

Naegleria fowleri: Pathogenesis, Diagnosis, and Treatment Options

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Naegleria fowleri has generated tremendous media attention over the last 5 years due to several high-profile cases. Several of these cases were followed very closely by the general public. *N. fowleri* is a eukaryotic, free-living amoeba belonging to the phylum Percolozoa. *Naegleria* amoebae are ubiquitous in the environment, being found in soil and bodies of freshwater, and feed on bacteria found in those locations. While *N. fowleri* infection appears to be quite rare compared to other diseases, the clinical manifestations of primary amoebic meningoencephalitis are devastating and nearly always fatal. Due to the rarity of *N. fowleri* infections in humans, there are no clinical trials to date that assess the efficacy of one treatment regimen over another. Most of the information regarding medication efficacy is based on either case reports or *in vitro* studies. This review will discuss the pathogenesis, diagnosis, pharmacotherapy, and prevention of *N. fowleri* infections in humans, including a brief review of all survivor cases in North America.

Naegleria fowleri has generated tremendous media attention over the last 5 years due to several high-profile cases. Several of these cases were followed very closely by the national news outlets, as well as the general public. Unfortunately, much of the media attention served to generate public fear rather than public education. In this review, we will discuss the etiology, pathogenesis, case studies, and treatment options for *N. fowleri*.

PATHOGENESIS

N. fowleri is an amphizoic amoeba, as it can survive in a free-living state in water, soil, or in the host, which can be the human central nervous system (CNS) (1). *N. fowleri* infections have been documented in healthy children and adults following recreational water activities, including swimming, diving, and water skiing. *N. fowleri* has been thought to infect the human body by entering the host through the nose when water is splashed or forced into the nasal cavity. Infectivity occurs first through attachment to the nasal mucosa, followed by locomotion along the olfactory nerve and through the cribriform plate (which is more porous in children and young adults) to reach the olfactory bulbs within the CNS (2). Once *N. fowleri* reaches the olfactory bulbs, it elicits a significant immune response through activation of the innate immune system, including macrophages and neutrophils (3, 4). *N. fowleri* enters the human body in the trophozoite form. Structures on the surface of trophozoites known as food cups enable the organism to ingest bacteria, fungi, and human tissue (3). In addition to tissue destruction by the food cup, the pathogenicity of *N. fowleri* is dependent upon the release of cytolytic molecules, including acid hydrolases, phospholipases, neuraminidases, and phospholipolytic enzymes that play a role in host cell and nerve destruction (1). The combination of the pathogenicity of *N. fowleri* and the intense immune response resulting from its presence results in significant nerve damage and subsequent CNS tissue damage, which often result in death.

DIAGNOSIS

Clinical symptoms and signs of infection with *N. fowleri* usually present within 2 to 8 days of infectivity, though some have been reported within 24 h (5, 6). Despite the absence of specific signs and symptoms indicating *N. fowleri* infection, the most common symptoms include severe headache, fever, chills, positive Brudz-

inski sign, positive Kernig sign, photophobia, confusion, seizures, and possible coma. In addition, cardiac rhythm abnormalities and myocardial necrosis have been observed in some cases (7). Perhaps most importantly, increases in intracranial pressure and cerebral spinal fluid (CSF) pressure have been directly associated with death. CSF pressures of 600 mm H₂O have been observed in patients with *N. fowleri* infection (5). CSF analysis has shown various abnormalities in color, ranging from gray in the early stages of infection to red in late-stage disease due to a significant increase in red blood cells (8, 9). Additional increases are seen in polymorphonuclear cell concentrations (as high as 26,000 mm³), as well as the presence of trophozoites in the CSF (using trichrome or Giemsa stain) (5, 7). Magnetic resonance imaging (MRI) of the brain often shows abnormalities in various regions of the brain, including the midbrain and subarachnoid space (5, 7).

TREATMENT OPTIONS

Due to the rarity of *N. fowleri* infections in humans, there are no clinical trials to date that assess the efficacy of one treatment regimen over another. Most of the information regarding medication efficacy is based on either case reports or *in vitro* studies. Perhaps the most-agreed-upon medication for the treatment of *N. fowleri* infection is amphotericin B, which has been studied *in vitro* and also used in several case reports. Other anti-infectives which have been used in case reports include fluconazole, miconazole, miltefosine, azithromycin, and rifampin. Various other agents have been studied *in vitro* and/or *in vivo*, including hygromycin, rokitamycin, clarithromycin, erythromycin, roxithromycin, and zeocin (10).

AMPHOTERICIN B

Amphotericin B has a minimal amoebicidal concentration of 0.01 µg/ml against *N. fowleri* (11, 12). However, *in vitro* studies have

Accepted manuscript posted online 10 August 2015

Citation Grace E, Asbill S, Virga K. 2015. *Naegleria fowleri*: pathogenesis, diagnosis, and treatment options. *Antimicrob Agents Chemother* 59:6677–6681. doi:10.1128/AAC.01293-15.

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shown that an amphotericin B concentration of at least 0.1 µg/ml was needed to suppress greater than 90% of growth, while 0.39 µg/ml was needed to completely suppress amoeba proliferation (11). The associated MIC of amphotericin B to kill 100% of the organisms in the *in vitro* studies was 0.78 µg/ml (12). Based on these findings and the limited data from survival cases of *N. fowleri*, amphotericin B, whether intravenously or intrathecally, is the cornerstone of therapy and should be used with or without other adjunctive therapies. *N. fowleri* studies in mice have shown that amphotericin B at doses of 7.5 mg/kg of body weight/day is needed to improve survival in mice infected with *N. fowleri* (11). Intravenous doses of amphotericin B of 0.25 to 1.5 mg/kg/day are recommended in adults, while doses ranging from 0.5 to 0.7 mg/kg/day are recommended in pediatric patients (13). The recommended duration of therapy with amphotericin B for the treatment of *N. fowleri* is 10 days (14, 15). The Centers for Disease Control and Prevention (CDC) recommends the conventional amphotericin B formulation over the liposomal formulation or amphotericin B methyl ester, as both these agents have been shown to have a significantly higher MIC against *N. fowleri* than conventional amphotericin B (13, 16). The CDC recommends intravenous conventional amphotericin B at doses of 1.5 mg/kg/day in 2 divided doses for 3 days followed by 1 mg/kg/day once daily for an additional 11 days (total of 14 days of therapy) (13). Intrathecal amphotericin B should also utilize the conventional amphotericin B formulation. The CDC-recommended dose of conventional amphotericin B intrathecally is 1.5 mg/day for 2 days followed by 1 mg/day for an additional 8 days (total of 10 days of therapy) (13).

Although amphotericin B has become the primary drug of choice for treating primary amoebic meningoencephalitis (PAM), its use is associated with multiple side effects, including use-limiting renal toxicity (17). Many of the problems with amphotericin B can be linked to its lack of aqueous solubility, which affects dissolution, compartmental concentration, and clearance. Recently, corifungin, which is described as a new drug entity, was granted orphan drug status for the treatment of PAM (18). Initial reports for the *in vivo* efficacy of corifungin in a mouse model of PAM showed activity superior to that of amphotericin B at equivalent dosing (18). Chemically, corifungin is the sodium salt of amphotericin B with excellent aqueous solubility (>100 mg/ml) (18). The increased solubility of corifungin is likely to account for the described increase in activity. Unfortunately, human studies that would determine whether the increased solubility will translate into therapeutic benefits such as enhanced blood-brain barrier penetration and/or reduced renal toxicity have not yet been reported.

MILTEFOSINE

In 2013, two patients infected with *N. fowleri* survived after being treated with miltefosine (discussed in further detail below). While these results are limited, they show promise for the use of miltefosine when early diagnosis is made. Chemically, miltefosine is a phospholipid with an attached choline, an alkylphosphocholine (Fig. 1). The overall molecule is amphiphilic, with a polar phosphocholine head region and an aliphatic tail. It also exists in the zwitterionic form, resulting from the permanently charged quaternary ammonium ion, as well as the anionic phosphate. The reported mechanism of action for miltefosine is inhibitory action against protein kinase B (PKB or Akt). This mechanism is plausi-

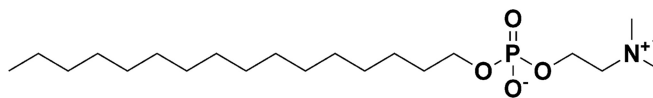


FIG 1 Miltefosine chemical structure.

ble, as miltefosine received interest and approval as an anticancer agent due to the role of phosphatidylinositol 3-kinase (PI3K) and PKB in cell survival (19). Miltefosine is still undergoing studies to determine absolute bioavailability in humans. It has demonstrated favorable (>80%) oral bioavailability in rodents and dogs (19). Evidence thus far indicates both a passive and an active transport mechanism of miltefosine absorption following oral administration (20). Miltefosine demonstrates a high level of plasma protein binding (>95%) and also has wide tissue distribution in rodent models, with the highest concentration of drug being found in organ tissues: lung, adrenal glands, spleen, and liver. It is difficult to rationalize the CNS penetration of miltefosine, as a permanently charged species, without an active transport mechanism. Specific studies of miltefosine concentrations in brain have not been described, but its effective use in *N. fowleri* infection demonstrates some level of penetration. It is not clearly understood if the blood-brain barrier of these patients has been in any way compromised due to the infection. Metabolism studies on human subjects have not been performed. However, miltefosine has been measured for oxidative metabolism against a panel of cytochrome P-450 (CYP450) isoforms, as well as the ability to induce isoforms of CYP3A. None of those evaluations predicted a significant degree of metabolism or potential for induction, suggesting that the risk for drug-drug interactions would be low (21). It is actually suspected that the breakdown products of miltefosine result from the activity of phospholipases. The primary metabolites produced in animal studies have been choline, phosphocholine, and cetyl alcohol. The cetyl alcohol is oxidized to palmitic acid (Fig. 2). Each of these metabolic by-products is naturally occurring and likely gets utilized in biosynthetic processes (19). Miltefosine has shown *in vitro* efficacy against *N. fowleri*, suggesting a possible benefit in treatment (22). *In vitro* studies have shown that *N. fowleri* amoebas have survived in miltefosine concentrations of 40 µg but not 80 µg (22). Using incremental increases in concentrations of miltefosine, a concentration of 40 µg can be considered amoebostatic (representing the MIC), while concentrations of 55 µg are amoebicidal (representing the minimum amoebicidal concentration), thus killing all amoebas exposed to this concentration of miltefosine (22). The CDC has made miltefosine available on a need basis through an investigational new drug (IND) protocol for the treatment of infections caused by free-living amoebas, which include *N. fowleri*, *Balamuthia mandrillaris*, and *Acanthamoeba* species (23). The CDC recommends miltefosine at doses of 50 mg orally two to three times daily (based on body weight) with a maximum dose of 1.5 mg/kg/day for a total of 28 days (23).

ADJUNCTIVE THERAPIES

Fluconazole. Fluconazole, an azole antifungal agent, has been used in conjunction with amphotericin B in the treatment of some cases of *N. fowleri* infection (24). The addition of fluconazole has been shown to provide some additional benefit to amphotericin B therapy (25–27). Fluconazole's efficacy may be due to its increased

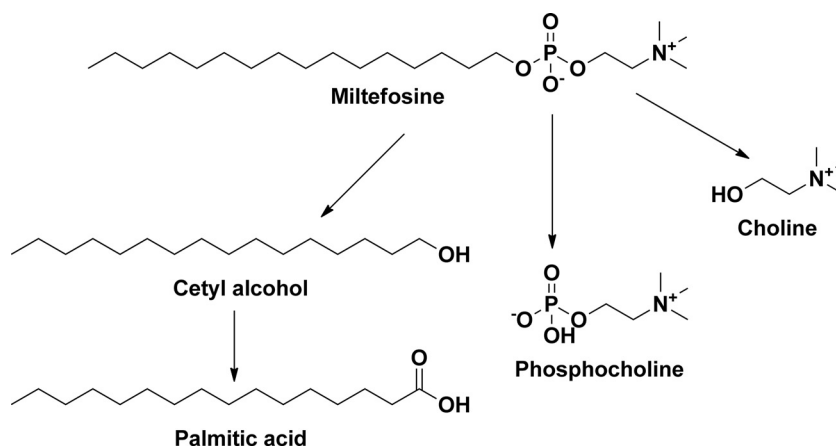


FIG 2 Metabolites of miltefosine.

penetration into the CNS. Fluconazole and amphotericin B in combination show synergistic effects on eradicating *N. fowleri* infection through recruitment of neutrophils (28). Based on these findings, fluconazole can be considered an add-on therapy to amphotericin B in patients with suspected *N. fowleri* infections. The CDC recommends intravenous fluconazole at a dose of 10 mg/kg/day once daily (maximum dose of 600 mg/day) for a total of 28 days (13). Voriconazole, a broader-spectrum azole antifungal agent, has been shown *in vitro* to effectively kill *N. fowleri* at concentrations of ≥ 1 $\mu\text{g/ml}$ (22).

Azithromycin. Azithromycin, a macrolide antibiotic, has been tested *in vitro* against *N. fowleri* and has been shown to suppress greater than 90% of organism growth at concentrations of 10 and 100 $\mu\text{g/ml}$ (21). Azithromycin has been shown to have a MIC of 10 $\mu\text{g/ml}$ against *N. fowleri* (11). *In vivo* studies in mice have shown that azithromycin administered at a dose of 75 mg/kg/day was needed to prevent death in mice infected with *N. fowleri*. The CDC recommends intravenous azithromycin at doses of 10 mg/kg/day once daily (maximum 500-mg daily dose) for 28 days (13).

Of note, other agents such as miconazole, hygromycin (aminoglycoside antibiotic; not available in the United States), rokitamycin (macrolide antibiotic; not available in the United States), clarithromycin (macrolide antibiotic), erythromycin (macrolide antibiotic), roxithromycin (macrolide antibiotic), chlorpromazine (antipsychotic), and rifampin (RNA polymerase inhibitor antibiotic) have been tested *in vitro* against *N. fowleri*, showing either a lack of efficacy or inconsistency with efficacy *in vitro* (10, 12).

Rifampin. Although rifampin has been used in all of the PAM survivor cases in the United States and Mexico (all three cases in the United States and one survivor in Mexico), its efficacy remains questionable (29, 30). The primary issue involves whether sufficient CNS penetration of rifampin at standard therapeutic dosing occurs. Multiple reports have demonstrated favorable concentrations of rifampin in the CNS as measured by drug concentrations in the CSF (31, 32). However, one report by Mindermann et al. investigated compartmental concentrations of rifampin in the CNS and found significant variations (33). Concentrations in the cerebral extracellular space and within normal brain tissue were measured at 0.32 ± 0.11 $\mu\text{g/ml}$ and 0.29 ± 0.15 $\mu\text{g/ml}$, respectively. These concentrations would exceed the required MIC for

most susceptible bacteria but may not be sufficient for eradicating *N. fowleri*. An initial report by Thong et al. in 1977 (34) showed that the natural product, rifamycin, delayed the growth of *N. fowleri* by 30 to 35% at concentrations of 10 $\mu\text{g/ml}$ over a 3-day incubation period. However, rifamycin had lost its ability to inhibit *N. fowleri* growth by the 6th day of incubation (34). Growth inhibition ($>80\%$) was sustained for the 6-day period only when higher concentrations of rifampin, a semisynthetic analogue of rifamycin (100 $\mu\text{g/ml}$), were used. A later report by Ondarza failed to demonstrate a MIC for rifampin against *N. fowleri* and showed a 50% inhibitory concentration (IC_{50}) of >32 $\mu\text{g/ml}$, which was the maximum concentration evaluated in the study (35). Based on these data, there is no evidence supporting the use of rifampin at standard doses for the treatment of PAM.

The secondary issue regarding the use of rifampin in the treatment of *N. fowleri* is the high potential for drug-drug interactions in combination therapy. Rifampin is a well-known inducer of the CYP2 and CYP3 family of monooxygenase enzymes, specifically, CYP2C9, CYP2C19, and CYP3A4 (36, 37). The greatest likelihood for interaction with rifampin lies with the 14 α -demethylase inhibitors, also known as the azole fungistatics. In a majority of the treatment cases, miconazole was initially used before giving way to fluconazole use in more recent cases. 14 α -Demethylase is a CYP450 isoform, and obvious interactions between rifampin and fluconazole have been reported (38, 39). Coadministration of the two drugs leads to significant changes in the pharmacokinetics of fluconazole: a $>20\%$ decrease in area under the concentration-time curve (AUC) and as much as a 50% decrease in critically ill patients, at least a 30% increase in clearance rate, and a 28% shorter half-life. Because synergy has been demonstrated between 14 α -demethylase inhibitors and amphotericin B against *N. fowleri*, it would appear that the addition of rifampin to the combination may be of little benefit and actually work against the maximum therapeutic effect of the other agents (40).

SURVIVOR CASES

To date, there have been only seven survivors worldwide, of whom four survivors were in North America, including three in the United States and one in Mexico. The first case of *N. fowleri* survival in North America was in the United States in 1978 (published in 1982), which involved a 9-year-old girl who had been swim-

ming in Deep Creek Hot Springs in the San Bernardino National Forest on two separate occasions. She was treated intravenously and intrathecally using both conventional amphotericin B and miconazole in addition to oral rifampin, intravenous dexamethasone, and oral phenytoin (41). In 2004, one survivor was reported in Mexico (30). This survivor was a 10-year-old male child who developed *N. fowleri* infection 1 week after swimming in an irrigation canal. The patient was successfully treated using intravenous amphotericin B for 14 days in combination with rifampin and fluconazole for 1 month. The patient was discharged from the hospital on day 23 of therapy when the brain computed tomography showed no evidence of infection. The two most recent U.S. cases both occurred in 2013 (29). The first case involved a 12-year-old girl who was diagnosed with *N. fowleri* infection 7 days after visiting a water park near Little Rock, Arkansas, and 2 days after the onset of symptoms (42). The patient was started on therapy on the same day that she presented to the emergency department, using amphotericin B (intravenously and intrathecally), miltefosine, fluconazole, rifampin, dexamethasone, and azithromycin. Additionally, her treatment included induced hypothermia to help decrease brain swelling. The patient made a full recovery following treatment (13, 42). The second case in 2013 involved an 8-year-old male in the United States who was treated with a combination of intrathecal and intravenous amphotericin B, rifampin, fluconazole, dexamethasone, azithromycin, and miltefosine (29). The patient survived the infection but suffered from brain damage secondary to the infection. The infection had been ongoing several days prior to seeking medical attention, and medically induced hypothermia was not used as in the previous case (13, 29).

PREVENTION

Steps which can be taken by individuals who participate in water-related sports in warmer climates include avoidance of exposure to freshwater bodies such as lakes, rivers, and ponds, especially during the summer months when the water temperature is higher. Both chlorinated and salt water significantly decrease the risk of *N. fowleri* infection due to the inability of *N. fowleri* to survive in such environments. If freshwater activities cannot be avoided, it is recommended that individuals avoid jumping into the body of water, splashing, or submerging their heads under the water in order to avoid *N. fowleri* entering the nasal passages. If such activities cannot be avoided, individuals should use nose clips to decrease the chance of contaminated water entering the nose. Some advocate rinsing the nose and nasal passages with clean water after swimming in fresh bodies of water; however, the effectiveness of this method is hypothetical and unknown at this time. If water is going to be used for sinus rinsing, the CDC recommends commercially available distilled or purified bottled water. In the absence of the abovementioned options, the CDC recommends treating water for sinus rinsing by either boiling or filtering the water using a filter with pores of 1 μm or smaller.

ACKNOWLEDGMENTS

We thank F. Marciano-Cabral and J. S. Yoder for their support and insight in reviewing the manuscript prior to submission.

None of the authors have any conflict of interest to disclose.

There are no funding sources to acknowledge.

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