Balamuthia mandrillaris Encephalitis: Survival of a Child With Severe Meningoencephalitis and Review of the Literature

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Balamuthia mandrillaris causes granulomatous amoebic encephalitis (GAE), which is frequently fatal. There are few reports of survival in children. A 4-year-old child developed severe meningoencephalitis with multiple intracranial ring-enhancing lesions. Empiric therapy was commenced after a biopsy was performed, and the patient had a good clinical response. Molecular testing and indirect immunofluorescence later confirmed the diagnosis of Balamuthia encephalitis. Diagnosis of Balamuthia encephalitis is often delayed. The literature is reviewed with particular reference to reported survival. Prompt tissue diagnosis and initiation of therapy are common features among survivors. In previous reports, miltefosine was not used to treat children, but it was well tolerated in this case and should be considered as a therapeutic option.

Key words. Balamuthia; Encephalitis; Amoeba; Miltefosine

Balamuthia mandrillaris is a mitochondria-bearing free-living amoeba, which has been known to cause a rare and usually fatal granulomatous amoebic encephalitis (GAE) in both immunosuppressed and immunocompetent individuals [1]. In addition, B mandrillaris has been isolated or identified in soil, dust, and water [2–4]. Balamuthia mandrillaris is believed to gain access to the body via broken skin or inhalation and later disseminate hematogenously to the central nervous system, causing GAE. The incubation period for Balamuthia GAE is not clearly known, but researchers believe that clinical symptoms may manifest from 2 months to 2 years after infection [1]. Balamuthia GAE has recently received much attention because of its manifestation within 20 days of transplantation in solid organ transplant recipients [5, 6]. We report this case to emphasize the importance of considering amoebic infection in cases of encephalitis, in which an aetiological agent is not established by first-line investigations, and to highlight our experience with the use of miltefosine in a child with GAE.

CASE HISTORY

A 4-year-old girl, with new onset seizures, a 2-month history of headaches, and intermittent gait abnormality, was referred to our institution from a regional Queensland hospital. She had been diagnosed with complex partial seizures and commenced on sodium valproate. Progression of her seizures prompted magnetic resonance imaging (MRI), which revealed multiple, well defined focal lesions, which were dispersed throughout the cerebral and cerebellar hemispheres and brain stem (Figure 1A). The child had no history of fever. The results of an eye examination revealed bilateral papilledema, but there was no other neurological abnormality. The Glasgow Coma Scale score was 15 and clinical examination was otherwise normal. There was no significant past medical history and she was fully immunized. The patient lived on a suburban property, in a large rural town with surrounding farm properties. The area and the property on which she lived underwent severe flooding 8 months before her presentation.
She had no significant travel history or any known infectious human or animal contacts.

Initial investigations were normal with normal acute phase reactants and normal hepatic and renal indices: hemoglobin 119 g/L; white cell count of $8.9 \times 10^9$/L (normal neutrophil and lymphocyte counts); C-reactive protein level of 1 mg/L; and an erythrocyte sedimentation rate of 25 mm/h. The differential diagnosis included infection and neoplastic and inflammatory causes, including demyelinating disease, but a broad range of further investigations did not provide a diagnosis. Empiric antibiotics were commenced for central nervous system (CNS) infection.

In view of the concern of raised intracranial pressure (ICP), a craniotomy and open brain biopsy was performed 4 days after admission. The dura was noted to be tight, and the brain had an inflamed, exudative appearance. Cerebrospinal fluid (CSF) analysis revealed mononuclear pleocytosis and slightly raised protein levels: leucocytes $59 \times 10^6$/L (100% mononuclear cells), erythrocytes $1 \times 10^6$/L, glucose 2.3 mmol/L, and protein 0.56 g/L. No organisms were seen, and staining for Cryptococcus was negative. Further testing of CSF looking for toxoplasma, mycobacterial, and fungi did not yield any positive results. Histology on biopsy specimens revealed chronic granulomatous inflammation, with necrosis and lymphocytic infiltrates. No organisms were seen.

A 3-day course of methylprednisolone was commenced after the biopsies, in view of the raised ICP and gross appearances in keeping with an inflammatory disorder, and because no infectious cause had been found. Three days after completion of methylprednisolone, the patient deteriorated, with fevers above 39°C, poor appetite, lethargy, and occasional seizures. She developed signs of raised intracranial pressure and became irritable and sleepy.

An infectious etiology was now believed to be most likely, and the possibility of an amoebic infection was considered. The patient was commenced on empiric treatment for *B. mandrillaris* amoebic encephalitis while testing was undertaken. Brain tissue samples and serology samples were sent for testing to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. [The decision to proceed with empiric treatment for *B. mandrillaris* encephalitis was prompted by the recollection of 2 similar cases in which the patients unfortunately succumbed, and in which diagnosis of *B. mandrillaris* encephalitis was made post mortem. One case was reported from Western Australia [11]. The second was a patient who attended our institution more than a decade ago. She was an 11-year-old girl, also from rural Queensland, who presented with persistent fever, headache, and mild meningism. She had a sudden deterioration with raised intracranial pressure, and a computed tomography scan showed a right parasaggital lesion. She progressed and died despite aggressive antimicrobial and neurosurgical intervention. Post mortem histology and IIF on the lesion revealed chronic granulomatous inflammation and *B. mandrillaris* amoebic trophozoites.] Biopsy samples were also sent to a local laboratory for polymerase chain reaction (PCR) testing for *B. mandrillaris*, using a species-specific assay [7].

Empiric treatment comprised 50 mg/kg flucytosine intravenously (iv) every 6 h, 12 mg/kg fluconazole iv daily, 10 mg/kg azithromycin iv daily, 4 mg/kg pentamidine iv daily, and 40 mg/kg sulfadiazine orally every 6 h. The patient improved within 48 h, with resolution of her fevers, lethargy, vomiting, and seizures.

Polymerase chain reaction for *B. mandrillaris* was positive. Serology and indirect immunofluorescence (IIF) on brain tissue, performed at the CDC subsequently, confirmed the diagnosis. After reviewing histological

![Figure 1. A, Magnetic resonance image showing multiple well defined cerebral lesions. B, Histology of the brain biopsy specimen, showing granulomatous inflammation and an amoebic trophozoite with characteristic double nucleoli (circled, magnification 100 x; arrow, magnification 1000x), and after immunofluorescence staining for *Balamuthia mandrillaris*.](image-url)
specimens at the CDC, the authors noted occasional amoebic trophozoites (Figure 1B).

Significant, persistent hypoglycaemia occurred as a side effect of pentamidine; this was ceased after 4 weeks, and 50 mg of miltefosine was administered orally per day. Over the subsequent weeks, treatment was further rationalized based on continued improvement, with discontinuation of sulfadiazine, and a switch to oral agents. The patient was discharged and given the following oral regimens: 10 mg/kg azithromycin per day, 6 mg/kg fluconazole per day, and 50 mg of miltefosine per day, all of which are ongoing 8 months after discharge. Serial magnetic resonance scans have shown a gradual reduction in the size of the lesions. The patient has no residual neurological signs at this stage and has returned to kindergarten, without any difficulties reported.

DISCUSSION

More than 150 cases of *B. mandrillaris* encephalitis have been reported in both immunocompromised and immunocompetent individuals, but there are few surviving cases and only 4 reports to date of survival in children [1,8,9, unpublished personal communication]. Cases of infection have been reported from around the world, most commonly the southern US and South America, but also from Europe, Asia, Japan, and Australia [10–14]. The 2 cases reported here were both from rural areas in Western Queensland.

Delay in diagnosis is a common feature in case reports and diagnosis is frequently made post mortem. Patients often present with advanced disease. The median survival following admission to hospital was 16.5 days in 1 case series [15]. Central nervous system imaging is usually abnormal at presentation, often with multiple ring-enhancing lesions, but hydrocephalus and ventriculomegaly have also been described [15].

Diagnosis is challenging for a number of reasons. This is a rare infection and empiric treatment for a variety of other conditions (eg, vasculitides, *Toxoplasma*, and neurocysticercosis) is often initiated before the diagnosis is made. Skin is thought to be site of entry of *B. mandrillaris* trophozoites, followed by haematogenous spread. In some cases, skin lesions, in which trophozoites have been identified by biopsy, have preceded CNS infection [10,16], but this is not always the case, and none were noted in these cases. Microbiological diagnosis is difficult and requires a high index of suspicion, requiring specific IIF staining of biopsy tissue for diagnosis [17]. The histological findings on brain biopsy are nonspecific, characterized by chronic granulomatous encephalitis with poorly formed granulomas and lymphocytic infiltrates. Amoebae were difficult to see on histology in this case and were not present in high numbers. If seen, amoebic trophozoites tended to be in perivascular locations [18].

The established standard for diagnosis of *B. mandrillaris* infection has been IIF on brain tissue sections [17]. In some cases, serology was also helpful [15]. More recently, molecular assays have been developed for identification of the organism [4,7,19], and this method offers potential for earlier diagnosis. Real-time PCR assays are less expensive and time-consuming than the standards, and they use smaller quantities of specimen. PCR is potentially more sensitive than IIF, targeting the 18s RNA gene, of which there are greater than 40 copies per cell. In this case, molecular testing was performed by a local laboratory using a single-plex assay, to preserve sample and allow triplicate sample testing. With advancing knowledge and experience with PCR, testing for unusual organisms at a local level may lead to early diagnosis. Caution with such testing is advisable, however, because validation of molecular assays can be difficult without frequent clinical samples. Confirmation of the diagnosis, in this case using the gold standard test, was critical to ongoing management.

Table 1 summarizes the demographics, clinical features, and treatment regimens of the 10 known surviving cases of *B. mandrillaris* encephalitis. In particular, we examined the timing of diagnostic testing and treatment after the onset of neurological symptoms in 7 case reports where there was sufficient information. It is notable that 5 of the 7 underwent brain biopsy or had re-examination of biopsies from pre-existing skin lesions within 1 week of onset of neurological symptoms, from which *B. mandrillaris* was identified, facilitating the early introduction of treatment. In the case reported here, empiric therapy for *B. mandrillaris* infection was initiated before identification of amoebae. To our knowledge, this is the only case where empiric treatment has been successfully introduced in *B. mandrillaris* encephalitis and may have contributed to the patient’s favourable outcome.

Data on efficacy of treatments of *B. mandrillaris* infection are lacking. Those successfully treated usually received combinations of fluconazole, pentamidine, sulfadiazine, flucytosine, thiordiazine, and macrolide antibiotics [5,6,20]. There is some evidence for amebicidal activity of azithromycin and the phenothiazines, but the toxicity profile of the latter has been a consistent problem [16]. Three Peruvian survivors were treated with albendazole, itraconazole, and trimethoprim-sulfamethoxazole [20], but progression from skin lesions to CNS disease has occurred in a patient on this regimen [15,19]. In 1 case, surgical excision of a single lesion in combination with antimicrobial therapy was associated with a successful outcome [10].
**Table 1. Features of Surviving Cases of *Balamuthia mandrillaris* Encephalitis Including Sites of Infection, Treatment Regimens, Demographics, and Timing of Diagnostic Testing and Treatment**

<table>
<thead>
<tr>
<th>Reference, year</th>
<th>Age</th>
<th>Country</th>
<th>Neurological Outcome</th>
<th>Treatment Regimen</th>
<th>Site of Infection</th>
<th>Time to Biopsy and Treatment After Neurological Symptoms</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deetz, 2003</td>
<td>64</td>
<td>US</td>
<td>Significant problems slowly improved after 5 years</td>
<td>8 g of fluocytosine per day, 400 mg of fluconazole per day, and 6 g of sulfadiazine per day for 5 years; 500 mg of clarithromycin per day for 2 years; and 4 mg/kg pentamidine isethionate per day and 20 mg of trimethoprim per day for 18 days</td>
<td>Skin, CNS</td>
<td>Brain biopsy 1 week: BM identified and treatment commenced 16 days</td>
<td>5 years</td>
</tr>
<tr>
<td>Deetz, 2003</td>
<td>5</td>
<td>US</td>
<td>Some performance problems but no gross neurological deficit</td>
<td>110 mg/kg fluocytosine per day and fluconazole 4 mg/kg per day for 2.4 years; 1 mg/kg pentamidine isethionate per day for 34 days; 14 mg/kg clarithromycin per day for 2.4 years; and 1 mg/kg sulfadiazine per day for 1.8 years</td>
<td>CNS</td>
<td>Excisional biopsy day 48. <em>Balamuthia Mandrillaris</em> identified (CDC) and treatment commenced.</td>
<td>2.4 years</td>
</tr>
<tr>
<td>Jung, 2004</td>
<td>72</td>
<td>US</td>
<td>Intact</td>
<td>400 mg of fluconazole per day, 6 g of sulfadiazine per day, and 1500 mg of clarithromycin per day; and 300 mg of pentamidine isethionate per day; duration of therapy is unknown</td>
<td>CNS</td>
<td>Early MRI and excisional biopsy. Commenced treatment and discharged home day 13</td>
<td>6 months</td>
</tr>
<tr>
<td>PC, 2006</td>
<td>8</td>
<td>Peru</td>
<td>Mild left hemiparesis Below average to average school performance.</td>
<td>400 mg of albendazole per day and 200 mg of itraconazole per day for 14 months</td>
<td>Skin lesions, CNS</td>
<td>Treatment commenced and discharged home day 13</td>
<td>3 years</td>
</tr>
<tr>
<td>PC, 2006</td>
<td>10</td>
<td>Peru</td>
<td>Intact</td>
<td>600 mg of albendazole per day, 100 mg of itraconazole per day, and 320 mg/1600 mg TMP-SMX per day for 6 months; surgical resection</td>
<td>Skin, CNS</td>
<td>No details</td>
<td>18 months</td>
</tr>
<tr>
<td>Schuster, 2009</td>
<td>35</td>
<td>US</td>
<td>Intact</td>
<td>Initial treatment regimen for skin lesions included itraconazole, albendazole, amphoterixin, trimethoprim-sulfamethoxazole, clarithromycin, and oral artesunate. A final regimen initiated after onset of neurological symptoms resulted in immediate clinical response: 800 mg of albendazole per day, 450 mg of itraconazole per day for 7.5 months, as well as 150 mg of miltefosine per day for 12 days and 100 mg of miltefosine per day for 7 months.</td>
<td>No details</td>
<td>Extensive skin lesions prior to brain lesion CNS</td>
<td>No details</td>
</tr>
<tr>
<td>Cary, 2010</td>
<td>2</td>
<td>US</td>
<td>Profoundly disabled at discharge but improving</td>
<td>4 mg/kg pentamidine per day (stopped after 2 months); 200 mg/kg sulfadiazine, fluocytosine, clarithromycin, and trimethoprim-sulfamethoxazole per day</td>
<td>CNS</td>
<td>Brain biopsy 7 days. Treatment commenced less than 2 weeks.</td>
<td>22 months</td>
</tr>
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</table>
Miltefosine is an alkylphosphocholine compound originally developed as an anticancer drug and is effective for therapy of leishmaniasis [20, 21]. The drug has in vitro amoebicidal activity against *B. mandrillaris* [22], crosses the blood-brain barrier, and concentrates in brain tissue [23]. Miltefosine has been used in 2 adult patients who have survived [5, 6, 24, 25]. The case presented here is the first reported use of miltefosine to treat *B. mandrillaris* encephalitis in a child. It has been very well tolerated, and this result provides further hope for its role as an effective and safe treatment. Most patients with GAE are treated with multiple and complex combinations of agents because of the poor prognosis associated with the infection. In this case, attempts were made to rationalize and simplify therapy as early as possible. The patient’s progress is satisfactory on 3 oral agents. Miltefosine has a favorable safety profile in children in doses similar to those used in this case [21], and it is delivered as a single daily dose, making it suitable for prolonged courses. Preferred duration of therapy is not established for this infection, but it is planned in this case that the patient will continue the current regimen until resolution of lesions is seen on MRI scan.

New diagnostic and treatment modalities will facilitate early diagnosis of *B. mandrillaris* infections. Consideration of this rare diagnosis, prompt tissue biopsy, and early empiric treatment were important factors in a successful outcome.

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Disclaimer. The findings and conclusion in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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