European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied
Website: https://farmclin.es/

Original Research Article

Novel Drug Delivery and Combination Approaches in the Pharmacological Management of Naegleria fowleri Infections

Article History:

Name of Author:
Rajarajeshwari[1], Nithya Varadarajan[2*], Surendhar[3], Preethi[4], Jasmine Charlotte[5] and Jeeva[6]

Corresponding Author:
Dr Nithya Varadarajan

How to cite this article:
Rajarajeshwari, *et, al.* Novel Drug Delivery and Combination Approaches in the Pharmacological Management of Naegleria fowleri Infections. *Eur J Clin Pharm.* 2025;7(1). 3954-3964.

Received: 12-10-2025
Revised: 05-11-2025
Accepted: 20-11-2025
Published: 30-12-2025

Abstract: Naegleria fowleri, also referred to as the brain-eating amoeba, causes a disease called primary amoebic meningoencephalitis (PAM) which is a serious, acute fulminant, and normally lethal central nervous system infection. This is primarily caused by late diagnosis, fast rate of illness occurrence, poor penetration of the blood-brain barrier (BBB) of the available drugs and in place of standardised treatment methods. Combination of amphotericin B, miltefosine, azoles, rifampicin and corticosteroids have been included in the current pharmacological therapy but these regimens are typically characterized by low CNS bioavailability and inconsistent efficacy. The most recent developments indicate that the nanotechnology-based pharmaceutical delivery approaches including liposomes, polymeric nanoparticles, solid lipid nanoparticles, and intranasal nano formulations have the potential of enhancing brain targeting and reducing toxicity. Recycled ant iparasitism / antibacterial agents, immunomodulatory supplements and multi-drug combinations are proving to be effective in improving survival rates. At the same time, there is increased discovery of new therapeutic targets and amoebic molecules that is accelerating with the help of computational drug discovery tools like virtual screening, molecular docking, and QSAR modelling. To enhance the management of PAM, this review identifies the perspectives in the future that include nanotechnology, combination therapy, and precise medication design, explains the existing challenges in pharmacology, and critically discusses the emerging treatment options.

Keywords: Naegleria fowleri, primary amoebic meningoencephalitis (PAM), multi-drug combinations, nanotechnology, precise medication.

INTRODUCTION

Primary amoebic meningoencephalitis (PAM) is a fast growing and frequently fatal, central nervous system infection caused by *Naegleria fowleri*, a thermophilic free-living amoeba. The infection takes place, as a rule, when infected warm freshwater gets into the nasal passages, and the amoeba spreads to the brain through the olfactory nerve. after CNS infection, the rate of PAM is rapid, its course is fatal to a person in 95-100% of cases, the amoeba dies 5-14 days after the start of symptoms.(1)

The current pharmacological medicines are scarce and weak. Amphotericin B is still the back bone of therapy, and is often given in large doses, and sometimes intrathecally, in a hope to reach the central nervous system. Nonetheless, amphotericin B does not have a high blood-brain barrier (BBB) penetration rate but is not depicted by low toxicity. Other additional drugs that are used in combination regimens include miltefosine, rifampicin, and azoles (including miconazole and fluconazole). The strategies have not had a significant impact historically; the success seems to be largely dependent on the diagnosis and treatment that is to be administered very early.(2)

Due to high mortality rate, velocity of the development of the disease, and difficulty in providing drugs to the central nervous system, new therapeutic methods are in an extreme need. There are two interesting areas of interest, including the new drug

delivery systems (including nano formulations or carrier conjugates) and the combination therapy (including the existing drugs alongside new agents, including natural products or inhibitors targeting certain amoebic pathways). New delivery systems might enhance BBB penetration, systemic toxicity, bioavailability, and long-term brain pharmaceutical concentrations. In the same way, the combination of treatment can enhance amoebicidal activity through synergistic effect and reduce the dose needed and unwanted adverse effects.(3)The recent research has yielded positive outcomes. Conjugates using amphotericin B or curcumin nanocarriers, such as those, have been proven to be more effective in killing N. fowleri trophozoites than the free medicines and also reduced host-cell cytotoxicity.(3)The in vitro activity of naphthyridine-chromenone hybrids has been demonstrated in a large number of N. fowleri strains by natural compounds that can cause programmed cell death, mitochondrial dysfunction, reactive oxygen species (ROS) generation, and membrane damage.(4)

Moreover, other molecular targets are obtained by studying the inhibitors that are expressed during cyst formation (including cysteine protease inhibitors) and possible inhibitors of parasite-host interaction (including extracellular matrix models).(5) Nevertheless, there are still severe impediments. There is a difficulty in early diagnosis of PAM; delays in diagnostics are common. It is difficult to translate good in vitro or animal-model results to human scenarios because of the infrequence of survivorship, ethical, and logistical limitations, and differences in CNS penetration of therapeutic agents. Consequently, the comprehensive evaluation of innovative delivery platforms, combination regimens optimisation (in time, dose, and route), and the combination of pharmacokinetics, toxicity, and target engagement should be adopted. In this paper, the recent advances in drug delivery technologies (e.g., nanocarriers and conjugates), combination therapy strategy, the mechanisms of action, and experimental data of such strategies in the case of N. fowleri infection will be discussed. The objective is to assimilate what is known, determine gaps, and suggest new directions that will result in more efficient therapy interventions that would diminish the horrific rate of mortality in PAM.(6)

In this paper, I will look at the existing developments in drug delivery systems (e.g. nanocarriers, conjugates), recent combination therapy plans, mechanisms of action, as well as experimental data concerning such plans in N. fowleri infection. The goal is to integrate existing knowledge, highlight gaps and suggest opportunities on how to develop more effective therapeutic designs that could have a lower devastating mortality of PAM.

1.1 Overview of PAM Pathogenesis and Clinical Features

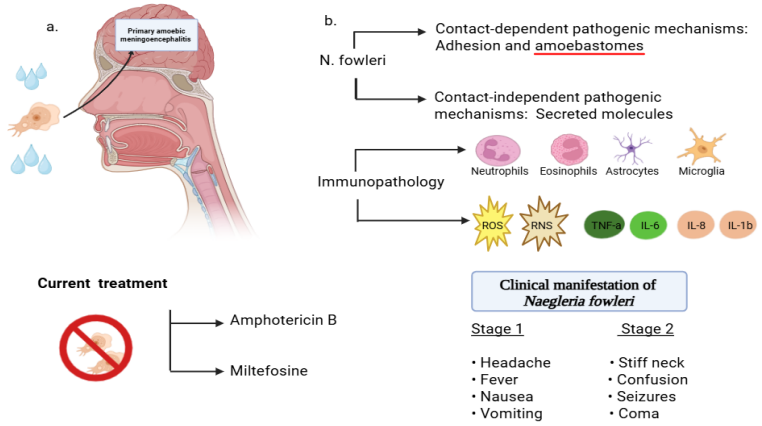

Figure 1. pathogenesis, current treatment, clinical manifestation of Naegleria fowleri infection.

a. The figure outlines how Naegleria fowleri enters through the nasal passages to cause primary amoebic meningoencephalitis (PAM). It illustrates both contact-dependent mechanisms (adhesion, amoebastome-mediated damage) and contact-independent mechanisms via secreted cytotoxic molecules.

b.The host immune response involving neutrophils, eosinophils, astrocytes, and microglia generates ROS/RNS and inflammatory cytokines (TNF-α, IL-6, IL-8, IL-1β), driving neuropathology. Clinical progression from early symptoms (headache, fever, nausea) to severe neurological signs (stiff neck, seizures, coma) is highlighted, along with current treatments—amphotericin B and miltefosine

Current Pharmacological Strategies

2.1 Overview of Standard-of-Care Drugs

Naegleria fowleri is the causative agent of primary amoebic meningoencephalitis (PAM), which is among the most severe infections of the central nervous system (CNS), and its survival rate is less than 5 percent despite the extensive treatment (7). The management plan is largely based on multidrug regimens that are used to facilitate amoebicidal activity and enhance the availability of CNS drugs. Amphetamine is used as the standard therapy in cerebral oedema and can be combined with supportive therapy, including dexamethasone and induced hypothermia.(8)

2.1.1 Amphotericin B

Amphotericin B (AmB) has been the treatment of choice of PAM since the first surviving patient was treated with it in 1978. It is a high amoebicidal polyene macrolide antifungal. Due to the low blood-brain barrier (BBB) permeability, AmB is normally administered intravenously and intrathecally to generate therapeutic levels in the cerebrospinal fluid (CSF).(9) AmB is not easily tolerated; although effective, it has a narrow therapeutic index and causes dose-limiting nephrotoxicity and electrolyte abnormalities and requires close surveillance. Lipid preparations (liposomal AmB and AmB lipid complex) have been developed to reduce systemic toxicity and increase brain tissue penetration, and have greater in vitro efficacy against N. fowleri trophozoites. (10)

Amphotericin B interacts with the ergosterol-like sterols on the N. fowleri plasma membrane, which results in the formation of ion-permeable pores, cellular leakage, osmotic imbalance, and cell death. It was shown by recent studies that AmB triggers apoptosis-like PCD pathways in amoebae, including mitochondrial depolarisation, ROS formation, DNA fragmentation and chromatin condensation.(10)

2.1.2 Miltefosine

Miltefosine, a phosphocholine alkyl phosphate, has been investigated as a broad-spectrum antiparasitic agent that has proved to be active in vitro against N. fowleri. It is among those few drugs that are effective against the trophozoite and cyst stage of free-living amoebas.(11) Amphotericin B, fluconazole, azithromycin, rifampicin, and miltefosine are recommended to the first survivor in the United States (12). The oral bioavailability of miltefosine, along with a moderate CNS penetration, potential to induce apoptosis-like cell death pathways, provides miltefosine with an advantage compared to conventional medicine. Nevertheless, it is not without gastrointestinal side effects and teratogenicity.(11)

The action of miltefosine is multifactorial and it acts on both membrane lipid metabolism and on cellular signaling. It interferes with the production of phosphatidylcholine and membrane integrity and causes mitochondrial depolarisation and apoptotic death.(11) Miltefosine is also able to regulate cell-surface receptors and activate stress-related kinases, stimulating the accumulation of ROS and caspase-like enzymes in the N. fowleri trophozoites. It has amphiphilic structure, which is advantageous to interact with amoebic membranes to increase uptake and intracellular delivery.

2.1.3 Azoles

Other significant agents of treatment are azole antifungals that include fluconazole, voriconazole, ketoconazole and miconazole. These triazoles and imidazoles restrict the growth of N. fowleri by attacking lanosterol 14a-demethylase (CYP51), a P450-dependent enzyme used by the amoebic plasma membrane to make sterols.(13) The disruption of membrane sterols does not only change the permeability but also interferes with the transport of nutrients and ultimately leads to cell death. Synergistic effects are sometimes achieved with amphotericin B used together with azoles. Amphotericin attaches to the sterols that are perturbed by azoles, and it increases membrane damage. Voriconazole and fluconazole should be used in CNS infections due to their better penetration of the BBB and reduced toxicity.(9)

Azoles block the ergosterol synthesis pathway by reducing the activity of CYP51 and reducing the process of demethylating lanosterol to ergosterol. The effect of sterol deficiency is the instability of membranes, high permeability, and lack of localisation of enzymes.(9) Moreover, a number of azoles block the production of ATP and mitochondrial activity. In vitro studies show that voriconazole and fluconazole inhibit the growth of the trophozoites at micromolar concentrations and that their combination with amphotericin B and miltefosine results in an additive or synergistic effect.(8)

2.1.4 Rifampicin

Rifampicin is an antibiotic of the rifamycin-class due to its potency to enter the BBB and block DNA-dependent RNA polymerase, which is capable of stopping transcription in bacteria and amoeba. (8)The amoebicidal effect of rifampicin is restricted in vitro, whereas combined therapy enhances the overall treatment effect through the alteration of metabolic processes and membrane transporters. It is also synergistic with amphotericin B and azoles on the experimental models.(9)

The Rifampicin binds to the b-subunit of DNA-dependent RNA polymerase and suppresses the transcription initiation and production. Its action in N. fowleri is believed to interfere with the vital metabolic process and gene expression. Although rifampicin is amoebic with a very low degree, it enhances the overall treatment efficacy in multidrug combinations and is effective in CNS tissue penetration.(13)

2.2 Other Adjunctive and Investigational Agents

Other drugs with a vitro effect, such as azithromycin, chlorpromazine, posaconazole, and auranofin are examined as supplementary or repurposed agents. The production of proteins is suppressed by azithromycin, which binds itself to the 50S ribosomal sub-unit, and it has been shown to work synergistically with amphotericin B. Posaconazole, a second-generation triazole, is more potent and penetrates the CNS more effectively than its predecessors. Auranofin, which is a drug of gold containing medicine capable of treating rheumatoid arthritis, inhibits thioredoxin reductase and alters redox balance, leading to mortality caused by oxidative stress in N. fowleri.(13)Carrying out of these medicines, however, is limited to anecdotal reports and preclinical studies.

Other experimental drugs, like chlorpromazine (a phenothiazine antipsychotic), interfere with membrane potential and endocytosis, and azithromycin inhibits protein synthesis as well as has immunomodulatory effects, potentially decreasing inflammation and consequent neuronal damage.(3)

2.3 Combination Therapy Rationale

Considering that the individual medications are effective in most cases only in part, the combination therapy is the predominant approach to the PAM treatment. The aim is to realize synergistic amoebicidal activity and reduce the individual drug dosage and at the same time attack different cellular activities. As an example, Amphotericin B (membrane disruption) and Azoles (sterol inhibition) appear to induce synergistic cell membrane damage.

When mitochondrial/apoptotic induction (miltefosine) is combined with Amphotericin B, the amoebic death rates increase.

- The use of rifampicin (transcription inhibitor) and amphotericin B will result in a greater metabolic inhibition.
- Targeted therapy has also managed to achieve a small number of reported survivors, which adds plausibility to the premise that multi-targeted treatments are beneficial when started at an early stage.(12)

3. Novel Drug Delivery Systems for Naegleria fowleri

Nanotechnology-based systems — liposomes, solid lipid nanoparticles, polymeric nanoparticles

Nanocarriers can be used to carry amoebicidal drugs with an attractive delivery to the brain and reduced system toxicity. The best known example of a lipid-based formulation which reduces nephrotoxicity in comparison to conventional amphotericin B is liposomal amphotericin B (LAmB; e.g., AmBisome(r) which has been used clinically in CNS fungal infections, and analogous liposomal formulations have been proposed and preclinically tested in N. fowleri to raise brain tissue levels and tolerability. Brain targeting with lipid-based carriers Bypassing the blood vessels of the blood-brain barrier, surface-modified carriers can enhance the solubility of hydrophobic drugs and protect the labile drugs by the rapid clearance. Experimental nanocarrier-drug conjugates (amphotericin B and natural chemicals such as curcumin) translationally target to support the use of nanoparticles as nano formulations of PAM, as these have been shown to have a greater amoebicidal effect in vitro than free drug and reduced host cytotoxicity in cell cultures. Polymeric nanoparticles (including PLGA) containing miltefosine or any other repurposed drug, with sustained release, improved cellular uptake, and reduced toxicity peaks, are also appealing adjuvant therapy options in both in vitro and in vivo settings.(14)

b. Intranasal and intrathecal delivery approaches

Direct CNS delivery may avoid the blood-brain barrier (BBB), deliver high local concentrations in a short period of time, and avoid systemic side effects; intranasal (transnasal/transcribriform) and intrathecal/intraventricular may be used in the list of therapeutically significant techniques.

Intranasal delivery involves the olfactory and trigeminal neuronal pathways to deliver drugs through the nasal mucosa to the olfactory bulb and the central nervous system which is particularly significant in N. fowleri because of its natural nasal-to-brain route. Preclinical and translational studies have shown that intranasal amphotericin B or aerosolised formulation can be used to attain therapeutic levels in the brain with reduced systemic toxicity, a number of reviews have suggested that clinical trials of nebulised/intranasal amphotericin B and other nano-formulations should be undertaken as an initial adjunct in suspected PAM. Repeated administration Intranasal dosage can also be administered repeatedly and minimally invasively in emergencies.(15)

History intrathecal/intraventricular delivery PAM has a history with intrathecal amphotericin B which is used to

raise levels of CSF medications when administered systemically is inadequate. Even though intrathecal administration results in high CSF concentrations, it is associated with risks of procedure (infection, haemorrhage), requires neurosurgical assistance, and does not always penetrate deep parenchymal foci. Intrathecal treatment can, therefore, be regarded as a salvage or add-on approach to a multidisciplinary critical-care approach.(16)

c. Hydrogels, microemulsions, and nanoemulsions — sustained and localized release platforms

Mucosal or parenchymal application can be developed using hydrogels and micro/ nanoemulsions which provide vehicles to achieve localised drug release in the long term. Microemulsion-loaded hydrogel systems (nanogels) demand high drug-loading capacity of lipophilic drugs (as amphotericin B) and can be used to antagonize extended residence time on mucosal surfaces, which increases absorption into perineural channels. Nanoemulsions when combined with thermoresponsive gels offer controlled delivery and can be administered by intranasal dosage repeatedly. They also increase solubilisation and nasal penetration, and they have been studied to be used as an antifungal agent across mucosal barriers. The hydrogel-nanoparticle composites also can be tailored to intracerebral depot implantation in preclinical neurosurgery models and can be limited to a specific intracerebral release to the infected regions. Even though direct data are scarce in PAM currently, the larger literature on antifungal and CNS delivery justifies the study of these platforms to treat N. fowleri.(9)

d. Targeted delivery strategies — ligand- or receptor-based targeting for amoebic cell uptake

Target delivery enhances both medicine concentration on the site of infection and also increases the absorption of pathogen or infected tissue. Brain-targeted ligands (transferrin, lactoferrin, angiopep-2, or peptide shuttles) are ligands that induce receptor-mediated transcytosis across the BBB and have been applied successfully to deliver nanoparticles to the CNS parenchyma in a variety of neuroinfectious and neurooncologic conditions. Selective uptake by N. fowleri trophozoites within the olfactory epithelium or CNS niches can also be enhanced by pathogen-directed targeting e.g. nanoparticles coated with lectins or antibodies that target amoebic surface molecules. Recent discussions of brain-targeted nanovesicles, computational/experimental studies of molecular targets of N. fowleri, offer a road map on how to make ligand-based systems and choice of surface markers to make targeted uptake. A possibility that is potentially, but technically challenging, is the possibility of coupling BBB-shuttle ligands to amoeba-specific targeting moieties.(9)

4. Synergistic and Combination Therapy Approaches

4.1 Rationale for Multi-Drug Regimens

Multi-drug regimens are used by clinicians to put the greatest likelihood of early amoebicidal activity due to the speed of progression form of primary amoebic meningoencephalitis (PAM) by Naegleria fowleri which is sometimes fatal in spite of treatment. Combination therapy is worth considering due to a number of reasons: (1) Combination therapy has the advantage of acting on different biochemical pathways (membrane integrity, sterol biosynthesis, mitochondrial function, transcription), which is more likely to produce quick clearance of the parasites; (2) synergistic effects can be seen with combinations, which reduces the doses needed, thereby reducing host toxicity; and (4) multi-target approaches reduce the theoretical risk of heterogeneous populations of parasites or life-cycle stages surviving monotherapy. These concepts are the basis of the current regimens adjoining amphotericin B and/or miltefosine and rifampicin or macrolides as auxiliaries.(17)

4.2 In Vitro and In Vivo Evidence for Drug Synergy

A growing body of preclinical work demonstrates additive or synergistic interactions among established and repurposed agents:

4.2.1 Amphotericin B + Azoles or Amphotericin B + Miltefosine. Amphotericin B in combination with azoles or miltefosine increases amoebic activity both in vitro and increases survival in numerous animal systems. It is a simple process in that the azoles drain membrane sterols and amphotericin B raises membrane permeability, leading to more severe membrane dysfunction. A number of in vivo mouse studies have demonstrated that combination therapies are more effective compared to monotherapy. (17)

4.2.2 Auranofin and Amphotericin B. Auranofin is a repurposed thioredoxin reductase inhibitor with rapid amoebic activity in vitro (low-micromolar EC50s), and has strong synergy with amphotericin B. Combinations of the two drugs result in much lower concentrations of each medicine having inhibitory activity in many N. fowleri genotypes. The partnership is impressive since the redox homeostasis of the auranofin and the membrane of the amphotericin B are complementary and form the death mechanisms.(17)

4.2.3 Posaconazole ± Azithromycin Phenotypic screening revealed that posaconazole was a very potent azole against N. fowleri, and in combination with azithromycin, survival was observed in a mouse infection model in improved comparison with controls, suggesting that there was significant in vivo synergy between some azole-macrolide

combinations.(18)

4.2.4 Azithromycin with Amphotericin B. Conventional microbiology studies indicate that some macrolides (azithromycin, rokitamycin) enhance action of amphotericin B on amoebae in in vitro, possibly by acting on protein synthesis and immunomodulation, which enhances susceptibility.

Altogether, these studies inform the clinical approach of early, aggressive multi-drug treatments and find promising combinations to be tested translatologically. Nonetheless, there is no data on human trials, and most of the information is preclinical or provided in case reports.(17)

Table 1. Synergistic Drug Combinations Against Naegleria fowleri

Drug Combination	Mechanism Basis of Synergy	Evidence Type	Key Findings
Amphotericin B + Azoles	Azoles deplete membrane sterols, increasing susceptibility to AmB-mediated pore formation and membrane leakage	In vitro & in vivo (mouse models)	Enhanced amoebicidal activity and improved survival over monotherapy. (17)
Amphotericin B + Miltefosine	AmB disrupts membrane integrity; miltefosine induces mitochondrial depolarization and apoptosis-like death	In vitro & animal studies	Increased trophozoite kill rates and extended survival in experimental model. (17)
Auranofin + Amphotericin B	Auranofin disrupts redox homeostasis (thioredoxin reductase inhibition); AmB damages membrane → multi-mechanistic collapse	In vitro (multiple genotypes)	Strong synergy; significantly reduced EC50s for both drugs when combined. (17)
Posaconazole + Azithromycin	Posaconazole inhibits sterol biosynthesis; azithromycin blocks protein synthesis and modulates inflammation	In vivo (mouse model)	Combined therapy improved survival vs. monotherapy controls.(18)
Azithromycin + Amphotericin B	Macrolides impair protein synthesis and may increase membrane susceptibility to AmB	In vitro	Enhanced amoebicidal effects; increased AmB potency. (17)

5. Role of Repurposed Drugs in Combination Therapy

Repurposing drugs accelerates the rapid discovery of partners in combinations who already have a license to use in other applications, reducing the time taken to use them in an emergency. Phenotypic screening has found amphotericin B and miltefosine synergetically active with a range of kinase inhibitors, including auranofin, Auranofin analogues, auranofin-like thioredoxin inhibitors, pitavastatin, chlorpromazine and other inhibitors. The idea of repurposed pharmaceuticals is attractive since their safety profiles and human pharmacology are well-known compared to new molecules, which means that they can be used under compassionate-use or emergency guidelines when time is a critical factor. Auranofin and posaconazole have often been found to be promising repurposing candidates in combo regimens due to recent high throughput and targeted screening.(18)

6. Pharmacodynamic and Pharmacokinetic Interactions in Combinations

Rational combination design requires integration of PK/PD considerations:

CNS penetration and Timing. The drugs need to get to the site of infection (the olfactory bulb and deeper parenchymal tissue) in therapeutic amounts. Due to its low levels of BBB penetration, amphotericin B should be administered intrathecally or intraventricularly, or in combination with more CNS-penetrating azoles (voriconazole, fluconazole, posaconazole) or systemically active miltefosine. Patterns of temporal concentration are significant: It is desirable to use longer-acting azoles, but faster acting drugs (like auranofin) can be more successful at the start.(17)

Synergy versus toxicity trade-off. Synergy can be used to reduce doses of toxic drugs (e.g. amphotericin B) although safe combinations may be restricted by

overlapping organ toxicities (hepato- and nephrotoxicity). As an example, the combination of amphotericin B (nephrotoxic) and other nephrotoxic or hepatotoxic drugs requires close monitoring and adjustments in dosage. Rifampicin induces cytochrome 450 enzymes, and may alter exposure to azoles (reducing azole levels); however, azoles inhibit cytochrome 450 enzymes and hence increase concentrations of co-administered drugs-the interactions should be anticipated.(17)

PD complementarit The combination of agents with diverse mechanisms (membrane disruption + redox stress + transcriptional inhibition) and compatible kill-kinetics should be optimal, and time-kill and chequerboard assays can help in measuring the level of synergy (fractional inhibitory concentration indices), and dosing strategies before animal or human experimentation.(19)

7. Emerging Experimental Combinations

Several promising combinations have surfaced recently and warrant prioritized translational evaluation:

1.Auranofin + Amphotericin B is highly synergistic in vitro across all genotypes and show fast kill kinetics. (17)
2.Posaconazole was combined with Azithromycin which improved the survival of mice. (18)
3.Amphotericin B + Miltefosine + Azoles: This triple therapy, which has miltefosine, has been demonstrated to be used in documented human survivorship as well as being the standard in many procedures.(20)
4.Amphotericin B or miltefosine can be employed together with a nanomolar potency kinase inhibitor to have a multi-mechanistic attack.(21)
5.By incorporating the amphotericin B and/or the miltefosine into nanocarriers, it is possible to decrease the systemic dosages without CNS exposure, resulting in safer combination regimens in vivo. Early formulation investigations are useful, however preclinical infection models are needed.(22)

8. Computational and Molecular Approaches in Drug Design

8.1 Virtual screening, molecular docking and QSAR for anti-amoebic discovery

Anti-amoebic discovery Virtual screening, molecular docking and QSAR Molecular docking and QSAR Virtual screening.

As virtual screening, molecular docking and quantitative structure-activity relationship (QSAR) modelling, computational methods are rapid and low cost methods used to prioritize compounds to experimental testing against N. fowleri. Their applications are of particular advantage to rare, high-mortality infections, where the traditional high-throughput screening is limited by both biosafety considerations and the existence of the models.

The screening against N. fowleri targets (e.g. CYP51) using virtual screening programs provided drug-like natural products, approved medicines and scaffold leads with predicted binding energies and interaction modes. A number of studies incorporated ligand-based pharmacophore modelling and structure-based docking, then molecular dynamics (MD) of poses to determine complex stability, hence selecting candidates to be tested in-vitro. As an example, docking/QSAR studies of CYP51 and other sterol-biosynthetic enzymes have identified flavonoids and miconazole-like scaffolds as good leads, and additional MD simulations confirmed that they were binding stable.

The complementary approach to prioritisation provided by QSAR approaches correlates amoebicidal efficacy with chemical descriptors (where possible). QSAR models have the potential to filter large libraries of physicochemically active molecules based on CNS penetration (lipophilicity, polar surface area) and minimal mammalian toxicity, thereby enhancing the translation emitted hits. New qsar + docking pipelines A variety of terpenoid and alkaloid candidates have been advanced by recent qsar + docking pipelines, and have shown good results in phenotypic testing. (23)

Important enabling resources are Curated N. fowleri protein models and experimental solved structures which have been used to create correct docking grids are also important enabling resources; their open access has promoted computational discovery. Combining MD simulation and free-energy calculations is also beneficial to hitting a target because a binding stability test and an off-target interaction test are conducted before a bench test.(17)

8.2 Network pharmacology to identify synergistic targets

Network pharmacology involves applying systems biology to map drug-target-pathway interactions and construct combinations to disrupt pathogen critical biological modules without pertaining to host pathways. In the case of N. fowleri, in which just a few single targets are validated, network strategies can be used to (a) prioritize multi-target strategies, (b) discover hub proteins whose concomitant inhibition may lead to synthetic lethality, and (c) propose host-pathogen interaction nodes that can be perturbed.

Combining N. fowleri omics (transcriptomes/proteomes) with orthology mapping and route databases can allow the researcher to generate pathogen interactomes and simulate the system-wide implications of blocking a large fraction of nodes. Sterol biosynthesis, redox homeostasis, and

cytoskeletal dynamics have all been found using network analyses to be high-value pathways to attack combinatorically, which is consistent with experimental synergy between amphotericin B (membrane/sterol disruption), auranofin (redox disruption), and tubulin-targeting chemotypes. Network models also inform the choice of repurposed pharmaceuticals to select that have a joint influence on non-overlapping modules and increase the chances of synergy.(17)

8.3 AI and bioinformatics to predict combinations and delivery systems

Modeling drug-target interactions and combinations. MLs that have been trained with chemical-biological interaction matrices and phenotypic screening data are capable of predicting potential medicines and therapeutic combinations that are synergistic. In the case of N. fowleri, the emphasis of such models was on repurposed drugs (e.g., auranofin, posaconazole) and suggested novel pairs, which subsequently were shown to have in vitro synergy. Together with PK/PD constraints (CNS penetration, half-life), AI ranking will be used to give preference to a combination that is more likely to be actionable clinically.(17)

Generative and de novo design. Generative models based on deep-learning have the potential to propose new chemotypes optimised to predicted bind to target pockets but satisfying CNS drug-likeness metrics. Compounds are prioritised using in silico retrosynthesis and ADMET predictors, which are compounds that are probable to be synthesizable and safe. Initial research work in the N. fowleri field explains computational algorithms that develop tubulin-targeting compounds that are anticipated to be selective against human analogs.(19)

Optimising delivery methods. Bioinformatics and machine learning may also assist engineers to design delivery vehicles, e.g. design peptide BBB-shuttles (e.g. angiopeps, transferrin mimics) that will maximise nanoparticle transcytosis or select lipid/polymer mixtures that achieve tradeoffs between CNS absorption and payload release. Simulations (computational fluid dynamics and pharmacokinetics) are useful in designing intranasal formulations that have the highest mucosal residence and perineural transfer to the olfactory bulb as this is essential because N. fowleri enters the body via the nose.(24)

8.4 Best practices and limitations

Computational methods accelerate the discovery of hits, but they should be intimately related to the validation of them experimentally. It has best practices: (1) validated protein structures or high-quality homology models; (2) CNS-penetration filters (e.g. predicted blood-brain partitioning) early to eliminate dead ends; (3) ensemble docking and MD to model protein flexibility; and (4) cross-validation of ML models on independent phenotypic datasets and estimation of applicability domains.

Weaknesses consist of insufficient structural coverage of N. fowleri proteome, lack of in vitro potency data of high quality to be used to train model and a possibility that predicted binding does not lead to amoebicidal action because of cell uptake or metabolic inactivation. Moreover, network models demand strong datasets of omics that are not currently available; as transcriptome and proteomic data regarding various life stages and genotypes continue to become increasingly available, the predictions offered by network pharmacology will be more accurate.(25)

8.5 Outlook and priorities

To maximise impact, computational efforts should prioritise: (a) integrating docking/QSAR hits with ML-predicted synergy and PK/PD constraints to nominate combinations for animal testing; (b) expanding curated N. fowleri structural and omics databases; (c) coupling network pharmacology with experimental perturbation (CRISPRi/RNAi where feasible) to validate predicted hubs; and (d) using AI-guided design to accelerate CNS-optimized small molecules and BBB-shuttle-enabled Computationally prioritised medicines and combinations, combined with quick intranasal or intrathecal administration techniques, have the potential to significantly reduce the journey from in silico discovery to clinical application for this severe disease.(25)

9. Future Perspectives & Emerging Therapeutic Trends

9.1 Nanocarrier–Drug Hybrids

Nanoparticles conjugated with amphotericin B or curcumin show stronger anti-N. fowleri effects and reduced human cell toxicity compared to free drugs.(26)

Other drug-nanoconjugate (e.g, sulfamethoxazole, metronidazole) also demonstrate potent amoebicidal activity with low cytotoxicity. (3)

9.2 Precision / Genotype-Guided Therapy

N. fowleri strains demonstrate diverse responses to azoles, which indicates the possibility of genotype-based and individualized treatment.(27)

Quick molecular diagnostics (e.g., PCR, metagenomic NGS) could reduce the time to individual therapy significantly, as demonstrated in clinical cases. (28)

9.3 BBB-Permeable & CNS-Targeted Agents

- Posaconazole is a fast acting azole that has good in vitro anti-amoebic and in vivo activity.

- Nanoparticles made of PLGA are a potential choice in CNS delivery: this is a biocompatible material that can be surface-modified to target the BBB. (25)
- Non-invasive delivery (e.g. through liposomes or nanoemulsions) is a method used to deliver drugs to the brain.(28)

9.4 Immunomodulation

N. fowleri infection triggers inflammasome NLRP3, resulting in IL-1b, which has the potential to contribute to neuropathology. (29)

The modulation of pro-inflammatory cytokines (IL-1, IL-6, TNF-a) is also being suggested as add-on therapy to curtail immune-mediated destruction.(30)

9.5 Integrated and Rapid-Response Therapeutic Platforms

An integrated solution - i.e. rapid diagnostics + nanocarrier delivery + synergistic drugs combinations - may become the cornerstone of uniform emergency treatment regimes.

Expanded-access pathways / preapproval will be important in converting these innovations into clinical practice, in particular in high-risk environments(31)
conclusion

The treatment of Naegleria fowleri will be based on the enhanced technology of drug delivery, targeted drugs, and host-directed therapy to address the shortcomings of the existing pharmacological therapy. Liposomes, polymeric nanoparticles and solid lipid nanoparticles are examples of nanocarrier-drug hybrid formulations that provide superior brain accessibility, controlled drug delivery and reduced toxicity at the systemic level. According to preclinical trials, amphotericin B and miltefosine nano formulations are more effective and safer as anti-amoebic agents.

These incorporate new personalised or precision-based approaches where medications are adjusted based on patient-specific PK and PD reactions, pharmacogenomic results and N. fowleri genotypes. Targeted, early intervention, which is crucial to lower mortality, may be achieved with the help of genome-based medication responses and prompt molecular diagnostics.

The parallel projects are to use structural optimisation, ligand-conjugated nanoparticles, intranasal or intrathecal delivery of drugs to develop blood-brain barrier (BBB)-permeable and CNS-targeted drugs. The approaches increase therapeutic levels within the central nervous system and reduce systemic toxicity. Immunomodulatory adjuncts regulating inflammatory effects are also under investigation to reduce the amount of neuronal damage but retain host defence.

All said and done, the clinical management of primary amoebic meningoencephalitis (PAM) can be radically changed through the incorporation of immunotherapy, precision medicine, and nanotechnology. Future research is needed to focus on combinatorial nanocarrier systems, rapid diagnostic-based therapeutic algorithms, and safe immunomodulatory interventions so as to deliver effective and timely treatment. These developments in combination with models of kind usage and translational animal models bring new hope to the war against this almost deadly infection.

REFERENCE:

1. Jahangeer M, Mahmood Z, Munir N, Waraich U e. A, Tahir IM, Akram M, et al. Naegleria fowleri: Sources of infection, pathophysiology, diagnosis, and management; a review. Vol. 47, Clinical and Experimental Pharmacology and Physiology. 2020.
2. Soltow SM, Brenner GM. Synergistic activities of azithromycin and amphotericin B against Naegleria fowleri in vitro and in a mouse model of primary amebic meningoencephalitis. Antimicrob Agents Chemother. 2007;51(1).
3. Siddiqui R, Boghossian A, Kawish M, Jabri T, Shah MR, Anuar TS, et al. Nanocarrier Drug Conjugates Exhibit Potent Anti-Naegleria fowleri and Anti-Balamuthia mandrillaris Properties. Diseases. 2023;11(2).
4. Rizo-Liendo A, Arberas-Jiménez I, Martin-Encinas E, Sifaoui I, Reyes-Batlle M, Chao-Pellicer J, et al. Naphthyridine derivatives induce programmed cell death in naegleria fowleri. Pharmaceuticals. 2021;14(10).
5. Chao-Pellicer J, Arberas-Jiménez I, Sifaoui I, Díaz-Marrero AR, Fernández JJ, Jamerson M, et al. Potential Inhibitors of Human–Naegleria fowleri Interactions: An In Vitro Extracellular Matrix-Based Model. Mar Drugs. 2025;23(8):1–11.
6. Haston JC, Cope JR. Amebic encephalitis and meningoencephalitis: An update on epidemiology, diagnostic methods, and treatment. Vol. 36, Current Opinion in Infectious Diseases. 2023.
7. Mungroo MR, Khan NA, Siddiqui R. Naegleria fowleri: diagnosis, treatment options and pathogenesis. Expert Opin Orphan Drugs. 2019;7(2):67–80.
8. Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, Da Silva AJ, et al. Successful treatment of an adolescent with naegleria fowleri primary amebic meningoencephalitis. Pediatrics. 2015;135(3).
9. Rice CA, Colon BL, Chen E, Hull M V., Kyle

DE. Discovery of repurposing drug candidates for the treatment of diseases caused by pathogenic free-living amoebae. PLoS Negl Trop Dis [Internet]. 2020;14(9):1–21. Available from: http://dx.doi.org/10.1371/journal.pntd.0008353

10. Cárdenas-Zúñiga R, Silva-Olivares A, Villalba-Magdaleno JDA, Sánchez-Monroy V, Serrano-Luna J, Shibayama M. Amphotericin B induces apoptosis-like programmed cell death in Naegleria fowleri and Naegleria gruberi. Microbiology (United Kingdom). 2017;163(7).
11. Advances in the treatment of infections due to brain-eating amoebae. :1.
12. Guarner J, Bartlett J, Shieh WJ, Paddock CD, Visvesvara GS, Zaki SR. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. Modern Pathology. 2007;20(12).
13. Debnath A, Calvet CM, Jennings G, Zhou W, Aksenov A, Luth MR, et al. CYP51 is an essential drug target for the treatment of primary amoebic meningoencephalitis (PAM). PLoS Negl Trop Dis. 2017;11(12).
14. Cavassin FB, Baú-Carneiro JL, Vilas-Boas RR, Queiroz-Telles F. Sixty years of Amphotericin B: An Overview of the Main Antifungal Agent Used to Treat Invasive Fungal Infections. Vol. 10, Infectious Diseases and Therapy. 2021.
15. Siddiqui R, Yee Ong TY, Maciver S, Khan NA. Can Amphotericin B-mediated effects be limited using intranasal versus intravenous route? Ther Deliv. 2023;14(8).
16. Ismail NN, Yusof H. Occurrence of the pathogenic amoeba naegleria fowleri, pathogenesis, diagnosis, and treatment options. Vol. 17, Malaysian Journal of Medicine and Health Sciences. 2021.
17. Aurongzeb M, Rashid Y, Naqvi SHA, Khatoon A, Haq SA, Azim MK, et al. Naegleria fowleri from Pakistan Has Type-2 Genotype. Iran J Parasitol. 2022;17(1):43–52.
18. Debnath A. Drug discovery for primary amebic meningoencephalitis: from screen to identification of leads. Expert Rev Anti Infect Ther. 2021;19(9):1099–106.
19. Rajendran K, Ahmed U, Meunier AC, Shaikh MF, Siddiqui R, Anwar A. Nanoparticle-Terpene Fusion: A Game-Changer in Combating Primary Amoebic Meningoencephalitis Caused by Naegleria fowleri. ACS Omega. 2024;9(10):11597–607.
20. Cope JR, Conrad DA, Cohen N, Cotilla M, Dasilva A, Jackson J, et al. Use of the Novel Therapeutic Agent Miltefosine for the Treatment of Primary Amebic Meningoencephalitis: Report of 1 Fatal and 1 Surviving Case. Clinical Infectious Diseases. 2015;62(6).
21. Yuan A, Fong H, Nguyen J V., Nguyen S, Norman P, Cullum R, et al. High-Throughput Screen of Microbial Metabolites Identifies F1FO ATP Synthase Inhibitors as New Leads for Naegleria fowleri Infection. ACS Infect Dis. 2023;9(12).
22. Kyle DE, Cassiopeia Russell A, Troth E V., Rice CA. Discovery of anti-amoebic inhibitors from screening the mmv pandemic response box on balamuthia mandrillaris, naegleria fowleri, and acanthamoeba castellanii. Pathogens. 2020;9(6).
23. Sharma V, Madia VN, Tudino V, Nguyen J V., Debnath A, Messore A, et al. Miconazole-like Scaffold is a Promising Lead for Naegleria fowleri-Specific CYP51 Inhibitors. J Med Chem. 2023;66(24).
24. Fong H, Leid ZH, Debnath A. Approaches for Targeting Naegleria fowleri Using Nanoparticles and Artificial Peptides. Pathogens. 2024;13(8).
25. Tillery L, Barrett K, Goldstein J, Lassner JW, Osterhout B, Tran NL, et al. Naegleria fowleri: Protein structures to facilitate drug discovery for the deadly, pathogenic free-living amoeba. PLoS One [Internet]. 2021;16(3 March):1–19. Available from: http://dx.doi.org/10.1371/journal.pone.0241738
26. Siddiqui R, Boghossian A, Kawish M, Jabri T, Shah MR, Anuar TS, et al. Nanocarrier Drug Conjugates Exhibit Potent Anti-Naegleria fowleri and Anti-Balamuthia mandrillaris Properties. Diseases. 2023;11(2).
27. Russell AC, Kyle DE. Differential Growth Rates and In Vitro Drug Susceptibility to Currently Used Drugs for Multiple Isolates of Naegleria fowleri . Microbiol Spectr. 2022;10(1).
28. Soontrapa P, Jitmuang A, Ruenchit P, Tiewcharoen S, Sarasombath PT, Rattanabannakit C. The First Molecular Genotyping of Naegleria fowleri Causing Primary Amebic Meningoencephalitis in Thailand With Epidemiology and Clinical Case Reviews. Front Cell Infect Microbiol. 2022;12.
29. Chen CW, Moseman EA. Pro-inflammatory cytokine responses to Naegleria fowleri infection. Vol. 3, Frontiers in Tropical Diseases. 2022.
30. Lê HG, Kang JM, Võ TC, Yoo WG, Na BK. Naegleria fowleri Extracellular Vesicles Induce Proinflammatory Immune Responses in BV-2 Microglial Cells. Int J Mol Sci. 2023;24(17).

31. Ahmad Zamzuri M 'Ammar I, Abd Majid FN, Mihat M, Ibrahim SS, Ismail M, Abd Aziz S, et al. Systematic Review of Brain-Eating Amoeba: A Decade Update. Int J Environ Res Public Health. 2023;20(4).

