Case Report

Meningitis due to Prototheca wickerhamii: rare case in China

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A rare case of Prototheca wickerhamii meningitis is reported in a patient without any underlying immunodeficient condition. Wet-mount microscopy and culture of cerebral spinal fluid specimens, along with temperature resistance, cycloheximide tolerance, carbohydrates assimilation, including API 20C AUX tests of the isolated etiologic agent were performed. In addition, transmission and scanning electron microscopy studies and in vitro antifungal susceptibility tests were conducted. Through the combination of these investigations, the isolate was identified as P. wickerhamii and the patient was successfully treated with intravenous amphotericin B and itraconazole. This is the first detailed report of meningitis caused by P. wickerhamii in China.

Keywords Prototheca wickerhamii, meningitis, amphotericin B, itraconazole

Introduction

Cases of meningitis due to Prototheca wickerhamii are rare with only two in the literature [1]. We report a case in China in a 24-year-old male without any underlying immunodeficient condition who presented with 9-month history of meningitis. The patient was successfully treated with intravenous amphotericin B and itraconazole.

Case report

A 24-year-old man presented with a 9-month history of fever and headache. His body temperature ranged from 37.5–39°C. The symptoms progressively aggravated from paroxysmal headache to persistent headache accompanied with ambiopia and difficulty in walking. The patient denied history of trauma, skin lesions, and intravenous drug use. Treatment with penicillin, dexamethasone, ciprofloxacin, rifampicin, pyrazinamide, and ethambutol failed to produce any clinical response.

Physical examination of heart, lungs and skin revealed no abnormality. Extensive laboratory tests, e.g., liver and renal function, CD3, CD4, CD8 cell counts; IgG, IgA and IgM levels were conducted but the results were within normal ranges. Serum antibody tests for syphilis and HIV were negative. Cranial magnetic resonance imaging showed dilation for both lateral ventricles. On lumbar puncture, opening pressure was 180 mmH2O. Cerebral spinal fluid (CSF) analysis showed; white blood cells 500–1000/μl (80% monocytes, 20% polymorphonuclear leukocytes), glucose 0.96 mmol/l, protein 2040 mg/l and cultures were negative for bacteria including tubercle bacillus.

Wet-mount microscopy of CSF showed spherical sporangia with sporangiospores, which were 6–10 μm in diameter (Fig. 1). CSF specimens collected on three different occasions were inoculated onto Sabouraud dextrose agar (SDA) and incubated at 25 and 37°C. We noted in 96 h, smooth, creamy white, yeastlike colonies (Fig. 2) composed of spherical to oval sporangia, containing many sporangiospores, 7.2–12 × 9.6–19.2 μm in diameter. While the organism did not form hyphae or blastoconidia (Fig. 3) nor grow at 40°C or in the presence of cycloheximide, it did form pink colonies on CHROMagar Candida. Carbohydrate assimilation tests showed the isolate could assimilate...
trehalose, but not sucrose and n-propanol. API 20C AUX (bioMérieux) as a commercial yeast identification system was used to confirm the identification of \( P. \) wickerhamii. Transmission electron microscopy showed spherical to oval sporangia with 2–6 sporangiospores, which reproduce asexually by internal separation and irregular cleavage (Fig. 4). Subsequent rupture and release of sporangiospores were observed by scanning electron microscopy (Fig. 5) and the spores were found to have uneven surfaces and were obvate in shape. These findings were in accordance with a previous report [1].

In vitro antifungal susceptibility tests were conducted with amphotericin B (AMB), 5-flucytosine (5FC), fluconazole (FCZ), ketoconazole (KTC), and itraconazole (ITZ). The minimal inhibitory concentration (MIC) was determined according to the guidelines of the National Committee for Clinical and Laboratory Standards reference broth microdilution method, M27-A [2]. The MIC values for individual antifungals were: AMB 0.5 \( \mu g/ml \), KTC 4 \( \mu g/ml \), 5FC 64 \( \mu g/ml \), FCZ 64 \( \mu g/ml \), and ITZ 16 \( \mu g/ml \). Disk diffusion susceptibility tests (ROSCO, Denmark) indicated that the isolate was susceptible to AMB, moderately susceptible KTC, and resistant to 5FC, FCZ and ITZ.

The patient was treated with intravenous AMB and ITZ. The dosage of AMB was gradually increased from 1 mg/d to 25 mg/d (75 kg, 0.33 mg/kg.d). Intravenous ITZ was given 200 mg bid for 2 days and then 200 mg qd for 12 days followed by treatment with a ITZ 200 mg solution bid for 3 months. The patient improved significantly within 2 weeks with the disappearance of both fever and headaches. After 4 weeks of treatment, CSF reexaminations showed white blood cell 2 \( \times \) 10/\( \mu l \),
protein 1875 mg/l, glucose 2.9 mmol/l. Microscopic examination and culture of CSF for fungi were negative. The cumulative dose of AMB was 2.0 g.

**Discussion**

*Prototheca* spp. have been found in water, sewage, soil, cow milk, cattle, dogs and fruit bats. In spite of their ubiquitous existence in nature, these unicellular algae are of low virulence and only produce chronic infection in humans [1]. Three species are recognized, i.e., *P. wickerhammi*, *P. zopfi* and *P. stagnora*, but only the first two have been known to cause infection in humans [3]. Prototheca infection is exogenous, non-transmissible and generally introduced via traumatic inoculation [4]. Until now, more than 100 cases of protothecosis have been reported. Most of the cases were in immunocompetent hosts and involved skin and subcutaneous tissue and presented with erythematous nodules, plaques or ulcers, verrucous or herpetiform lesions. Olecranal bursa may be involved, presumably as a result of antecedent trauma or surgical procedures and subsequent contamination, manifested as swelling, mild erythema, and occasional drainage in the vicinity of the elbow. Only a few cases of systemic protothecosis have been documented [1] and involved patients with cancer, AIDS, diabetes mellitus, renal transplantation, steroids or other immunosuppressive therapy. Most protothecal infections are attributed to local inoculation at sites of skin defects or trauma and its incubation period has been speculated to be several weeks [5,6].

Only two cases of meningitis due to *Prototheca* spp. have been reported. The first was in a 25-year-old woman with AIDS [7]. The patient was a heavy drinker and an intravenous drug user and died 5 months after diagnosis despite intensive treatment. The second case was a 20-year-old man with anemia, liver abscess and meningitis [8]. The liver abscess was cured with intravenous miconazole and AMB but *P. wickerhammi* could not be eliminated from CSF and the infection persisted for 6 years. The patient was observed with no drug treatment.

We present the third case of *Prototheca* meningitis and in this instance, an immunocompetent young man. He was a maintenance worker and was unable to trace the portal of entry of the organism. We speculate that his infection resulted by contacting something contaminated by *Prototheca* or skin trauma. It has been reported that people who experience frequent mechanical traumas are more susceptible to *Prototheca* infection. This patient had a 9-month history of fever and headache before diagnosis. Various treatment regimens have been attempted in recent years, but AMB and azole still are the first choices [9,10]. Our patient was successfully treated with the combination of AMB and ITZ.

Although the MIC to ITZ of the isolate from our case was high, AMB combined with ITZ was used in our case in accord with the ‘90–60 rule’ presented by Rex et al. [11]. This rule observes that infections due to susceptible isolates respond to appropriate therapy about 90% of the time, whereas infections due to resistant isolates respond about 60% of the time. Our results from susceptibility testing of the isolate recovered in our case are in accord with the report of Beatriz [12], who found that *P. wickerhammi* was sensitive to AMB and resistant to 5FC, FCZ and ITZ.

Considering all three cases, the patients were in their 20s. While the first case in an individual with AIDS showed poor prognosis, the other two in immunocompetent patients manifested as chronic infections with mild to moderate symptoms. Combining AMB with azoles seems to be the first choice for treatment. In identifying the etiologic agent, the sporangiospores may be confused with the white blood cells in CSF smears and require 48–72 h incubation in the API 20C AUX [13].

This case is the rare report of meningitis caused by *P. wickerhammi* in China.

**References**


