Naegleria fowleri after 50 years: is it a neglected pathogen?

Moisés Martínez-Castillo,1 Roberto Cárdenas-Zúñiga,1 Daniel Coronado-Velázquez,1 Anjan Debnath,2 Jesús Serrano-Luna3 and Mineko Shibayama1

1Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies of the National Polytechnic Institute, Av. IPN 2508, Mexico City 07360, Mexico
2Center for Discovery and Innovation in Parasitic Diseases, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA
3Department of Cell Biology, Center for Research and Advanced Studies of the National Polytechnic Institute, Av. IPN 2508, Mexico City 07360, Mexico

It has been 50 years since the first case of primary amoebic meningoencephalitis (PAM), an acute and rapidly fatal disease of the central nervous system (CNS), was reported in Australia. It is now known that the aetiological agent of PAM is Naegleria fowleri, an amoeba that is commonly known as ‘the brain-eating amoeba’. N. fowleri infects humans of different ages who are in contact with water contaminated with this micro-organism. N. fowleri is distributed worldwide and is found growing in bodies of freshwater in tropical and subtropical environments. The number of PAM cases has recently increased, and the rate of recovery from PAM has been estimated at only 5%. Amphotericin B has been used to treat patients with PAM. However, it is important to note that there is no specific treatment for PAM. Moreover, this amoeba is considered a neglected microorganism. Researchers have exerted great effort to design effective drugs to treat PAM and to understand the pathogenesis of PAM over the past 50 years, such as its pathology, molecular and cellular biology, diagnosis and prevention, and its biological implications, including its pathogenic genotypes, its distribution and its ecology. Given the rapid progression of PAM and its high mortality rate, it is important that investigations continue and that researchers collaborate to gain better understanding of the pathogenesis of this disease and, consequently, to improve the diagnosis and treatment of this devastating infection of the CNS.

Introduction

Protozoal infections of the central nervous system (CNS) are major causes of morbidity and mortality worldwide, second only to HIV infection (Mishra et al., 2009). Recently, diseases caused by members of the free-living amoebae (FLA) group have been included in these statistics (Mishra et al., 2009; WHO, 2009). FLA are protists that are distributed worldwide. Naegleria fowleri, Acanthamoeba spp. and Balamuthia mandrillaris are the most common FLAs with medical implications; these micro-organisms can produce severe and fatal infections in the CNS of humans and other mammals (Rodriguez-Zaragoza, 1994). In this review, we will focus on the pathogenic behaviour of N. fowleri, which is the aetiological agent of primary amoebic meningoencephalitis (PAM), an acute and fulminant disease of the CNS (Schuster & Visvesvara, 2004). N. fowleri infections have been reported in healthy children and young adults who have recently participated in swimming activities in water sources contaminated with this amoeba (Marciano-Cabral, 1988) and in developing countries with a lack of control procedures or preventive information against N. fowleri (Siddiqui & Khan, 2014).

CNS infection by N. fowleri occurs by the amoebae passing through the nasal cavity, penetrating the olfactory neuroepithelium, migrating through the olfactory nerves (Fig. 1a) and crossing the cribriform plate until they reach the olfactory bulbs (OBs) (Martinez et al., 1973; Jarolim et al., 2000; Rojas-Hernández et al., 2004). Once the amoebae reach the brain, they can proliferate and induce an acute inflammatory reaction (Fig. 1b), leading to patient death in approximately 1 week (Cervantes-Sandoval...
et al., 2008a). The clinical symptoms consist of several bifrontal headaches, neck stiffness, vomiting and coma. *N. fowleri* can be isolated from soil, tap water, swimming pools, freshwater lakes and thermal springs (Marciano-Cabral, 1988). Pathogenic strains have been isolated from water at 10–45 °C, pond mud at 16 °C and soil at 8 °C; amoebae found in soil have been reported to serve as vectors for other microorganisms (Tyndall & Domingue,
The interaction between *N. fowleri* and humans occurs accidentally, given that this amoeba is not considered an obligate parasite. More recently, the infection has been associated with religious and cultural practices (Siddiqui & Khan, 2014) and, in some cases, with hygienic procedures, such as sinus irrigation using contaminated water (Diaz & Boudreaux, 2013). When an infection of the protozoan occurs, it can damage tissues after the invasion to the CNS. A broad battery of mechanisms, such as pore-forming proteins, proteases and adhesion-mediating glycoproteins, among others, are involved in the pathogenic mechanisms through which *N. fowleri* acts (Aldape et al., 1994; Herbst et al., 2002; Serrano-Luna et al., 2007; Shibayama et al., 2013) (Fig. 1a). However, to date, not all of the biological mechanisms utilized by *N. fowleri* have been elucidated, and many studies are required to provide a better understanding of its molecular pathogenesis. It is important to mention that, in the past few years, the death rate of the reported cases has been greater than 95%. This situation may be due to the symptoms and signs of PAM being similar to those of bacterial or viral meningitis or to the lack of a timely diagnosis and, therefore, the lack of a specific and appropriate treatment (Heggie, 2010).

Taxonomy

The classification system for protozoal unicellular eukaryotes developed by Levine et al. (1980) was based primarily on ultrastructural studies. The new system for classifying unicellular eukaryotes utilizes modern data obtained using morphological approaches, biochemical-pathway analysis and molecular phylogeny (Adl et al., 2005). In this classification system, *Naegleria* spp. are included in the paraphyletic supergroup Excavata (Simpson, 2003; Cavalier-Smith et al., 2015), in the group Heterolobosea (Adl et al., 2005) and the family Vahlkampfiidae (De Jonckheere, 2004; Adl et al., 2005). The *N. fowleri* genome is not yet available; however, some studies aimed at classifying *Naegleria* spp. have been performed, particularly focusing on their molecular and genetic characteristics. The origin and evolution of *Naegleria* have been evaluated: this genus includes more than 40 species, but *N. fowleri* is the only species that is known to infect and cause disease in humans (De Jonckheere, 2011). The most accepted system for the identification of *N. fowleri* species was created by De Jonckheere (2011). This molecular typing system, which is based on the sequences of internal transcribed spacers (ITS1) and 5.8S rDNA, revealed the existence of at least eight different genotypes of *N. fowleri*. These genotypes are unevenly distributed on different continents: there are three genotypes (I, II and III) in America, seven genotypes (II, III, IV, V, VI, VII and VIII) in Europe, one genotype (V) in Oceania and two genotypes (II and III) in Asia. Of these eight genotypes, only four have been identified in patients (types I, II, III and V) (De Jonckheere, 2011, 2014). Recent findings regarding the molecular machinery and biochemical pathways of *Naegleria gruberi* could provide important information that will allow phylogenetic reorganization of members of the *Naegleria* genus (Fritz-Laylin et al., 2010, 2011).

Ecology and morphology

Members of the genus *Naegleria* are distributed worldwide in soil and water (De Jonckheere, 2012) and have been isolated from fresh and warm-water lakes, streams, spas, heated but non-chlorinated swimming pools, hot springs, hydrotherapy and remedial pools, aquaria, sewage and even the nasal passages and throats of healthy individuals (Rodríguez-Zaragoza, 1994; Trabelsi et al., 2012). This amoeba has also been isolated from various animals, including reptiles, amphibians and fishes (Dyková et al., 2001; Pantchev & Tappe, 2011). However, this micro-organism has not been recovered from seawater, suggesting its sensitivity to elevated osmolarity. The vertical distribution of *N. fowleri* in water has been correlated with the presence of cyanobacteria and eubacteria; therefore, it is possible that the natural function of *Naegleria* genus is regulating bacterial populations (Kyle & Noblet, 1985). Additionally, the distribution of *Naegleria* has been associated with the concentrations of manganese and iron in the water column (Kyle & Noblet, 1985; Martinez-Castillo et al., 2015). *N. fowleri* is thermophilic and can survive temperatures of up to 45°C (Kyle & Noblet, 1987). Therefore, these amoebae proliferate mainly during the summer months, when the environmental temperature is likely to be high (Sfuentes et al., 2014).

The length of trophozoites is approximately 15–25 µm. In an axenic culture, cytoplasmic lobopodia, which are used for locomotion, are present (Fig. 2a). In addition, these organisms have cytoplasmic projections called food cups that allow them to phagocytose bacteria, yeast, erythrocytes and cellular debris (Fig. 3) (Scaglia et al., 1991).

Ultrastructural morphology of trophozoites revealed the typical features of eukaryotic cells (Schuster, 1963). Ancestral proteins related to centrioles have recently been identified (Fritz-Laylin & Cande, 2010). The cytoplasmic membrane is approximately 10 nm thick. The cytoplasm contains a large number of free ribosomes, along with ribosomes that are associated with the membranes that form
the rough endoplasmic reticulum. The cytoplasm also contains a smooth endoplasmic reticulum. Other membranes that are associated with a large number of vesicles and are organized similarly to the Golgi apparatus have been identified. In addition, abundant vacuoles of different sizes, either empty or containing different types of materials, have been observed. The mitochondria of these micro-organisms have a characteristic curved ‘dumb-bell’ shape. Lysosomes have also been identified using histochemical staining for acid phosphatases (Feldman, 1977). The presence of contractile vacuoles has been reported in several species of Naegleria (Chávez-Munguía et al., 2009).

The most evident organelle is the nucleus because of its conspicuous nucleolus (Fig. 4). The nucleus has a double membrane and a large number of pores, and the outer nuclear membrane has associated ribosomes. When N. fowleri trophozoites are incubated in solutions free of nutrients (saline solution), they can differentiate into transitional flagellates that cannot divide or feed (Fig. 2b). This flagellate differentiation involves a change in the cell shape from the pleomorphic trophozoites to a pear-shaped form with a pair or more flagella at the distal end.

The mature flagellar apparatus has the canonical 9+2 structure and is surrounded by a cytoplasmic membrane sheet (Patterson et al., 1981; Fritz-Laylin & Cande, 2010). Finally, the resistant form of N. fowleri, the cyst, is usually spherical, smooth, double walled and refractive, and measures approximately 20 µm in diameter (Fig. 2c); the wall is composed mainly of polysaccharides (Chávez-Munguía et al., 2009; Lee et al., 2014). The cysts contain pores that are sealed by a thin mucoid layer. During the early stages of cyst formation, elongated mitochondria and an endoplasmic reticulum with widened cisterns are also observed. The material of the cyst wall is synthesized and packaged by the rough endoplasmic reticulum. Once the cyst is mature, its nucleus and nucleolus are less pronounced than they were during the trophozoite stage (Marciano-Cabral, 1988; Chávez-Munguía et al., 2009, 2011).

**Epidemiology**

The amoeboid flagellate N. fowleri has attracted attention because PAM is a rapidly fatal disease. The number of reports worldwide is unclear; some authors have reported 235 cases (De Jonckheere, 2011), whereas others have reported 300 cases (Trabelsi et al., 2012). Unfortunately, only a few epidemiological studies have focused on determining its geographic distribution (De Jonckheere, 2011, 2014).

The first case of PAM was reported in 1965 in southern Australia, where the patient died of an unknown acute pyogenic meningitis (Fowler & Carter, 1965). Three more patients with a similar medical history were also mentioned. The authors described the presence of amoebic forms distinct from Entamoeba histolytica and similar to FLA (Fowler & Carter, 1965). Although the aetiological agent was not identified by autopsy, later, the scientific community considered that N. fowleri was the aetiological agent (Carter, 1969; Carter et al., 1981; Marciano-Cabral, 1988). After these reports, many cases of PAM were reported in different countries, such as the USA (Butt et al., 1968; Marciano-Cabral & Cabral, 2007; Yoder et al., 2010), Australia (Fowler & Carter, 1965; Norton et al., 2010) and the Czech Republic (Cerva & Novak, 1968). A retrospective study of PAM showed that approximately 16 cases were identified using histological samples between 1962 and 1968 (Cerva & Novak, 1968; Cerva et al., 1968; Cerva, 1969).

In 1982, 108 cases were reported in different countries of Europe, Africa, Oceania and America (John, 1982). Almost all of the cases have been mainly reported from the USA and Europe; however, the disease has spread to all of the
continents except Antarctica (Valenzuela et al., 1984; Lares-Villa et al., 1993; Cogo et al., 2004; Cubero-Menéndez & Cubero-Regó, 2004; Hara & Fukuma, 2005; Jaffar-Bandjee et al., 2005; De Jonckheere, 2011; Siddiqui & Khan, 2014). The first case in Mexico was reported in 1989 in Baja California (López-Corella et al., 1989). Since then, more than 30 cases of PAM have been reported in this country (Valenzuela et al., 1984; López-Corella et al., 1989; Lares-Villa et al., 1993; Lares-Villa, 2001; Lares-Villa & Hernández-Peña, 2010).

Recently, several clinical cases of PAM have been reported in Pakistan. These cases were not related to recreational swimming but rather to performing ablutions (Shakoor et al., 2011). In other work, it was reported that 38% of domestic water samples were positive for pathogenic FLA, of which 30% contained N. fowleri (Yousuf et al., 2013). Although many PAM cases have been reported in the literature, the incidence of this disease is considered to be underestimated (De Jonckheere, 2011). It is important to note that the epidemiological information consists mainly of clinical and research reports. Therefore, it is believed that more specific and complete epidemiological studies are necessary to determine the rate of N. fowleri infection.

Pathogenesis

N. fowleri causes PAM, an acute, severe and fatal disease in humans. To understand the interplay between the amoeba and its host, several in vivo studies have been conducted (Martinez et al., 1971, 1973; Jarolim et al., 2000; Rojas-Hernández et al., 2004; Cervantes-Sandoval et al., 2008a), in which the mouse is the most common animal model used to study the different stages of PAM. The process of N. fowleri invasion through the neuroepithelium was described in 1973. Transmission electron microscopic studies showed that N. fowleri trophozoites cross through the intercellular junctions of sustentacular cells to reach the olfactory nerve plexus, moving through the mesaxonal spaces of the Schwann cells. This invasion occurs without causing an inflammatory reaction (Martinez et al., 1971, 1973). Investigations of the early stages of PAM demonstrated that 1 h after instillation of the amoeba, they interacted with the mucus present in the nasal cavity and that, at 6 h post-inoculation, the trophozoites were surrounded by an acute inflammatory reaction, mainly consisting of neutrophils (Cervantes-Sandoval et al., 2008a); however, this innate response appeared to be insufficient to eliminate N. fowleri. Then, at 12 h post-inoculation, the amoebae attached to and penetrated the olfactory neuroepithelium (Rojas-Hernández et al., 2004; Cervantes-Sandoval et al., 2008a; Shibayama et al., 2013). Furthermore, at 30 h post-infection, trophozoites were found in the cribriform plate, and at 48–72 h post-infection, amoebae reached the OBs without causing an inflammatory reaction (Jarolim et al., 2000; Rojas-Hernández et al., 2004). At 102 h post-infection, a severe inflammatory focus consisting of eosinophils and neutrophils was observed, and the number of trophozoites in the OBs was increased. Finally, during the later stages of infection (5–7 days), extensive areas of lytic necrosis and haemorrhaging were observed, and red blood cells were found within the amoeba, suggesting that erythrophagocytosis had occurred (Rivera-Aguilar et al., 2000) (Fig. 5).

Mechanisms of pathogenicity

One of the first events that occurs during pathogen invasion is adhesion. Much effort has been exerted to identify the specific adhesion molecules of N. fowleri. For example, an integrin-like protein of 60 kDa was found in its outer membrane (Han et al., 2004). Recently, the capacity of N. fowleri to bind to extracellular proteins, such as collagen type I, fibronectin and laminin-I, was evaluated. Interestingly, the authors demonstrated that N. fowleri and Naegleria lovanensis (a non-pathogenic amoeba) expressed a differential pattern of adhesion (Jamerson et al., 2012). Another in vitro study demonstrated the differential carbohydrate expression of N. fowleri and N. gruberi (Cervantes-Sandoval et al., 2010). These findings were supported by those of Carrasco-Yepez et al. (2013), who reported that mannose residues are essential for N. fowleri to adhere to mouse nasal mucosa.

Proteases play crucial roles in parasite biology and pathogenesis. Although N. fowleri is not considered a strict parasite, proteases are involved in PAM progression. One of the first reports of its protease activity was published by Martinez et al. (1971), in which the authors proposed that ‘the destruction and lysis of the olfactory epithelium may occur from yet-to-be-defined cytolytic substances produced by amoebae’. Another report describing the secretion of proteolytic enzymes was published by Chang (1979). Herein, the author reported that pathogenic Naegleria degraded sphingomyelin that was attributed to its phospholipolytic enzymes. Three years later, phospholipases were identified in amoebic secretion products (Hysmith & Franson, 1982). During the same year, studies of isoenzyme expression revealed the presence of phosphatase.

![Fig. 5. Histopathology of the OBs infected with N. fowleri (mouse model). (a) Sections of OBs 6 days after instillation with the amoebae (arrows). Trophozoites appear to be ingested erythrocytes (arrowheads); lytic necrosis is also seen (N). Magnification, ×40. (b) Important inflammatory reaction is observed (IR). Amoebae are observed inside the inflammation areas (arrowheads). Magnification, ×60.](image-url)
and leucine aminopeptidase (De Jonckheere, 1982). The first isolated and partially characterized protease released by *N. fowleri* is a member of the cysteine protease family. This protease, which has an MW of 30 kDa, has a cytotoxic effect on BHK cells. This degradative effect was abrogated by Z-Phe-Ala fluoromethyl ketone, an irreversible cysteine protease inhibitor (Aldape et al., 1994). Other important proteolytic proteins include the naegleriapores A and B (N-A and N-B), which are toxic to human cells. During the biochemical processing of N-A and N-B, the participation of cysteine proteases was essential (Herbst et al., 2002).

In 2004, two groups of researchers demonstrated the presence of cysteine proteolytic activities in both total crude extracts and the secretion products of *N. fowleri* using gelatin zymograms. However, the specific substrate was not identified, and it was not possible to correlate these activities with PAM development (Mat Amin, 2004; Tiewcharoen et al., 2004). Another study showed the presence of a differential pattern of degradation between *N. fowleri* and *N. gruberi* in total crude extracts and in the conditioned medium. The authors found mainly cysteine proteases and small amounts of serine proteases in *N. fowleri* (Serrano-Luna et al., 2007). The same group demonstrated a mucinase activity in total crude extracts of *N. fowleri* (Cervantes-Sandoval et al., 2008b). Recently, cathepsin B and cathepsin-B-like cysteine proteases have been cloned and purified. These proteases can degrade a variety of human substrates, such as IgA, IgG, IgM, collagen, fibronectin, haemoglobin and albumin (Lee et al., 2014). Considering all of these findings, the proteases of *N. fowleri* might be excellent targets of chemotherapeutic agents directed against this pathogen (Klemba & Goldberg, 2002; Sajid & McKerrow, 2002; McKerrow et al., 2008).

Other important pathogenic mechanisms that are associated with the capacity of *N. fowleri* to invade the CNS are the active locomotion (Fulton, 1977) and phagocytosis of various host cells, including erythrocytes, microglial and neuroblastoma cells (Marciano-Cabral & John, 1983; Alonso & Zubiaur, 1985). Recently, NF-actin was associated with phagocytosis (Lee et al., 2007). This protein was localized mainly in phagosome-cup structures (amoebostomes) and in the cytoplasm and pseudopodia during phagocytosis (Sohn et al., 2010). Similarly, contact-independent lysis triggered by *N. fowleri* trophozoites has been reported (Kim et al., 2008b). This lysis was performed in a non-contact system, and the results showed morphological changes, such as cell membrane destruction and a reduction in the number of human microglial cells, due to the secretion of *N. fowleri* proteins. Moreover, a significant increase in the percentage of apoptotic cells (16%) was observed in the non-contact system compared with that in *N. fowleri* lysates (Kim et al., 2008b). Another important molecule described is NF-cHSP70: the biological roles of this protein are not clear, but it is thought that it may protect amoebae from environmental damage, particularly that caused by high temperatures. Likewise, NF-cHSP70 has been associated with the pathogenicity and proliferation of *N. fowleri* (Song et al., 2007, 2008). Additionally, *N. fowleri* utilizes mechanisms to evade the immune response, such as capping formation, and this strategy is sufficient for evading immunoglobulins (IgA and IgG) (Shibayama et al., 2003). *N. fowleri* can also avoid complement-mediated lysis, a property that has been associated with the presence of a ‘CD59-like’ molecule (Ferrante & Thong, 1979). All of the molecules and mechanisms described above have been correlated with the capacity of *N. fowleri* to produce damage. Investigations of this amoeba conducted by different groups throughout the world can contribute to a better understanding of the physiopathology of PAM and improve the management and treatment of this devastating CNS infection.

**Clinical features**

The typical symptoms of PAM appear during the first week after infection with *N. fowleri* trophozoites. There are no distinctive clinical features to differentiate PAM from other types of meningitis. Therefore, it is very important that physicians obtain a detailed clinical history of the patients (Jain et al., 2002; Naqi & Azeemuddin, 2013). The earliest symptoms include severe headache, a high fever and neck stiffness, followed by anorexia, vomiting, irritability, photophobia and neurological abnormalities, including diplopia, lethargy, seizures and coma. Cranial nerve palsies may indicate brain oedema (Trabelsi et al., 2012; Budge et al., 2013). Death occurs between the third and seventh days after symptom onset (Valenzuela et al., 1984; Yoder et al., 2012). Autopsies of PAM patients have revealed brain inflammation with severe tissue damage throughout the area of invasion, with ulceration of the olfactory mucosa and necrosis of the olfactory nerves (Sugita et al., 1999; Visvesvara, 2013). Microscopically, the OBs were almost completely disorganized by fibrin-purulent exudates and by haemorrhaging from necrotic blood vessels, and the adjacent frontal cortex exhibited the invasion of a considerable number of amoebae (Hannisch & Hallagan, 1997).

**Diagnosis**

To develop an appropriate therapy for the rapidly fatal PAM, accurate and early diagnosis is necessary because PAM is often misdiagnosed as was previously mentioned (Da Rocha-Azevedo et al., 2009). Therefore, it is imperative to perform a complete and precise clinical history. Physicians should obtain information regarding any recent patient contact with freshwater, including hot springs, and data regarding rhinitis, allergies and other diseases of the upper respiratory tract.

Computed tomography or magnetic resonance imaging studies of the brains of patients with PAM showed multicentric parenchymal lesions, pseudotumoural lesions, meningial exudates, haemorrhagic infarcts and necrosis in the brain. In addition, Kidney & Kim (1998) reported oedema...
and hydrocephalus in patients with PAM. However, these methodologies cannot differentiate among cases of meningitis with different aetiologies. A correct and prompt diagnosis can be reached by a microscopic examination of the cerebrospinal fluid (CSF) to detect motile trophozoites. The colour of the CSF of a PAM patient may range from greyish to yellowish-white, and the CSF is sometimes tinged red due to the presence of a few erythrocytes (250 cells mm⁻³). However, the red blood cell count increases during the later stages of the disease (24 600 cells mm⁻³) (Visvesvara et al., 2007).

The microscopic examination of the CSF can be supported by staining using Giemsa, haematoxylin and eosin, periodic acid-Schiff and Wright stains. However, the Gram stain is not useful in identifying Naegleria trophozoites (Martinez & Visvesvara, 1997; CDC, 2013). Under the microscope, N. fowleri trophozoites exhibit a typical amoeboid form. To corroborate this morphological observation, it is necessary to perform differential identification using the flagellation test (FT). To perform the FT, the CSF is incubated in an isotonic saline solution for 2 h. The FT is a useful tool because other amoeboid species, such as E. histolytica, Acanthamoeba spp., Sapininia spp. and B. mandrillaris, can also infect the CNS. Some N. fowleri isolates are poorly flagellated under laboratory conditions, causing their misidentification (Beheits et al., 2003). In this case, immunofluorescence techniques or ELISAs using specific antibodies can be used to reach the proper diagnosis (Martinez & Visvesvara, 1997). Different modalities of the PCR (real-time, nested and multiplex PCR) can also be employed in clinical diagnostic and research laboratories. Sensitive PCR assays to detect the presence of N. fowleri in clinical and environmental samples with high specificity have been utilized (Réveillé et al., 2002). A nested-PCR assay has been developed to detect N. fowleri amoebae in environmental samples. This method is based on the amplification of a 166 bp fragment of the Mp2Cl5 gene, which is related to the virulence of the amoeba (Réveillé et al., 2002; Maclean et al., 2004).

Other researchers developed an ITS-based PCR assay that allows the identification of Naegleria spp. (Pélandakis et al., 2000; Hara & Fukuma, 2005; Robinson et al., 2006; Madaróva et al., 2010). To improve the clinical diagnosis, a multiplex real-time PCR assay using probes specific for 185 rRNA has been developed, which allows the simultaneous detection of N. fowleri, B. mandrillaris and Acanthamoeba in the same sample (Qvarnstrom et al., 2009). However, despite the development of various sensitive PCR assays, they cannot be widely applied, and most clinical cases are confirmed by post-mortem biopsies employing haematoxylin and eosin staining (Martinez & Visvesvara, 1991). Another procedure for identifying N. fowleri trophozoites is culturing CSF samples in nutritive agars to obtain axenic cultures of amoebae (Carter, 1970). More recently, it has been proposed that a diagnosis could be made by recovering motile trophozoites from the nasal cavity by washing it with a saline solution (Baig & Khan, 2015).

**Treatment**

It is important to highlight that an appropriate diagnosis is the key to choosing an appropriate treatment. However, PAM is not commonly confirmed during the early stages of infection, and most people infected with this organism die. Because of the high mortality rate, more effective drugs are urgently needed. Drug discovery research has improved since the first report of PAM (Fowler & Carter, 1965; Carter, 1969; Rice et al., 2015). In 1969, Carter employed several drugs; he found that only amphotericin B (AmB) had an amoebicidal effect in vitro and a protective effect in vivo (Carter, 1969). Since then, AmB has been employed alone or in combination with other drugs to the treatment of PAM (Table 1). The effect of AmB on *Naegleria* was corroborated by Schuster & Rechthand (1975). They reported that the effects of AmB differed depending on the stage of the cultures, observing an amoebicidal effect during the lag phase and a proliferation-inhibitory effect during the log phase (Schuster & Rechthand, 1975). It is important to note that the AmB does not specifically target against *N. fowleri*. The biochemical mechanism underlying the effect of AmB involves lysis of the cell membrane, specifically through interaction with sterols in the membrane, even those of human cells (and more efficiently through interaction with ergosterol in fungal cells) (Brajtburg & Bolard, 1996), which is why AmB is considered toxic, mainly causing renal toxicity. AmB is generally employed at low concentrations for the treatment of fungal infections. However, clinical doses in the range 0.25–1.5 mg kg⁻¹ day⁻¹ have been employed to treat PAM (Apley et al., 1970; Poungvarin & Jariya, 1991; Tiphine et al., 1999).

The first report of clinical cases of humans for whom AmB was employed to treat PAM appeared in 1970 (Apley et al., 1970). In this report, three cases involving children in Great Britain were reported. One of the cases was fatal, despite early diagnosis and treatment, whereas the treatment was successful for the other two patients. However, one of the patients was initially diagnosed with a severe sore throat and was treated with oral penicillin. Subsequently, pyogenic meningitis was diagnosed, and the treatment was changed to intravenous administration of AmB at a dose of 0.25–1 mg kg⁻¹ plus sulfadiazine at a dose of 750 mg kg⁻¹. Unfortunately, the patient died approximately 2 weeks after admission. In contrast, the other two patients were treated with the same regimen of AmB and sulfadiazine but not with penicillin. They had a complete symptom-free recovery (Apley et al., 1970). Later, AmB was continuously employed during the treatment of PAM, even when used in combination with other drugs. In 2002, a 26-year-old female was diagnosed with PAM and was treated with AmB at a dosage of 1 mg kg⁻¹ daily, rifampicin at 450 mg kg⁻¹ daily and ornidazole at a dosage of 1500 mg day⁻¹ until she recovered completely, which took 3 weeks (Jain et al., 2002). To date, AmB is the antibiotic for which there is the most clinical evidence of the successful treatment of humans with PAM. However, only 15 cases of recovery have been reported worldwide (Apley et al., 1970; Duma et al., 1971; Anderson & Jamieson, 1972; Brown, 1991;
Table 1. Clinical reports of PAM associated with AmB treatment

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Treatment</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apley et al.</td>
<td>1970</td>
<td>AmB, sulphanilamide</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Duma et al.</td>
<td>1971</td>
<td>AmB</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Anderson &amp; Jamieson</td>
<td>1972</td>
<td>AmB</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Brown</td>
<td>1991</td>
<td>AmB</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Poungvarin &amp; Jariya</td>
<td>1991</td>
<td>AmB, rifampicin, ketoconazole</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Loschiavo et al.</td>
<td>1993</td>
<td>AmB</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>1993</td>
<td>AmB, rifampicin, chloramphenicol</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Jain et al.</td>
<td>2002</td>
<td>AmB, rifampicin and ornidazole</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Vargas-Zepeda et al.</td>
<td>2005</td>
<td>AmB, dexamethasone, fluconazole, rifampicin</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Yadav et al.</td>
<td>2013</td>
<td>AmB, rifampicin, fluconazole</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Sood et al.</td>
<td>2014</td>
<td>AmB, rifampicin, fluconazole</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>2015</td>
<td>AmB</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Linam et al.</td>
<td>2015</td>
<td>AmB, rifampin, fluconazole, azithromycin, miltefosine, dexamethasone</td>
<td>Clinical report</td>
</tr>
<tr>
<td>Cope et al.</td>
<td>2016</td>
<td>AmB, rifampin, fluconazole, azithromycin, miltefosine, dexamethasone</td>
<td>Clinical report</td>
</tr>
</tbody>
</table>

Poungvarin & Jariya, 1991; Loschiavo et al., 1993; Wang et al., 1993; Jain et al., 2002; Vargas-Zepeda et al., 2005; Yadav et al., 2013; Sood et al., 2014; Linam et al., 2015; Sharma et al., 2015; Cope et al., 2016). Recently, an investigational breast cancer and anti-Leishmania drug, miltefosine (Dorlo et al., 2012; Kaur et al., 2015), has shown promise when used in combination with other drugs, and a PAM patient was successfully treated using miltefosine and hypothermia; however, the patient suffered permanent brain damage (Cope et al., 2016). Because of the toxicity of AmB and the low rate of recovery from PAM (5%), researchers are interested in finding new and more effective treatments for this disease. Additionally, some adjuvative therapies have been employed in the treatment of PAM such as the use of anti-inflammatory agents (e.g. dexamethasone) and non-pharmacological procedures like CSF drainage, hyperosmolar therapy, moderate hyperventilation and hypothermia (Cope et al., 2016). Some of the studies aimed at finding novel therapeutic drugs focussed on compounds such as acrolein, isoflavans, rokitomicin, miltefosine/chlorpromazine, corifungin and certain amidino derivatives that showed amoebicidal effects in Naegleria cultures and conferred protection in a mouse model (Zhang et al., 1988; Belofsky et al., 2006; Kim et al., 2008a, c; Debnath et al., 2012; Rice et al., 2015).

Conclusion

PAM is an acute and fatal disease that has recently become more common in both developed and underdeveloped countries. The number of PAM cases may increase due to global warming, global overpopulation and increased industrial activities. It is urgent that the health community, including medical and diagnostic laboratory technicians, be aware of this disease in order to make timely diagnosis that could save patients’ lives. The knowledge of the biology and pathogenesis of N. fowleri in the past 50 years could be used to make faster diagnosis and design new drugs against specific targets to eliminate the amoeba and increase the survival of the patients.

Acknowledgements

We are grateful to Angélica Silva-Olivares for her technical assistance in obtaining the transmission and scanning electron microscopy images of N. fowleri. This study was supported by CONACyT grant number 237523.

References


Carter, R. F. (1970). Description of a Naegleria sp. isolated from two cases of primary amebic meningoencephalitis, and of the experimental pathological changes induced by it. J Pathol 100, 217–244.


