

REVIEW

Recent advances in primary amoebic meningoencephalitis: a comprehensive review of therapeutic compounds and vaccine prospects

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ABSTRACT

This review article explores the latest developments in the study of primary amoebic meningoencephalitis. It emphasizes the importance of vaccinations as a possibly groundbreaking preventive measure that could revolutionize the fight against and elimination of primary amoebic meningoencephalitis. The importance of prospective applications of phytochemicals from different natural sources is further emphasized. This review provides an extensive overview of the frontiers in primary amoebic meningoencephalitis treatment and prevention by integrating the most recent research with potential future directions.

Keywords: *Naegleria fowleri*; Amebic Meningoencephalitis, Primary; Vaccines; Preventive Health Services; Public Health (Source: MeSH)

Avances recientes en la meningoencefalitis amebiana primaria: revisión exhaustiva de compuestos terapéuticos y perspectivas de vacunas

RESUMEN

Este artículo de revisión explora los últimos avances en el estudio de la meningoencefalitis amebiana primaria. Se destaca la importancia de las vacunas como posible medida preventiva innovadora que podría revolucionar la lucha contra la meningoencefalitis amebiana primaria y su eliminación. Además, se hace hincapié en la importancia de las aplicaciones prospectivas de los fitoquímicos procedentes de distintas fuentes naturales. Esta revisión ofrece un amplio panorama de las fronteras en el tratamiento y la prevención de la meningoencefalitis amebiana primaria, integrando las investigaciones más recientes con las posibles direcciones futuras.

Palabras clave: *Naegleria fowleri*; Meningoencefalitis Amebiana Primaria; Vacunas; Servicios Preventivos de Salud; Salud Pública (Fuente: DeCS)

INTRODUCTION

The amoeba *Naegleria fowleri* causes the uncommon but fatal brain infection known as primary amoebic meningoencephalitis (PAM). Swimming in warm freshwater rivers, lakes, and hot springs may spread *N. fowleri* through the nose into the body, leading to infection or disease. Once in the brain, the amoeba causes inflammation and brain tissue destruction by ascending the olfactory nerve (1).

PAM primarily affects children and young adults and is more prevalent in tropical and subtropical regions. Thirty-nine cases of PAM were reported globally between 2011 and 2020,

per a recent study by Güémez *et al.* (2). According to the place of origin, the United States was where most cases occurred, followed by Pakistan, Mexico, the Czech Republic, and India (2,3). Only a few individuals have ever survived PAM, with a mortality rate of over 98 % (4). Although there isn't a vaccine to protect against PAM in humans, researchers are working on various immunization approaches that may one day be utilized to stop this fatal illness (5).

Naegleria fowleri, commonly known as the "brain-eating amoeba," is a parasitic amoebic organism found in freshwater, ponds, and lakes. It belongs to the family Vahlkampfiidea, order Schizopyrenida and phylum Percolozoa. Among the 47 species within the *Naegleria* genus, *N. fowleri* is the sole pathogenic species. This parasite exhibits a global distribution on almost every continent except Antarctica. It thrives in temperatures exceeding 45 degrees Celsius and can endure temperatures ranging from 4 to over 50 degrees Celsius (6).

N. fowleri primarily exists in the trophozoite form (Figure 1), reproducing rapidly and mainly feeding on gram-negative bacteria. Under incredibly unfriendly circumstances, the trophozoite changes into a cyst stage, which is profoundly safe and fit for enduring temperatures as low as 4 degrees Celsius (7). Consequently, this parasite can thrive in various locations worldwide, excluding polar regions. When faced with unfavorable conditions, the amoeba transitions into a flagella-like shape that retains amoebic characteristics but is neither reproductive nor infective. Under normal conditions, the parasite reverts to the trophozoite stage. In this stage, it has a cup-shaped structure that enables it to feed on bacteria. This phase is when the parasite is most reproductive and infective, becoming pathogenic and easily entering the human body through the nasal passage during recreational or religious activities in warm freshwater bodies (8).

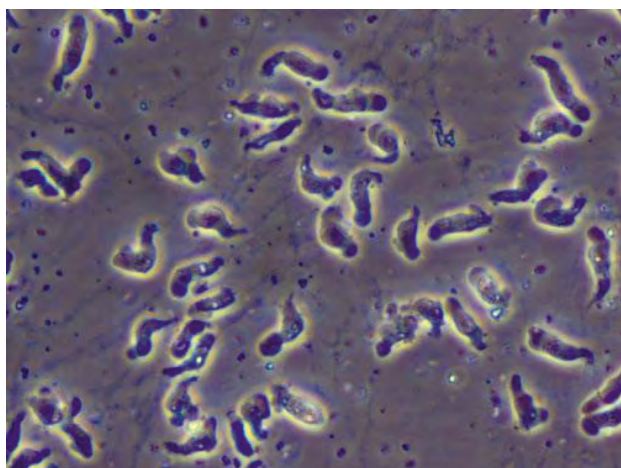


Figure 1. A wet mount of *N. fowleri* trophozoites cultured from the CSF of a patient with PAM (9). Source: Centers for Disease Control and Prevention (CDC), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED).

Moreover, the genome of *Naegleria fowleri* has been sequenced, revealing insights into its evolutionary history and potential targets for intervention. This hereditary data has prepared for developing novel inoculation procedures to prevent the devastating impact of *Naegleria fowleri* infections (10).

PATHOPHYSIOLOGY

Naegleria fowleri, generally known as the "brain-eating amoeba," initiates its pathophysiological course when individuals encounter warm freshwater contaminated with the amoebae, which get sufficient access through the nasal entries (11). Using the olfactory nerve, these amoebae navigate the ethmoid bone's cribriform plate, bypassing the blood-brain barrier (BBB) (12).

Once in the brain, they predominantly target the olfactory bulb and frontal lobes, primarily in the trophozoite form, releasing cytotoxic compounds such as pore-forming proteins, proteases, and phospholipases. These compounds contribute to host cell damage by forming membrane pores, breaking down proteins, and disrupting phospholipids within cell membranes (13). The damage inflicted by *N. fowleri* involves several mechanisms: *N. fowleri* amoebae secrete proteins that can form pores or holes in host cell membranes. This disrupts the integrity of host cells and allows the entry of ions and molecules, leading to cell dysfunction and death. Within host cells, proteases can degrade proteins, disintegrating cellular components and eventual cell death (14). Furthermore, phospholipases are enzymes that degrade phospholipids, which are critical components of cell membranes, hence contributing to cell death by altering the structure of cell membranes (15).

In addition, the body's immune response to *Naegleria fowleri* releases inflammatory molecules and activates immune cells, which can further damage nerve cells (13). Those infected with this "brain-eating amoeba" experience severe neurological manifestations and an elevated mortality rate because of the immune system's inflammatory responses contributing to the damage (16). Diagnosing and treating PAM early improves survival chances (17).

Clinical Manifestations

Naegleria fowleri is an amoeba found in warm freshwater that causes PAM, a rare but severe disease. After exposure to the amoeba, people usually develop symptoms that resemble the flu, such as headaches, fever, nausea, and vomiting within a week. As the disease progresses, symptoms can include loss of appetite, irritability, neck stiffness, tiredness, sensitivity to light, and seizures. In extreme cases, patients can fall into a coma. Diagnosing PAM is difficult due to its generic initial symptoms, and sometimes confirmation only comes after a post-mortem examination that reveals the amoeba's impact on the brain (18,19).

DIAGNOSIS AND TREATMENT

PAM is a severe infection caused by *Naegleria fowleri* and is typically diagnosed in advanced stages due to overt clinical symptoms. Diagnostic procedures include brain imaging through CT scans or MRIs to detect signs of brain swelling or inflammation (Figure 2). The definitive diagnosis is confirmed via a brain biopsy, which can detect the amoeba directly. CSF analysis through lumbar puncture also plays a role in the diagnostic process. Treatment of PAM involves a multidisciplinary approach with a combination of antifungal and antibiotic medications, including miltefosine, amphotericin B, rifampicin, fluconazole, azithromycin, and dexamethasone. Despite these efforts, PAM has a high mortality rate, and treatment success varies (20,21). Recent studies have found that a drug combination has led to the successful treatment of a small number of patients, suggesting that posaconazole may be more effective than other antifungals like ketoconazole or fluconazole, pointing toward the necessity of personalized treatment regimens (22,23).

Table 1 outlines the medication regimen administered used in a successful treatment of PAM in the United States in 2013. Along with this treatment, the patient underwent regular cerebrospinal fluid (CSF) drainage and hyperosmolar therapy using Mannitol. Despite achieving patient survival, significant mental deficits were observed 18 months post-discharge, as indicated in Table 1 (24).

Table 1. Successful treatments: the dosage regimen of the drugs used to treat PAM

Sr. N°	DRUG	REGIMEN
1	Amphotericin B	1 mg/kg/day; IV (19 days-0.1 mg); IT (5 days)
2	Rifampin	12 mg/kg/day PO for 19 days
3	Fluconazole	12 mg/kg/day loading dose, then 9 mg/kg/day IV for 19 days
4	Azithromycin	10 mg/kg/day PO for 19 days
5	Miltefosine	150 mg PO in 3 divided doses for 19 days
6	Dexamethasone	0.6 mg/kg/day IV as adjuvant therapy

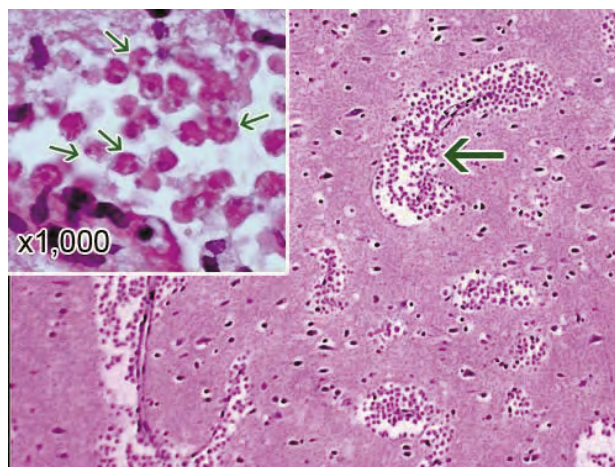


Figure 2. A hematoxylin and eosin-stained cerebral section of a PAM patient's brain indicates a substantial number of *N. fowleri* trophozoites and damage to normal brain tissue (9). Source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED).

Phytochemical Therapy: Novel Therapeutics

Plants have long been recognized as valuable sources of medicinal compounds with the potential to treat various diseases. Compared to synthetic compounds, more bioactive compounds exist in natural products. Research has focused on identifying phytochemicals with antifungal and anti-parasitic properties for treating diseases caused by *N. fowleri*. In a recent study evaluating plant-derived compounds' potential against amoebas, specifically *N. fowleri* and *B. mandrillaris*, three compounds demonstrated noteworthy anti-amoebic activity. After a 24-hour treatment period with these compounds, ursolic acid left 49.30 % of *N. fowleri* viable, betulinic acid left 28.19 %, and betulin resulted in 14.82 % viable amoebas. These findings underscore the therapeutic potential of plant-based compounds, particularly ursolic acid, betulinic acid, and betulin, in targeting *N. fowleri* infections (25,26). A study analyzed ten chamigrane-type sesquiterpenes extracted from the *Laurencia dendroidea* found on the southeastern Brazilian coast. Of these, three compounds, (+)-elatol, (-)-elatol, and (-)-rogiolol, displayed significant activity against *N. fowleri* strains, outperforming the reference drug miltefosine. Remarkably, (+)-elatol demonstrated potency comparable to amphotericin B and effectively targeted the amoeba's trophozoite and cyst stages without toxicity at low concentrations (27).

Six lignan compounds from *Larrea tridentata* displayed activity against various pathogens, including *Naegleria fowleri*. Notably, nordihydroguaiaretic acid (NDGA) and 3'-O-methyl-NDGA showed significant efficacy against *N. fowleri*, with EC50 values of 36 μM and 38 μM, respectively. These results outperformed the standard drug miltefosine, which had an EC50 of 54.5 μM. The action mechanism for these compounds against *N. fowleri* is hypothesized to involve the modulation of cysteine protease activity in the trophozoites (28).

A pivotal discovery in this regard is the identification of heat shock proteins Hsp90 in *N. fowleri* as essential drug targets. Bergenin and epigallocatechin gallate have emerged as the most potent binders to Hsp90, making them promising candidates for phytochemical therapy (29). Kaempferol, a flavonoid in various edible plants such as tea, broccoli, and tomatoes, has garnered attention due to its diverse therapeutic properties, including anti-cancer, antioxidant, anti-inflammatory, neuroprotective, and cardioprotective effects. A recent study conducted by Le *et al.* (30) demonstrated that kaempferol induces apoptosis in *Naegleria fowleri*, suggesting its potential as a promising drug molecule for the treatment of primary amoebic encephalitis caused by *N. fowleri*.

Sesquiterpene lactones, derived from plants and aquatic algae, have exhibited notable anti-ameboid properties. Among these compounds, Anhydroartemonin stands out due to its enhanced blood-brain barrier penetration compared to miltefosin and a superior selectivity index. These attributes, coupled with its low molecular weight, position Anhydroartemonin as a promising therapeutic agent for central nervous system diseases (31). Furthermore, debromolaurinterol, derived from sesquiterpene cyclouranes found in the marine source *Laurencia*, has shown remarkable specificity in targeting ATPases. This compound exhibits an impressive ATP inhibition rate of 99.98 % and demonstrates potent amoebicidal activity against *Naegleria fowleri* (32). Additionally, *in silico* studies predict that salicylic acid, carvacrol, curcuminol, curcumenol, thymol, and dehydroxyisocalamendiol are natural inhibitors of the CYP51 protein in *N. fowleri* (33).

Prevention and Control

Naegleria fowleri infects people when water contaminated with the amoeba enters their nasal passages. This infrequent contamination typically occurs during activities such as swimming or diving in warm freshwater areas like lakes and rivers. It's important for people to avoid submerging their heads completely underwater, to abstain from cleaning their noses during religious practices, and to refrain from irrigating their sinuses with tap water that may be contaminated. *Naegleria fowleri* cultivates itself in pipes, water heaters, and closed water systems, including public drinking sources water systems. You cannot get sick from drinking contaminated water, but your health can be affected only when contaminated water goes up your nose. It is enigmatic why specific individuals get infected with the amoebae, whereas many other people who swam in the same warm water as the infected people did not get sick. This includes people who were swimming right next to the infected people. Scientists have attempted to determine the threshold of *Naegleria fowleri* in the environment that poses a significant risk (34). In any case, no strategy exists to precisely and reproducibly gauge the quantities of amoebae in the water. This makes it indistinct how a standard may be set to safeguard human well-being and how general well-being authorities would quantify and uphold such a norm. In any case, the gamble of *Naegleria fowleri* disease is exceptionally reduced. Between 2013 and 2022, 29 cases of water contamination were reported in the US, despite numerous water sports events annually (3,35).

The primary preventive measure is to avoid activities that involve open water sources, such as swimming in lakes, ponds, rivers, and even hot springs. This is because *N. fowleri* is mainly found in warm freshwater environments. Individuals can take steps to ensure safe washing practices, particularly when using water for nasal irrigation or religious rituals. It is crucial to avoid using water sources that may be contaminated with *N. fowleri*. Instead, using sterile or treated water is recommended (3,7).

VACCINE MODELS: A DETAILED ANALYSIS

A vaccine, i.e., a biological product, is used to safely induce protection against pathogens by initiating an immune response and forming memory cells. The cutting-edge advancements in vaccine development involve viral vector vaccines, nucleic acid vaccines, bacterial vaccines, and the use of antigen-presenting cells, like dendritic cells and T-cells. Researchers are exploring some of these technologies and using different models to develop a vaccine against PAM (36).

Model 1: DNA Vaccines and *Nfa1* Gene Expression

The significance of DNA vaccines has been escalating in recent times. They have showcased efficacy against various pathogens. However, their potential against parasites is an area of ongoing exploration. A DNA vaccine was developed using viral vectors expressing the *nfa1* gene, which augmented the expression of multiple IgG subclasses, specifically IgG1 and IgG2 (37). The *Nfa1* protein is predominantly present on the pseudopodia of the parasite, playing an instrumental role in its phagocytic activity, essential for its survival and proliferation (38). To ascertain the effectiveness of this vaccine, mice were administered intra-peritoneally and intra-nasally doses. The latter proved more effective, resulting in heightened expression of IgG antibodies and induction of cytokines such as Th1, IFN- γ , and the regulatory cytokine IL-10 (37). Moreover, in another experimental setup, it was discerned that the anti-*Nfa1* antibody attenuated the cytotoxic impact of *N. fowleri* trophozoites on CHO cells (38).

Model 2: cDNA Library and *Mp2CL5* Expression

The immunoprotective response of two vaccine antigens against the causative agent of primary amoebic meningoencephalitis, *Naegleria fowleri*, has been previously reported (39). In the study published by Gutiérrez-Sánchez *et al.* (39), mice were given the antigen 19 kDa polypeptide, an immunogenic peptide from membrane protein MP2CL5 intranasally, and cholera toxin as an adjuvant. The nasal cavity and serum showed increased immune response markers like T and B lymphocytes, integrin $\alpha4\beta1$, and immunoglobulins, along with significant protection (up to 100 % with the 19 kDa polypeptide and CT). Based on these results, these antigens may be helpful as vaccine candidates to prevent infections caused by *N. fowleri*.

A novel membrane protein called *Mp2CL5* has been discovered by Reveiller *et al.* (40) and is only expressed specifically in pathogenic strains. Produced in *Escherichia*

coli, this 23-kDa recombinant protein matches the natural 17-kDa protein on the plasma membrane of *N. fowleri* trophozoites, indicating that it plays a crucial role in the pathogenicity of the organism. Interestingly, Mp2CL5 is only expressed in pathogenic *N. fowleri*, while it is not expressed in non-pathogenic *Naegleria* species or *Acanthamoeba*. The increased production of the protein during the amoeba's logarithmic to stationary growth phases is highlighted in the study, which may indicate that it is a virulence factor. To further highlight the adaptive mechanisms of *N. fowleri* in avoiding host defenses and establishing infection, pathogenic *N. fowleri* also demonstrates chemotactic responses to nerve cell components, a feature lacking in non-pathogenic counterparts. These results provide important new information about the pathogenesis and treatment of primary amoebic meningoencephalitis by highlighting the possible role of Mp2CL5 as a virulence factor.

Model 3: Cholera toxin with amoeba lysates

Carrasco-Yepes *et al.* (41) inoculated mice intranasally with cholera toxin and *N. fowleri* lysates, which resulted in enhanced survival, production of antibodies (IgA, IgG), and polymorphonuclear cell activation. This response was improved by up-regulating genes for antibody formation and particular cytokines (IL-10, IL-6, IFN- γ) while decreasing TNF- α production. The results indicated that this vaccination approach successfully blocks *N. fowleri* infection by establishing a robust immunological atmosphere in the nasal cavity.

Optimal Route of Administration: The Nasal Pathway

The exploration for the optimal vaccine delivery route has encompassed diverse animal models, including mice, guinea pigs, and rabbits. Among these, farm animals were found to have the closest anatomical resemblance to humans, making them prime candidates for testing (42,43). Given that the parasite in question infiltrates the human body through the nostrils, progressing to the brain via the olfactory nerves, researchers have pivoted towards understanding the human olfactory system. This system, in fact, is susceptible to various infectious agents, including influenza, herpes, and coronavirus. A pivotal study by Carrasco-Yepes *et al.* (41) highlighted that nasal mucosa vaccination resulted in heightened levels of specific IgA and IgG antibodies. These antibodies, once bound to the trophozoite surfaces, inhibited their movement. The associated polymorph nucleotides (PMNs) prevented the parasite's entry and subsequent infection of the olfactory epithelium, positioning this mechanism as a primary protective barrier against the parasitic infection. Consequently, the nasal route emerges as the most effective mode of vaccination to counteract PAM (44).

Future Horizons in the Treatment and Prevention of PAM

PAM has persistently posed significant challenges to the global health community. A principal reason behind this is the low permeability of most drugs across the blood-brain barrier, which impedes effective treatment (45). Furthermore, the late manifestation of its symptoms often results in delayed diagnosis and treatment, exacerbating

its impact (46). Recent strides in research have uncovered potential therapeutic avenues sourced from marine and terrestrial flora. Compounds such as bergenin, kaempferol, and sesquiterpenes, extracted from select marine sources and plants, have demonstrated promising results in preliminary studies (30). However, a comprehensive understanding of their therapeutic potential and pharmacokinetics is essential to establish them as primary therapeutic agents. As with any promising therapy, rigorous research is needed to delineate their safety, efficacy, and potential side effects. Parallel to therapeutic advancements, prophylactic measures, particularly vaccines, stand out as the most promising avenue for the prevention and potential eradication of PAM. Vaccines are inherently proactive, aiming to pre-emptively defend the host against the causative agent, *Naegleria fowleri*. Their utilization can simplify the complexity associated with PAM treatment regimens and considerably decrease the overall disease burden.

Conclusion

Primary Amoebic Meningoencephalitis represents a profound medical challenge due to its clinical complexities and the limitations of current therapeutic interventions. However, recent advancements spotlight a promising horizon. The exploration of marine and terrestrial-derived compounds offers potential novel therapeutic avenues that could revolutionize the treatment of PAM. Meanwhile, the research and development of vaccines against the causative agent, *Naegleria fowleri*, offer an optimistic avenue for not just the treatment but potentially the eradication of PAM. Moving forward, integrated research approaches, combining both therapeutic and preventive strategies, will be pivotal in curbing the impact of this disease and enhancing global health outcomes.

Author's contribution

Conceptualization: SUR; data collection, management, and curation: SUR, NI, FUR, MWM, SS; original version's redaction: SUR, NI, FUR, MWM, SS; results interpretation: SUR, NI, FUR, MWM, SS; final version's review and redaction: SUR, NI, FUR, MWM, SS.

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Ethical aspects

Not applicable.

Interests' conflicts

The authors have no conflict of interest associated with the material presented in the manuscript.

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