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Primary Amoebic Meningoencephalitis: Neurochemotaxis and Neurotropic Preferences of *Naegleria fowleri*

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ABSTRACT: *Naegleria fowleri* causes one of the most devastating necrotic meningoencephalitis in humans. The infection caused by this free-living amoeba is universally fatal within a week of onset of the signs and symptoms of the disease called primary amoebic meningoencephalitis (PAM). In all the affected patients, there is always a history of entry of water into the nose. Even though the diagnostic and treatment protocols have been revised and improved, the obstinate nature of the disease can be gauged by the fact that the mortality rate has persisted around ∼95% over the past 60 years. Some of the unanswered questions regarding PAM are is there a neurochemical basis of the chemotaxis of *N. fowleri* to the brain? What immune evasion means occurs preceding the neurotropic invasion? What is the contribution of the acute inflammatory response in the fatal cases? Can a combination of anti-amoebic drugs with antagonism of the acute inflammation help save the patient's life? As prevention remains the most valuable safeguard against *N. fowleri*, a quicker diagnosis, better understanding of the pathogenesis of PAM coupled with testing of newer and safer drugs could improve the chances of survival in patients affected with PAM.

KEYWORDS: *Naegleria fowleri, neurochemotaxis, primary amoebic meningoencephalitis, neuroinflammation, parasitic brain diseases, M1 receptors, CHRM1*

Naegleria fowleri is a free-living amoeba that is the cause of a fulminating infection of the brain and its covering meninges called PAM. *N. fowleri* survives in freshwater environments by feeding on microbes like bacteria. The incidences of PAM mostly occur worldwide in summers when the mercury touches 40 °C or higher, which favors the infective stage of *N. fowleri* called trophozoites. This pathogen grows at higher temperatures up to 46 °C. The patients affected by PAM have a history of nasal entry of water (Figure 1A) during water related sports such as swimming,[1] water boarding in warm freshwater places, like lakes and rivers, or during nasal cleansing with neti pots and performing ablution. These incidences and practices lead to the introduction of *Naegleria fowleri* into the nasal cavity, which increases the chances of PAM (Figure 1B). In very exceptional cases, PAM could also develop when contaminated water from inadequately chlorinated swimming pool or contaminated tap water enters the nose. Very little is known regarding the host−parasite interaction before *N. fowleri* invades the neural tissue. In most of the fatal cases, the postmortem findings reveal intense necrosis of the neural tissue and hemorrhage within the olfactory bulb and the brain.

NOSE MAP OF *N. FOWLERI* TO THE BRAIN

The host−parasite interaction in lower part of the nasal cavity remains obscure. Does the nasal cavity lack adequate innate immune resistance to combat the invasion of this parasite or does *N. fowleri* elude the innate immunity in the nasal cavity? These are some straightforward, but unanswered questions. Whatever the mechanism, it appears that the phagocytic tissue damage by *N. fowleri* does not begin within the non-olfactory parts of the nasal cavity. The patients affected by PAM do not show any evident clinical signs and symptoms of nasal inflammation, like nasal bleeding, pain, tenderness at the bridge of the nose, sneezing, and/or persistent rhinorrhea before developing signs of meningitis in PAM. Additionally, postmortem findings reveal damage and destruction mostly in the olfactory mucosa, olfactory bulb, and adjacent areas of the brain (Figure 1B), but a similar destruction is not observed in the region of non-olfactory mucosa of the nasal cavity. This finding hints toward an important feature in the pathogenesis of PAM that is the intention of the trophozoite of *N. fowleri* is more neurotropic than nasotropic. The facts that these trophozoites cross a sieve like bone called "cribriform plate" to reach the olfactory bulb and frontal lobe of the brain reflects the choice of the trophozoite for this ascent (Figure 1E). If the mobility of the pathogen had been due to chemokinesis and not chemotaxis, there would have been damages within the nasal mucosa, like the ones seen in the olfactory bulb and the frontal lobe of the brain in patients affected by PAM. The chemotactic mobility of the trophozoites across the olfactory mucosa to the cribriform plate and then to the brain tissue takes the complex pathogenetic process to the next level and generate a complex query, which is the identity of the chemical that generates a neurochemical gradient for the chemotaxis of *N. fowleri* toward the brain.

THE RECEPTOR AND CHEMICAL FOR NAEGLERIAL NEUROCHEMOTAXIS

The finding of a chemical that serves as a lure for *N. fowleri* to show chemotaxis toward the brain has unarguably remained the most challenging and fascinating query for the public, in general, and scientists doing research on amoeba worldwide, in particular. Answering this question via a real-time study in humans done by intranasal introduction of trophozoites of *N.*

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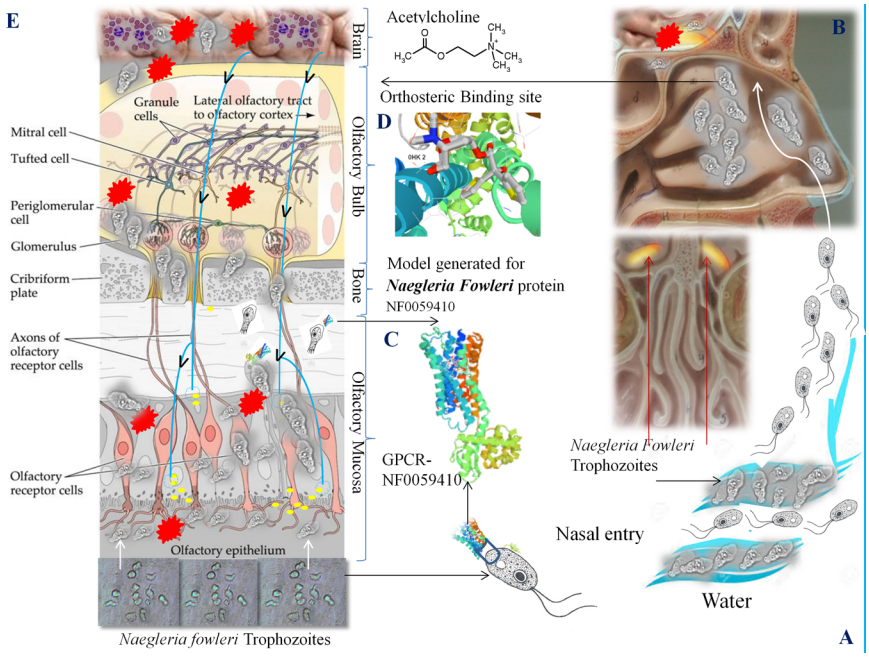

Figure 1. (A) Route of *N. fowleri* entry into the nose. (B) Olfactory mucosa and olfactory bulb (yellow) are targets of *N. fowleri*. (C) Trophozoites of *N. fowleri* express a GPCR NF0059410 that is a structural homologue of human muscarinic M1 receptor. (D) Binding of acetylcholine to this GPCR in olfactory mucosa can set up a neurotropic chemotaxis in the trophozoites that move upward across the cribriform plate (E). Destruction seen at postmortem sites is shown as red spots. Note the blue lines with black arrowheads represent the cholinergic olfactory modulating nerves that reach the olfactory mucosa to secrete acetylcholine (yellow dots). The prefrontal cortex shown above (E) is rich in acetylcholine secreting neurons (see text), which could further favor the neurochemotaxis of the *N. fowleri* trophozoites. The 3D models (C, D) were generated by submitting FASTA sequence of amino acids of the *N. fowleri* protein NF0059410 to Swiss Model.

fowleri is not possible. In addition, animal models cannot be a replica for humans for such a study, as the anatomy and histology of the nasal cavity and olfactory mucosal differs considerably in herbivores and carnivores (mostly macrosmatic mammals) and humans (microsmatic mammals). Moreover, the innate immune response of other species selected for experiments would be different from that of humans. Alternatively, the mystery of the reason why a selective roadmap is taken by *N. fowleri* toward the brain could be solved by providing evidence or justification of a chemoattractant driven craving of *N. fowleri* for the olfactory region and cerebral neurons. Like human white blood cells, validation of the presence of a cell surface receptor in *N. fowleri* that binds a specific chemoattractant as ligand with an incentive for its proliferation and mobility could explain the selective chemotaxis of trophozoites of *N. fowleri* toward the neural tissue. Additionally, downstream signaling originating from the such a receptor, if it promotes the actin and microfilament assembly, could further fortify the chemical basis of neurochemotaxis shown by this free-living amoeba.

In addition to its function as neurotransmitter, studies have shown that acetylcholine also acts as a chemoattractant for eukaryotic cells like neurons, neutrophils, and smooth muscles.[2] The olfactory region and frontal lobe of the brain are neurological tissue well-known for secretion of acetylcholine. Recent studies have reported the presence of cholinergic and adrenergic nerves in olfactory mucosa secreting olfactory modulating chemical ligand like noradrenaline and acetylcholine, respectively[3] (Figure 1E). Regarding acetylcholine release in the cortical areas of the brain, the basal forebrain (area involved in PAM), is a region composed of several cholinergic nuclei.[4] Of few known GPCRs in *N. fowleri*, a cell surface G-protein coupled receptor (GPCR) that has a structural homology to the acetylcholine binding human M1 muscarinic receptor subtype (mAChR1) is shown (Figure 1C). This finding, coupled with the fact that downstream signaling from this receptor promotes actin assembly, an event important for chemotaxis, reinforces the neurochemotaxis postulate. Though other chemicals and neurotransmitters like dopamine, glutamate, and 5HT could also be involved, the presence of these receptor types and their potential to dock a chemotatic ligand is yet to be determined in *N. fowleri*. During the erosion of the olfactory mucosa, the exposure of *N. fowleri* trophozoites to chemotaxis inducers and growth promoting chemicals like acetylcholine and related neurotransmitters could initiate a chemotactic craving in the trophozoites by binding to allosteric or orthosteric ligand binding site (Figure 1C,D), thus causing their locomotion oriented along a chemical gradient toward the brain (Figure 1E). Recently, the findings of amoebistatic and amoebicidal effects of antimuscarinic drugs (Figure 1D) on *N. fowleri* that target muscarinic M1 receptor subtype in particular further augments the role of this receptor in survival, growth, and motility of this free-living amoeba. More research is needed in this area to validate other receptors, their ligand, and chemotatic potentials of chemicals capable of setting a neurotropic locomotion in *N. fowleri*.

CHALLENGES IN THE DIAGNOSIS AND MANAGEMENT OF PAM

Signs and symptoms of PAM usually include severe headache; sometimes an altered sense of smell, fever, and vomiting[1] precede symptoms like stiff neck, seizures, apprehension, hallucinations, and coma. The disease causes the death of the affected patient within an average of 5 days after onset of signs

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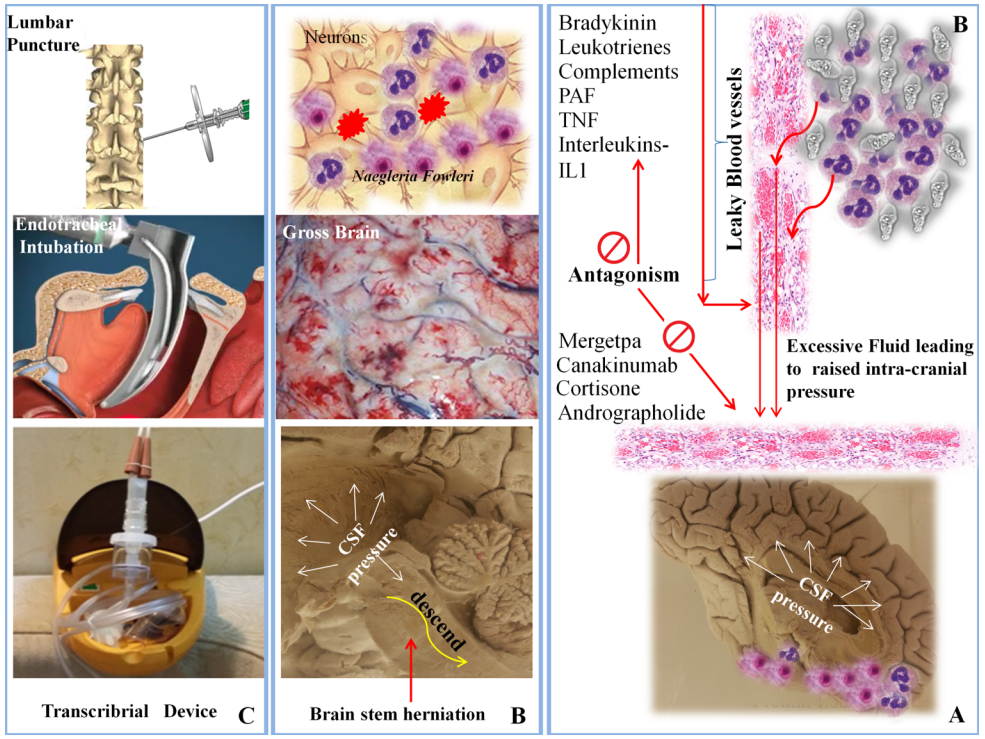

Figure 2. (A) Top right shows *N. fowleri* attacked by WBC that commence an acute inflammation and the secretion of mediators, which causes the plasma to leak from microcirculation, resulting in a raised intracranial pressure (ICP) in PAM patients. Antagonism of these chemical mediators by pharmacological agents could prove life-saving in PAM. (B) From top to bottom shows the neuronal damage, the appearance of the brain at postmortem, and the dangers of the raised ICP. (C) Lumbar puncture at L4−L5 level to obtain CSF for diagnosis, and an endotracheal intubation which is often needed in PAM patients. Also shown is the "transcribrial device" that can deliver the drug at the epicentre of *N. fowleri* proliferation

and symptoms. Usually the diagnosis is established after the patient's death as the disease is thought to be due to bacterial meningitis, to which it resembles clinically. In the majority of the cases, it is only when conventional antibiotics fail to improve the patient's condition, an unconventional cause like *N. fowleri* is given consideration. Even when PAM is suspected, a sample of cerebrospinal fluid collection (Figure 2C) to detect *N. fowleri* becomes a challenge because of the raised intracranial pressure (Figure 2A,B), as the latter is a contraindication for lumbar puncture. Deeper irrigation of the upper part of the nasal cavity with saline in patients with PAM to obtain nasal fluid for diagnostic purposes has been proposed, but this method has not been tested yet.[5] Along with antiparasitic drugs, maneuvers to reduce the intracranial pressure and attempts to preserve brain function are done. A ventriculo-peritoneal shunt is placed to drain the excess CSF from the brain to the abdominal cavity.[1] Simple aspiration, endoscopic evacuation, and stereotactic aspiration have been done when the CT scans reveal substantial intracerebral hemorrhages. The choice of procedure is the call of an experienced neurosurgeon. Recently, other methods like transnasal evaporative cooling are being evaluated for preventing brain damage in PAM patients. Induction of hypothermia may prove beneficial in PAM patients,[1] but it is not a routine practice in the management of PAM in many countries. Drug therapy to reduce the intracranial pressure also mandates careful monitoring of the intracranial pressure with drugs like mannitol that could potentiate cerebral edema in PAM patients, in the presence of intracerebral bleeding,[5] thus acting as a double-edged sword.

DRUG TREATMENT OF PAM

As of 50 years with drugs used in the treatment of PAM, there appears to be very limited success if any, as the mortality remains above ∼95%. Only four survivors have been reported with two of them being very recently, which is possibly because they were affected by a less virulent strain of *N. fowleri* or an early diagnosis. The successful management of these patients and an ambitious uneventful recovery depend upon the surgical maneuvers and a combination of antiparasitic drug therapy that targets the trophozoites at least two or more cellular targets.[1] Few drugs have been tested with in vitro and in vivo animal models with an exceptional kill rate of *N. fowleri* trophozoites, but are awaiting human trials, and could prove beneficial in PAM patients once their safety and efficacy is established. Alternatively, an aggressive approach could be the use of antagonist of acute inflammatory mediators (Figure 2A) that are involved in the formation of excessive fluid in the extracellular spaces and therefore are the cause of the raised intracranial pressure and cerebral edema in PAM. The possible role of the acute inflammatory mediators and their suggested antagonism is shown in (Figure 2A). The antagonism of the mediators of acute inflammation is expected to lower the irtacranial pressure, as it would slow down the basic pathogenetic process responsible for causing leakiness in the cerebral microcirculation and therefore prevent or delay the brainstem herniation that becomes the cause of death in patients with PAM. In an event of a delay in herniation of the brainstem, there would be a ray of optimism because it would give an adequate amount of time for the antiparasitic drugs to extert their amoebicidal effects on trophozoites of *N. fowleri*.

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The drug delivery route is also a challenge when it comes to the treatment of PAM patients. We at our laboratories have assembled a device (Figure 2C) that takes a novel transcribrial route for the delivery of the antiparasitic drugs which otherwise do not cross the blood-brain barrier when given by intravenous and other parenteral routes.[6] This device has to be tested in experimental animal models and in humans suffering PAM to gauge its benefits, if any.

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Notes

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REFERENCES

(1) Linam, W. M., Ahmed, M., Cope, J. R., Chu, C., Visvesvara, G. S., da Silva, A. J., Qvarnstrom, Y., and Green, J. (2015) Successful treatment of an adolescent with Naegleria fowleri primary amebic meningoencephalitis. *Pediatrics 135* (3), e744−8.

(2) Gerthoffer, W. T., Schaafsma, D., Sharma, P., Ghavami, S., and Halayko, A. J. (2012) Motility, Survival and Proliferation. Compr Physiol. *Compr. Physiol. 2* (1), 255−281.

(3) Hall, R. A. (2011) Autonomic modulation of olfactory signaling. *Sci. Signaling 4* (155), pe1.

(4) Bloem, B., Poorthuis, R. B., and Mansvelder, H. D. (2014) Cholinergic modulation of the Medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. *Front. Neural Circuits*, DOI: 10.3389/fncir.2014.00017.

(5) Baig, A. M., and Khan, N. A. (2015) Tackling infection owing to brain-eating amoeba. *Acta Trop. 142*, 86−88.

(6) Baig, A. M., and Khan, N. A. (2014) Novel chemotherapeutic strategies in the management of primary amoebic meningoencephalitis due to Naegleria fowleri. *CNS Neurosci. Ther. 20* (3), 289−290.

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