implicated along the lines proposed for chloramphenicol[40].

The mechanism of antianaerobic and antiprotozoal action of metronidazole has been exhaustively investigated by several groups of workers[16]. Particular mention must be made of the work of the group of Edwards[41] which has shown that metronidazole is selectively toxic to anaerobic bacteria and protozoa, because it can accept electrons at the level of pyruvate metabolism at potentials which are incapable of being generated in aerobic cells. The species which is lethal to the parasite/anaerobe is a transient one formed by the addition of one electron to metronidazole which damages the parasite DNA by helix destabilisation and strand breakage, releasing specifically thymine nucleotides. In keeping with this specificity, parasites and anaerobes which have nucleosides with higher A + T than G + C content are more sensitive to metronidazole than those with higher G + C content. Metronidazole has also been shown to inhibit the uptake of thymine by *T. vaginalis*[42]. Interestingly, a recent publication[43] provides ESR evidence for the formation of the metronidazole nitro anion radical in the reduction of metronidazole by intact *T. foetus* cells. The activity is related to cellular contents of reducing substrates,

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e.g. glucose and pyruvate. Such radicals are not detectable under anaerobic conditions. The group of Goldman in USA[44] has also provided massive evidence for a transient lethal species produced from metronidazole in various organisms but casts doubts on the specificity of the toxicity to protozoa and anaerobes. In another recent publication[45], yet another possible mode of action of metronidazole has been postulated, namely the removal by nucleophilic addition of an aminoacid like glutathione specifically needed for the growth of the organism. Experimentally, cysteamine has been shown to add to metronidazole under physiological conditions.

Some of the recent developments in the biochemistry of *E. histolytica* may now be surveyed. A recent major discovery in the glycolytic pathway of *E. histolytica* has been that in the reversible interconversion of fructose-6-phosphate to fructose-1, 6-diphosphate, the amoebal phosphofructokinase (E.C. 2.7.1.90) uniquely uses inorganic pyrophosphate (PPi) rather than ATP as phosphate donor[46]. Some alkane diphosphonates, e.g. 1-hydroxynonane-1, 1-diphosphonate have been found to inhibit this parasite-specific enzyme and also the axenic growth of *E. histolytica*[47].

One of the possibilities of interrupting the life cycle of *E. histolytica* would be to prevent the encystation of the trophozoite. Inhibitors of chitin synthesis like the pyrimidine nucleosides, polyxin D and nikkomycin have been shown to prevent the incorporation of the chitin precursor N-acetyl glucosamine by *E. invadens*, a snake parasite and inhibit the formation of detergent-resistant cysts. Since the cysts of *E. invadens* and *E. histolytica* have morphological similarities and chitin is present in their walls, this finding offers a lead for designing cysticidal drugs[48].

A third possible approach lies in understanding the cytopathogenicity of *E. histolytica*. This has been shown to be prevented by ion-channel inhibitors like bepredil and verapamil. The inhibitory action which must affect amoebic microfilament function can derive from a combination of properties—interference with carbohydrate-specific adherence of amoeba to target cells (bepredil) and lysis of adherent target cells (bepredil and verapamil)[49]. An ion channel inhibitor absorbed by the parasite but not by host offers the possibility of rendering *E. histolytica* trophozoites avirulent. These and other future discoveries of potential parasite-specific targets can be exploited to provide the antiamoebic drugs of the future.

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