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MINIREVIEW

The Development of Drugs against *Acanthamoeba* Infections

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For the past several decades, there has been little improvement in the morbidity and mortality associated with *Acanthamoeba* keratitis and *Acanthamoeba* encephalitis, respectively. The discovery of a plethora of antiacanthamoebic compounds has not yielded effective marketed chemotherapeutics. The rate of development of novel antiacanthamoebic chemotherapies of translational value and the lack of interest of the pharmaceutical industry in developing such chemotherapies have been disappointing. On the other hand, the market for contact lenses/contact lens disinfectants is a multi-billion-dollar industry and has been successful and profitable. A better understanding of drugs, their targets, and mechanisms of action will facilitate the development of more-effective chemotherapies. Here, we review the progress toward phenotypic drug discovery, emphasizing the shortcomings of useable therapies.

AQ:A Antimicrobial chemotherapy is the most widely used method of treating infections due to *Acanthamoeba*. Despite advances in antimicrobial chemotherapy, the morbidity and mortality associated with *Acanthamoeba* keratitis and *Acanthamoeba* encephalitis, respectively, have remained high. For example, the mortality rate of granulomatous amoebic encephalitis due to pathogenic *Acanthamoeba* spp. is more than 90%, even with treatment with various combinations of drugs such as amphotericin B, rifampin, trimethoprim-sulfamethoxazole, ketoconazole, fluconazole, sulfadiazine, miltefosine, albendazole, etc. (reviewed in references 1, 2, 3, 4, 5, 6, 7, and 8). Current treatment of *Acanthamoeba* keratitis involves chlorhexidine, in combination with diamidines and neomycin, and can last up to a year, and even then infection recurrence occurs in approximately 10% of cases (reviewed in references 1, 2, 3, 4, 5, 6, 7, and 8). In part, this is due to our incomplete understanding of the biology of the parasite and of the pathogenesis and pathophysiology of the disease, as well as to the lack of effective chemotherapeutic agents and/or the lack of clinical testing of the potential targets that have been shown to play an important role in the virulence of pathogenic *Acanthamoeba*. This is despite the fact that a plethora of drugs, some of which show potent antiacanthamoebic effects, have been tested; however, their translational value in the treatment of *Acanthamoeba* infections remains unclear (reviewed in reference 9). Many of the drugs tested target functional aspects of *Acanthamoeba*, as it is "easier to erase function of an organism than its structure" (9). However, there are disadvantages to this approach. Being eukaryotes, *Acanthamoeba* species share functional homologies with mammalian cells. Consequently, many of the available drugs cannot be prescribed at effective concentrations due to their unwanted side effects. This is particularly relevant for treatment of amoebal brain infection, in which drugs are given intravenously and are expected to cross the blood-brain barrier to access the central nervous system to target the intracerebral parasite. In this process, drugs penetrate many tissues and can affect their physiology before reaching the target site at an effective concentration. Hence, there is a need to develop a targeted therapeutic approach, i.e., to identify drugs that can affect *Acanthamoeba* viability without affecting the host cells. The purpose of this review is to classify the tested antiacanthamoebic agents into functional groups to identify drugs and/or chemotherapeutic approaches of potential value for further work.

The knowledge of the mode of action of the majority of drugs tested against *Acanthamoeba* is largely derived from studies conducted in bacterial, fungal, or protozoan pathogens. These are indicated here for information; however, future studies are needed to determine and/or confirm their mechanism of action against *Acanthamoeba*.

MEMBRANE-ACTING AGENTS

Being the outermost surface of the cell, the outer plasma membrane and its constituents provide a logical target(s) as it is easier to access. With the actively growing infective trophozoite that undergoes binary fission, the properties and charge of the cell membrane and its biosynthesis and modulation offer chemotherapeutic opportunities. Repurposing drugs with known modes of action for the aforementioned targets and/or agents with growth-inhibitory effects has been a useful avenue, but this approach lacks specificity, produces host cell toxicity, and may not be appropriate for the dormant cyst stage and thus poses a challenge in wider application of such drugs against *Acanthamoeba* infections. For example, chlorhexidine is positively charged and ionic with the negatively charged plasma membrane of the parasite, resulting in structural and permeability changes, ionic leakage, and cytoplasmic disruptions causing cellular damage and cell death, although AQ: B those effects have not curtailed the use of the compound. It exhibits potent amoebicidal properties as well as cysticidal properties at 200 μg per ml (0.02%), and it is used clinically against *Acanthamoeba* keratitis but is not a drug of choice for ocular and brain infection (10–21) (Table 1). Similarly, biguanide compounds T1 (polyhexamethylene biguanide [polyhexadine or polyaminopropyl biguanide]) are known to interact with membrane phospholipids, affecting membrane fluidity and conformation and leading to ionic leakage and cell death at 200 μg per ml (0.02%), and are used clinically against *Acanthamoeba* keratitis but may exhibit side

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TABLE 1 List of antiacanthamoebic agents

Classification and agent no.	Drug(s)	Mode of action	Description and effects on *Acanthamoeba*
Membrane-acting agents			
1	Chlorhexidine	• Chlorhexidine is positively charged and ionic with the negatively charged plasma membrane of the parasite, resulting in structural and permeability changes, and ionic leakage, cytoplasmic disruptions causing cellular damage and cell death.	• Amoebicidal and cysticidal concn is 200 μg per ml (0.02%).
2	Polyhexamethylenebiguanide (PHMB) (polyhexadine or polyaminopropylbiguanide; alexidine)	• PHMB interacts with membrane phospholipids, affecting the membrane fluidity and conformation that leads to ionic leakage and cell death.	• PHMB exhibits amoebicidal and cysticidal properties at 200 μg per ml (0.02%), while alexidine shows antiacanthamoebic properties at 200 μg per ml but is less toxic *in vivo*.
3	Antibacterials (polymyxin B; cefazolin; meropenem)	• Several antibacterials have been tested, including polymyxin B (binds to negatively charged membranes and disrupts the membrane integrity), cefazolin (binds to penicillin binding proteins present in the cell wall, thus inhibiting cell wall synthesis), and meropenem (inhibits penicillin binding proteins).	• Limited antiamoebic effects are observed.
4	Azole compounds (miconazole nitrate; ketoconazole; clotrimazole; itraconazole; fluconazole; voriconazole)	• Azole compounds target 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol, inhibiting phospholipid and triglyceride synthesis and affecting oxidative and peroxidative enzyme activities that cause deterioration subcellular components, leading to cell necrosis. • In addition to ergosterol biosynthesis, clotrimazole is known to inhibit endogenous respiration by impairing triglyceride and phospholipid biosynthesis as well as to inhibit cellular calcium homeostasis and calcium ATPases. Itraconazole inhibits ergosterol biosynthesis and has been shown to inhibit endogenous respiration, interact with membrane phospholipids, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Fluconazole inhibits ergosterol biosynthesis and is known to inhibit endogenous respiration, interact with membrane phospholipids, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.	• The majority of azole compounds can show amoebicidal effects at as low as 10 μg per ml *in vitro*, but cysticidal properties and host cell toxicity are observed at a far higher concn of ~1,000 μg per ml. • For example, 200 mg ketoconazole twice daily is prescribed in *Acanthamoeba* keratitis cases, and the recommended concn in GAE[a] is 5 mg per kg, body wt. • In keratitis cases, clotrimazole is recommended at 10 mg per ml.
5	Amphotericin B/natamycin	• Amphotericin B, a polyene, binds irreversibly to ergosterol, resulting in disruption of membrane integrity and ultimately in cell death. • Natamycin binds to ergosterol in the plasma membrane, preventing ergosterol-dependent fusion of vacuoles, as well as membrane fusion and fission.	• Amphotericin B has been shown to possess antiacanthamoebic properties at 100 μg per ml, while natamycin exhibits effects at far higher concn (10–50 mg per ml).

6	Caspofungin	• Caspofungin inhibits beta-(1,3)-glucan synthase, inhibiting the synthesis of beta-(1,3)-D-glucan.	• Caspofungin shows amoebicidal effects at 250 μg per ml, and cysticidal properties are observed at 500 μg per ml.
7	Mannose-linked or anti-MBP antibody-linked photoactivated agents	• Mannose-linked or anti-MBP antibody-linked photoactivated agents can selectively bind to *Acanthamoeba*, and exposure to light of the appropriate wavelength produces reactive oxygen species, targeting the parasite.	• Amoebicidal properties were observed at <50 μM, while excystation was abolished at this concn.
Intracellular targeting agents			
8	Quaternary ammonium compounds and their derivatives (cetyltrimethylammonium bromide; cetylpyridiniumbromide) (insertion of the ethyl phosphate group into the molecule of cetyltrimethylammonium bromide leads to the formation of alkylphosphocholines [hexadecylphosphocholine/miltefosine]).	• These quaternary ammonium compounds and their derivatives inactivate energy-producing enzymes, denature essential cell proteins, and disrupt the cell membrane.	• These quaternary ammonium compounds exhibit antiacanthamoebic activity (at <20 μM) compared with alkylphosphocholines compounds, which show activity at >60 μM.
9	Quaternary ammonium compounds (benzethonium chloride)	• These quaternary ammonium compounds induce apoptosis by activating caspases, inducing loss of mitochondrial membrane potential, and increasing cytosolic calcium concn, leading to cell death.	• These quaternary ammonium compounds show antiacanthamoebic effects at >500 μM.
10	Calcium modulating agents (amlodipine; loperamide; amiodarone; trifluoperazine dihydrochloride; chlorpromazine dihydrochloride).	• Amlodipine inhibits the transmembrane influx of calcium ions, loperamide inhibits calcium channel activity and calmodulin binding, amiodarone shows calcium blocker-like activity, and trifluoperazine dihydrochloride and chlorpromazine dihydrochloride inhibit calmodulin.	• These agents showed antiacanthamoebic effects at >500 μg per ml *in vitro*. • Chlorpromazine and trifluoperazine showed amoebicidal and cysticidal effects at concn in the micromolar range.
Nucleic acid-acting drugs			
11	Proflavine hemisulfate	• Proflavine hemisulfate is known to exhibit mutagenic effects on DNA by intercalating between nucleic acid base pairs and causes base pair deletions and insertions.	• Proflavine hemisulfate is reported to exhibit amoebicidal effects at 100 μg per ml and cysticidal effects at 1,000 μg per ml.
12	Hydroxystilbamidine isethionate	• Hydroxystilbamidine isethionate binds extensively and selectively to kinetoplastic DNA, inhibiting cell division and reproduction. It has also been shown to bind to RNA and is a significant inhibitor of ribonucleases as well as being taken up in lysosomes and is associated with a significant increase in the no. of lysosome-like bodies and secretion granules.	• Hydroxystilbamidine isethionate exhibits amoebicidal properties at 100 μg per ml and cysticidal properties at 1,000 μg per ml.
13	Trimethoprim	• Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid, which is important in the thymidine synthesis pathway for DNA synthesis.	• Trimethoprim has been shown to exhibit amoebicidal effects at 100 μg per ml.
14	5-Fluorocytosine and moxifloxacin	• 5-Fluorocytosine is a competitive inhibitor of purine and pyrimidine uptake. • Moxifloxacin is an inhibitor of DNA gyrase, a type II topoisomerase, and topoisomerase IV, required for DNA replication.	• Limited antiamoebic effects are observed.

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TABLE 1 (Continued)

Classification and agent no.	Drug(s)	Mode of action	Description and effects on *Acanthamoeba*
		• Moxifloxacin is an inhibitor of DNA gyrase, a type II topoisomerase, and topoisomerase IV, required for DNA replication.	
15	Pyrimethamine-sulformethoxine combination and trimethoprim-sulfamethoxazole	• Pyrimethamine is dihydrofolate reductase inhibitor to block biosynthesis of purines and pyrimidines), sulformethoxine targets dihydropteroate synthase and dihydrofolate reductase and competes with para-aminobenzoic acid for incorporation into folic acid.	• Both combinations showed amoebicidal effects at 100 μg per ml.
16	Pentamidine isethionate/propamidine isethionate	• Pentamidine isethionate and propamidine isethionate inhibit synthesis of DNA, RNA, phospholipids, and proteins.	• Pentamidine isethionate and propamidine isethionate show amoebicidal and cysticidal properties at ~100–200 μg per ml, while propamidine isethionate is used clinically against keratitis at a concn of 1 mg per ml.
17	Diminazene aceturate	• Diminazene aceturate binds to the groove between the complementary strands of DNA at regular intervals and thus distorts the helical structure. It is also known to affect synthesis of phospholipids and also interferes with the glycolytic pathway.	• Diminazene aceturate has been shown to exhibit amoebicidal and cysticidal properties at ~100–200 μg per ml.
18	Clinically useful protein synthesis inhibitors (including paromomycin sulfate, tobramycin, and neomycin sulfate)	• Paromomycin sulfate inhibits initiation and elongation steps of protein synthesis. • Tobramycin inhibits protein synthesis by binding to ribosomes and preventing mRNA translation, leading to cell death, while neomycin sulfate binds to four nucleotides of 16S rRNA and a single amino acid of protein S12 and interferes with the initiation complex, leading to misreading of mRNA such that incorrect amino acids are inserted into the polypeptide, leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes.	• Paromomycin sulfate has been shown to exhibit amoebicidal and cysticidal properties at >100 μg per ml, while the other agents exhibit anti-amoebic effects at >250 μg per ml, but cysticidal properties are observed at >500 μg per ml.
19	Prednisolone, beta-methasone phosphate, linezolid, co-trimoxazole.	• Prednisolone irreversibly binds with glucocorticoid receptors, inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthase, beta-methasone phosphate binds to plasma transcortin and becomes active when it is not bound to transcortin, linezolid inhibits the formation of subunits of ribosome, and co-trimoxazole is known to inhibit folic acid synthesis.	• These protein synthesis inhibitors show amoebicidal properties but have limited cysticidal effects and are of limited value as antiacanthamoebic agents.

[a] GAE, granulomatous amebic encephalitis.

effects and are not ideal for the treatment of ocular or brain infections, albeit they can be used in combination with chlorhexidine (10–13, 22–36). More recently, alexidine, an amphipathic bisbiguanide, has shown amoebicidal activity at 10 μg per ml and cysticidal activity at 100 μg per ml (37). The cytotoxic activities of alexidine are comparable to those of chlorhexidine; however, alexidine appeared less toxic *in vivo* (37). Several antibacterials have been tested in *Acanthamoeba* infection, including polymyxin B (binds to negatively charged membranes and disrupts membrane integrity) (18, 38–40), cefazolin (18), and meropenem (inhibits penicillin binding proteins) (41), but limited antiamoebic effects were observed at physiologically tolerable concentrations.

Despite similarities to the host's cell plasma membrane, ergosterol, mannose-binding protein (MBP), and laminin-binding protein have been identified as useful components for the targeted killing of amoebae. As the presence of ergosterol is limited to fungi and protists, while human cells contain cholesterol, this is considered a useful target, but anti-ergosterol biosynthesis strategies have shown limited value against *Acanthamoeba* infections. For example, several azole compounds, including miconazole nitrate, ketoconazole, clotrimazole, fluconazole, and voriconazole, have been tested that target 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol, inhibiting phospholipid and triglyceride synthesis and affecting oxidative and peroxidative enzyme activities, resulting in deterioration of subcellular components and leading to cell necrosis. Although several azole compounds showed amoebicidal effects at a concentration as low as 10 μg per ml *in vitro*, they showed cysticidal properties at a far higher concentration of 1,000 μg per ml (19, 20, 27, 31, 33, 40, 42–61). Among the ergosterol inhibitors, amphotericin B has been tested and has been shown to bind to ergosterol, forming a transmembrane channel that leads to monovalent ion leakage, which is the primary effect leading to cell death. The effective concentration against *Acanthamoeba* is reported to be 100 μg per ml (39, 40, 55, 59–63). Additionally, natamycin, which targets ergosterol in the plasma membrane, preventing ergosterol-dependent fusion of vacuoles as well as membrane fusion and fission, has shown limited amoebicidal properties at physiologically relevant concentrations (14, 27, 43, 64). In part, this is due to the fact that the present compounds targeting ergosterol or its synthetic pathway lack specificity and produce inconsistent results against various strains/species of *Acanthamoeba* and may also target the host cell P450 enzymes, resulting in side effects (60–65). Additionally, the effects of these compounds are often amoebistatic, rather than amoebicidal (65); hence, prolonged clinical application is needed, which could result in the emergence of drug-resistant strains, as seen in yeast, where azole resistance emerging through increased function of efflux mechanisms or through changes in the azole targets, e.g., C14 demethylase, or through changes in the biosynthetic steps of ergosterol synthesis has been observed. Similar mechanisms may explain variations in antimicrobial sensitivity among various isolates of *Acanthamoeba* (66). Overall, the ergosterol biosynthesis pathway is a potential target in the rational development of targeted therapeutic interventions against *Acanthamoeba*, as long as specificity is achieved to optimize the antiparasitic effects.

AQ: C

Within the plasma membrane, mannose-binding protein (MBP) has been identified as a key adhesin in *Acanthamoeba*-mediated host cell binding and cytotoxicity. The MBP protein consists of a signal peptide (amino acids 1 to 21), an extracellular cysteine (C)-rich region covering amino acid positions 274 to 615, and a single predicted transmembrane region (amino acids 733 to 755). Expression of MBP is linked with the pathogenicity of *Acanthamoeba* and is associated with binding to and cytotoxicity of host cells. Notably, immunization with recombinant MBP (rMBP) protects animals against subsequent challenge with pathogenic *Acanthamoeba* species (5). Although rMBP is not applicable as a vaccine tool given the rarity of the disease, these findings have highlighted MBP as an important chemotherapeutic target. For example, recent studies showed that MBP-targeted chemotherapy can effectively eradicate amoebae *in vitro* and protect host cells against amoeba-mediated damage (66). Although studies are needed to prove the value of this approach *in vivo*, it has been suggested that mannose-conjugated porphyrin may have an application for targeted photodynamic chemotherapy against *Acanthamoeba* infections and should be explored for potential clinical applications in future investigations (66). Other drugs targeting the membrane include caspofungin, which is known to inhibit the synthesis of beta-(1,3)-D-glucan (59, 67). Caspofungin shows amoebicidal properties *in vitro* at 250 μg per ml and is cysticidal at 500 μg per ml (59, 67) and is thus of limited value in clinical applications.

INTRACELLULAR TARGETING AGENTS

Calcium channels play a critical role in the viability of *Acanthamoeba*. For example, the viability of trophozoites depends on their amoeboid movement in search and uptake of food particles, encystation or excystation, and asexual reproduction. These processes involve myosin contractility, activation of actin filament, inhibition of actin cross-linking by alpha-actinin, or binding to calmodulin. Other low-molecular-weight calcium-binding proteins and calpain, actophorin, actobindin, calcium-sensitive actin gelation protein, actin bundling protein (AhABP), and calcium-dependent extracellular proteases play important roles in its physiology. Thus, drugs affecting these functions would have deleterious effects on the viability of *Acanthamoeba*. Notably, calcium antagonists such as amlodipine (inhibits the transmembrane influx of calcium ions), loperamide (inhibits calcium channel activity and calmodulin binding), amiodarone (calcium blocker-like activity), and trifluoperazine dihydrochloride and chlorpromazine dihydrochloride (inhibit calmodulin) exhibit amoebicidal effects *in vitro* (68). Although the majority of drugs are used clinically, they exhibit antiamoebic effects at a relatively high concentration of 500 μg per ml. However, two neuroleptic agents, chlorpromazine and trifluoperazine, show amoebicidal and cysticidal effects in the micromolar range of concentrations *in vitro* (69). Furthermore, the combination of chlorpromazine with rokitamycin or amphotericin B enhances protection of host cells against the parasite (69), suggesting the need for future studies to test the clinical relevance of these drugs against *Acanthamoeba* infections in experimental models as well as in patients.

Quaternary ammonium compounds, including cetyltrimethylammonium bromide and cetylpyridinium bromide, have been tested for antiacanthamoebic properties (54). Their effects are known to result in inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane. Insertion of the ethyl phosphate group into the molecule of cetyltrimethylammonium bromide leads to the formation of alkylphosphocholines (hexadecylphosphocholine/miltefosine). *In vitro* studies showed that quaternary ammonium compounds ex-

hibit higher antiacanthamoebic properties (at concentrations of <20 μM) than hexadecylphosphocholine/miltefosine (>60 μM) (54) and are promising agents. Another quaternary ammonium compound, benzethonium chloride (mode of action, induction of apoptosis) shows amoebicidal effects at >500 μg per ml (54) and is of limited utility. More recently, prochlorperazine (a known antagonist of dopamine [D2] receptor, muscarinic receptor, and histamine antagonist) and corticosteroids were shown to exhibit amoebicidal effects at 250 μg per ml *in vitro*, but determinations of their usefulness require further studies (47, 64, 68, 70).

NUCLEIC ACID-ACTING DRUGS

Nucleic acid inhibitors inhibit DNA/RNA synthesis, prevent DNA from functioning as a template, affect the function of polymerases involved in the replication and transcription of DNA, or intercalate into the DNA. The antibacterial properties of nucleic inhibitors are well known, making this pathway a useful target, but the lack of specificity against eukaryotic *Acanthamoeba*, together with the high concentrations required to target cysts and the observation that the majority of nucleic acid inhibitors are toxic or carcinogenic, suggests that, with the exception of few compounds, nucleic acid inhibitors are clinically inappropriate or not ideal candidates as antiacanthamoebic compounds. For example, proflavine hemisulfate exhibits mutagenic effects on DNA by intercalating between nucleic acid base pairs and causes base pair deletions and insertions. It has been reported to exhibit amoebicidal effects at 100 μg per ml and cysticidal effects at 1,000 μg per ml (40). The mode of action of hydroxystilbamidine isethionate involves binding extensively and selectively to kinetoplastic DNA, inhibiting cell division and reproduction. It has also been shown to bind to RNA and is a significant inhibitor of ribonucleases, and it is taken up in lysosomes, leading to a significant increase in the number of lysosome-like bodies and secretion granules. It exhibits amoebicidal properties at 100 μg per ml and cysticidal properties at 1,000 μg per ml (40). Other compounds tested include trimethoprim, which binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid, which is important in the thymidine synthesis pathway for DNA synthesis. It has been shown to exhibit amoebicidal effects at 100 μg per ml (39), while 5-fluorocytosine (mode of action, competitive inhibition of purine and pyrimidine uptake) and moxifloxacin (inhibitor of DNA gyrase, a type II topoisomerase, and topoisomerase IV, required for DNA replication), have shown limited value in the treatment of granulomatous amoebic encephalitis (41, 63). Given the nonselective nature of these compounds and their associated toxicity, several studies have tested combinations of nucleic acid synthesis inhibitors against *Acanthamoeba*. When pyrimethamine (a dihydrofolate reductase inhibitor blocking biosynthesis of purines and pyrimidines) and sulformethoxine (targeting dihydropteroate synthase and dihydrofolate reductase and competing with para-aminobenzoic acid for incorporation into folic acid) were tested in combination, amoebicidal properties were observed at 100 μg per ml (39). Similarly, trimethoprim plus pyrimethamine and trimethoprim plus sulfamethoxazole (inhibitor of folic acid synthesis) showed amoebicidal effects at 100 μg per ml (39, 50, 51, 71, 72).

Among the effective compounds tested, pentamidine isethionate inhibited synthesis of DNA, RNA, phospholipids, and proteins, with amoebicidal and cysticidal properties seen at ~100 to 200 μg per ml with variable results (11, 19, 73), while propamidine isethionate (DNA synthesis inhibitor) is used clinically against keratitis at a concentration of up to 1 mg per ml (13, 16, 17, 23, 27, 32, 33, 35, 36, 38, 42, 46, 53, 70, 74–79). Other drugs tested included diminazene aceturate, which binds to the groove between the complementary strands of DNA at regular intervals and thus distorts the helical structure. It is also known to affect phospholipids synthesis and also interferes with the glycolytic pathway of the parasite. It has been shown to exhibit amoebicidal and cysticidal properties at ~100 to 200 μg per ml (11). For treatment of brain infections, rifampin is promising as an additive drug, as it is lipophilic, a property that makes it a good candidate for treatment of infections of the central nervous system, which requires distribution to the central nervous system by penetration through the blood-brain barrier. The mode of action is inhibition of DNA-dependent RNA polymerase by binding to its beta-subunit, thus preventing transcription of RNA and subsequent translation to proteins. It has been shown to exhibit amoebicidal properties but is of limited value in treatments (50, 51, 64, 71).

PROTEIN SYNTHESIS-INHIBITING DRUGS

Inhibition of protein synthesis has been one of the key targets for many of the available antibiotics, mostly taking advantage of differences in prokaryotic and eukaryotic ribosome structures and functions. The majority of protein synthesis inhibitors block mRNA translation into proteins, e.g., initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination. As for other pathways and structures of eukaryotes, in homology with host mammalian cells, selective targeting of protein synthesis remains a challenge and the use of protein synthesis inhibitors is often associated with host cell toxicity. Given that amoebae are actively growing in their infective states, such compounds can be used to block reproduction with tolerable toxicities. For example, paromomycin sulfate (inhibitor of the initiation and elongation steps of protein synthesis) has been shown to exhibit amoebistatic, amoebicidal, and cysticidal properties at more than 100 μg per ml (33, 40, 54). Tobramycin (inhibitor of protein synthesis by binding to ribosomes and preventing mRNA translation, leading to cell death) has shown amoebicidal properties at more than 250 μg per ml (25, 80).

Similarly, neomycin sulfate, which binds to four nucleotides of 16S rRNA and a single amino acid of protein S12 and interferes with the initiation complex, leading to misreading of mRNA such that incorrect amino acids are inserted into the polypeptide, resulting in nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes, has been shown to exhibit antiamoebic effects at 250 μg per ml, but cysticidal properties are observed at >500 μg per ml (30, 32, 35, 38, 40, 70, 74). Among the drug combinations tested, neomycin plus polymyxin B (33, 42, 45, 46, 52) and neomycin sulfate plus polymyxin B sulfate plus gramicidin (cation detergent) exhibited amoebicidal properties (42, 45, 53). In contrast, the combination of neomycin plus polymyxin B plus bacitracin exhibited amoebistatic and amoebicidal as well as cysticidal properties (18, 40, 51, 65, 76).

Several other protein synthesis inhibitors tested show amoebicidal properties but have limited cysticidal effects. These include prednisolone (irreversibly binds with glucocorticoid receptors, inhibiting gene transcription for cytochrome oxidase 2 [COX-2], cytokines, cell adhesion molecules, and inducible NO synthase)

(52), beta-methasone phosphate (binds to plasma transcortin and becomes active when it is not bound to transcortin) (81), and linezolid (inhibits the formation of subunits of ribosome) (39, 41, 50, 51, 71, 72).

ENZYME-ACTING AGENTS

As described above, quaternary ammonium compounds (such as cetyltrimethylammonium bromide and cetylpyridinium bromide) and alkylphosphocholines (such as miltefosine) are promising candidates against *Acanthamoeba* infections. The mode of action of miltefosine is induction of apoptosis-like cell death by acting as an inhibitor of proteinase kinase B. It has been shown to exhibit amoebicidal properties (61, 82–84). Notably, miltefosine (at 65.12 μg per ml) was tested in combination with polyhexamethylene biguanide, chlorhexidine, and propamidine isethionate in a rat model for the topical treatment of *Acanthamoeba* keratitis (83). The results revealed that the miltefosine-polyhexamethylene biguanide combination gave the best treatment results, and approximately 86% of the eyes were cleared of amoebae. It is also recommended as part of the treatment regimen against human brain infection due to *Acanthamoeba* (85). Future studies of the combination and effective delivery of quaternary ammonium compounds and their derivatives to the target site will determine the clinical usefulness. Other drugs tested showed amoebicidal effects but limited cysticidal effects. These include sulfadiazine (inhibitor of dihydropteroate synthetase) (86, 87), flurbiprofen (nonselective cytochrome oxidase [COX] inhibitor of pathway responsible for the conversion of arachidonic acid into prostaglandin G2 into prostaglandin H2) (77), riboflavin (targeting riboflavin hydrogenase, riboflavin kinase, and riboflavin synthase) (88), diclofenac (inhibitor of prostaglandin synthesis by inhibiting COX) (13), albendazole (targeting the colchicine-sensitive site of tubulin, inhibiting its polymerization into microtubules and leading to impaired uptake of glucose and depletion of glycogen stores) (89), and digoxin, which binds to the sodium/potassium-transporting ATPase alpha-1 chain (68).

Given the rarity of the disease and availability of a number of compounds with various effects against *Acanthamoeba* trophozoites and cysts, there is a need to test various combinations to prove their clinical usefulness with tolerable toxicities and acceptable pharmacokinetics profiles, safety margins, etc. In the absence of targeted therapy, this would provide the logical avenues for further research in clinical practice that may provide strategies for chemotherapy against this difficult-to-treat infection.

THE WAY FORWARD

The search for safe and effective antiacanthamoebic drugs remains a challenge. Research over the past few decades has identified a large number of compounds that have therapeutic potential, but their translational value has not been explored. As discussed above, independent laboratories have done the groundwork in identifying several molecular targets and have identified several drugs of potential therapeutic value and used lead compounds for synthesis of derivatives; however, that work did not gain the attention of the major pharmaceutical companies, whose participation is needed to carry out the expensive *in vivo* studies as well as the clinical trials. Although the lack of interest of the pharmaceutical industry in finding cures for parasitic infections is well known, it is worth noting that eye infection due to the *Acanthamoeba* parasite occurs in contact lens wearers. The number of contact lens wearers was estimated at 125 million in 2004, worldwide, with approximately 35 million contact lens wearers in the United States alone (90). The contact lens market was estimated at $6.1 billion in 2010, and it was estimated that the global market would reach $11.7 billion by 2015 (91). For a multi-billion-dollar industry, it is puzzling that pharmaceutical companies are not investing in this research, especially as novel molecules/inhibitors/drugs and their clinical applications can be patented, which offers tremendous commercial value. Notably, several companies have agreed to pay billions of dollars to settle lawsuits and have also withdrawn contact lenses/disinfectants from the market for being ineffective against *Acanthamoeba* or *Fusarium*. This makes no financial sense. It is far more economical to develop effective contact lens disinfectants against *Acanthamoeba*. Although the ability of amoebae to switch phenotypes into a dormant cyst form is a major hindrance in the development of effective contact lens disinfectants and/or chemotherapeutic approaches, recent studies have shown that the addition of cellulase enzyme to disrupt cyst wall structure renders amoeba cysts susceptible to the effects of antiamoebic drugs (92). The combination of antiamoebic agent and cellulase enzyme was shown to abolish the viability of both cysts and trophozoites. Notably, none of the agents, when tested alone, completely destroyed cysts and trophozoites, suggesting that the use of cellulose-degrading molecules is a useful avenue for targeted killing of amoebae. As cellulose synthesis is absent in mammalian cells, the use of cellulose-degrading molecules in contact lens disinfectants as well as in drug formulations in the treatment of *Acanthamoeba* infection needs to be explored.

It is hoped that the recent completion of the *Acanthamoeba* genome (93) will expedite identification of novel drug targets further, through genomics, proteomics, and bioinformatics. Among the existing drugs/disinfectants, given that they are limited in efficacy, there is a need to find ways to enhance their efficacy. The constituents of contact lens disinfectants must be carefully selected to target the cyst stage of amoebae. For example, the recall of Complete MoisturePlus contact lens disinfectant (AMO, Santa Ana, CA) following an outbreak of *Acanthamoeba* keratitis revealed that one of the constituents of the solution, propylene glycol, induced encystation in *Acanthamoeba*, resulting in the formation of cysts, which are resistant to the majority of contact lens disinfectants. Similarly, treatment is problematic due to specificity and parasite dormancy. The use of a carrier for antiacanthamoebic drug delivery is an important avenue that could yield promising results without affecting host cell viability. For eye infections, mannose- or antibody- or Fab-conjugated drugs should allow specific targeting of drugs to *Acanthamoeba*. Notably, recent studies showed that conjugation of mannose with photodynamic compounds allows specific targeting of *Acanthamoeba* and enhances their antiacanthamoebic effects (66). These findings suggested that specific antibodies or antiacanthamoebic agents, coupled with selective cytotoxic agents, could be useful in the treatment of *Acanthamoeba* infections, as they can be minimally invasive and minimally toxic to the host cells. Alternatively, parasite-specific pathways, such as ergosterol biosynthesis or cellulose biosynthesis, and the underlying enzymes that are required for the makeup of these molecules offer important targets for the rational development of therapeutic interventions.

Additionally, liposome-complexed antiamoebic drugs have shown promising *in vitro* results in enhanced killing of pathogenic *Acanthamoeba* compared with the use of the drugs alone (94).

Moreover, liposomal ergosterol-pentamidine proved effective in preventing parasite-mediated host cell cytotoxicity *in vitro* (94), suggesting that ergosterol-formulated liposomes hold promise in the targeted delivery of drugs. The pace of research in identifying and characterizing novel targets has yielded promising results; however, the translational value for therapeutic interventions requires further investigation. The recent research shift to phenotypic screening against the whole parasite, as well as to repurposing of drugs, i.e., screening of FDA-approved drugs to identify those with antiacanthamoebic activity (68), is auspicious and has the potential to open several avenues for further research. Once active compounds are identified, the approval process can be expedited, as the drugs are already being used for clinical applications against other diseases. Moreover, several animal-based, plant-based, and microbe-based molecules have been identified that show antiacanthamoebic effects. Some of the aforementioned components represent appealing therapeutic targets that need to be exploited in future studies. With the availability of relevant disease models and of assays for target validation, there is an urgent need to develop translational research by encouraging academia-industry partnerships, which offer tremendous opportunities of commercial and scientific value.

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