Note

Cerebral infection caused by *Cryptococcus gattii:* a case report and antifungal susceptibility testing

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Summary

We report a clinical case of cerebral infection caused by *Cryptococcus gattii* in a 10 year-old boy. Clinical and laboratory exams did not demonstrate any apparent immunosuppressed state (HIV antibody and the tuberculin skin tests, both negative, were performed; blood cells count and immunoglobulin levels were within normality). Treatment was begun with amphotericin B-deoxycholate but renal toxicity signs led to its substitution by fluconazole. The infection proceeded even after treatment with fluconazole. In vitro determination of minimum inhibitory concentration values were high for itraconazole (≥ 2 µg/ml), fluconazole and 5-flucytosine (≥ 64 µg/ml) and low for amphotericin B (1.0 µg/ml). Renal toxicity signs, induced by amphotericin B, progression of infection after fluconazole, and likely in vivo resistance to this triazole made this case difficult to treat. In vitro drug interaction tests confirmed probable synergism between amphotericin B and 5-flucytosine (frational inhibitory concentration - FIC = 0.375). In contrast, a probable additive effect was observed for amphotericin B and fluconazole (FIC = 0.75). Initial treatment of persistent high intracranial pressure was insufficient and neurological surgery was necessary. Antifungal susceptibility tests and *Cryptococcus* species identification were important in selecting appropriate antifungal therapy.

Key words *Cryptococcus gattii*, Antifungal therapy, Amphotericin B, Antifungal susceptibility testing.

Infección cerebral causada por *Cryptococcus gattii:* caso clínico y sensibilidad a los antifúngicos

Resumen

Relatamos un caso clínico de infección cerebral provocada por *Cryptococcus gattii* en un niño de 10 años. Los exámenes clínicos y de laboratorio no demostraban ningún estado aparente de inmunosupresión (anticuerpos VIH y prueba de tuberculina negativos, recuento sanguíneo y niveles de inmunoglobulinas dentro de la normalidad). El tratamiento comenzó con anfotericina B desoxicolato, pero la toxicidad renal condujo a su sustitución por fluconazol. La infección persistió después del tratamiento con fluconazol. La determinación in vitro de los valores de la concentración inhibitoria mínima mostraron valores altos para el itraconazol (≥ 2 µg/ml), el fluconazol y la 5-fluorocitosina (≥ 64 µg/ml), y bajos para la anfotericina B (1 µg/ml). Los signos de intoxicación renal inducidos por la anfotericina B, el avance de la infección después del tratamiento con fluconazol y probablemente la resistencia in vivo a este triazol hacen que éste sea un caso difícil de tratar. Las pruebas de interacción in vitro entre las drogas confirman un probable sinergismo entre la anfotericina B y la 5-fluorocitosina (concentración inhibitoria fraccionaria - CIF = 0,375). Sin embargo, se ha observado un probable efecto aditivo para la anfotericina B y fluconazol (CIF = 0,75). El tratamiento inicial de la alta presión intracraneal persistente fue insuficiente y fue necesaria cirugía neurológica. Las pruebas de sensibilidad antifúngica y de identificación de las especies de *Cryptococcus* fueron importantes en la selección de la terapia antifúngica apropiada.

Palabras clave *Cryptococcus gattii*, Terapia antifúngica, Anfotericina B, Sensibilidad antifúngica

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Cryptococcus gattii has emerged as a primary pathogen of healthy hosts showing meningitis signs, focal features in the cerebrum and lungs, prolonged symptoms, extended therapy, and neurological sequelae which require surgery [3,30,31].

A 10 year-old boy sought medical assistance at the Pronto Socorro João XXIII Hospital, at Belo Horizonte, State of Minas Gerais, Brazil, in December, 2004, presenting with daily, progressive headaches, nuchal rigidity, seizures and nocturnal fever. The lumbar puncture and cerebrospinal fluid exams showed: leukocytes at 3,671 cells/ml (neutrophils 39%, lymphocytes 53%, monocytes 8%), erythrocytes at 48 cells/ml, protein at 23 mg/ml, glucose at 59 mg/ml, and direct mycological inspection demonstrated numerous budding encapsulated yeast-like cells. Treatment was begun with amphotericin B-deoxycholate (AMB) at 0.5 mg/kg/day. After being transferred to the Centro Geral de Pediatria, tests were done to assess the patient immune system. His HIV antibody and the tuberculin skin tests were both negative, blood cells count and immunoglobulin levels were within normal limits, and not defects in cell-mediated immunity were detected, confirming that the patient was not immunocompromised. The fundoscopy showed a slight blur of the temporal papilla.

An increase in the blood urea nitrogen (60 mg) and creatinine (1.9 mg) levels was observed after 20 days therapy with AMB, leading to its substitution with fluconazole, 150 mg every 48 hours (dose adjusted according to the patient's renal clearance). The fundoscopy showed a bilateral papilloedema.

Ten days after initiation of fluconazole therapy, the patient presented with headaches and fever. Budding encapsulated yeast-like cells were still observed in the CSF. A sample cultured onto Sabouraud dextrose agar grew *C. gattii* identified at the Instituto Adolfo Lutz, São Paulo, Brazil by characterizing growth in Creatinine-Dextrose-Bromothymol blue and Canavanine-Glycine-Bromothymol blue medium and by the D-proline assimilation test [13,15,24].

C. gattii infections have been previously reported in Brazil and have been characterized by focal involvement with the central nervous system in an immunocompetent host, meningismum, papilloedema, headaches, fever, neurological unstability, high intracranial pressure, prolonged symptoms, without dissemination to other organs, and with frequent neurological sequelae. Patients died (rates of mortality of 12.5% - 55.5%, according to Brazilian studies) [4,5,18] due to this yeast infection in spite to the long time of treatment using AMB alone or in combination with 5-flucytosine and fluconazole. Antifungal susceptibility tests were not usually provided in these reports [4-6,14, 18,19,22,27]. It is interesting to observe that cryptococcosis is the second leading cause of mortality by a systemic mycosis in Brazil, with 91 deaths occurring due to *Cryptococcus* spp., in 2005 [29].

Antifungal susceptibility testing of the *C. gattii* isolate to AMB (Sigma, USA), 5-flucytosine (Hoffmann - La Roche, UK), fluconazole (Pfizer, USA) and itraconazole (JANSSEN-CILAG, Belgium) was performed in pairs by a checkerboard technique, and minimum inhibitory concentrations (MIC) were determined as described elsewhere [7,21,23,35]. Individual MIC values were high for itraconazole (≥ 2 µg/ml), fluconazole and 5-flucytosine (≥ 64 µg/ml) and low for AMB (1.0 µg/ml). Modes of interaction between drugs were classified as synergism, additivism, or antagonism based on the profile of the interaction drug curves and by means of the fractional inhibitory concentration index (FIC). The interaction was defined as synergistic when the FIC was ≤ 0.5, additivism when the FIC was > 0.5 but < 4.0 and antagonism when the FIC was > 4 [8]. Results also demonstrated in vitro synergistic effect for AMB plus 5-flucytosine (FIC = 0.375) and additive effect for AMB plus fluconazole (FIC = 0.75). When analyzing the curves (Figure), it is possible to note that an increase in the AMB concentration occurred concomitantly with the reduction of the 5-flucytosine concentration and vice-versa. This was not true when AMB was tested in combination with fluconazole, specifically when the concentration of this drug was increased from 16 to 32 µg/ml.

Fluconazole was discontinued and AMB was restarted with close renal monitoring. However, 32 days after his admission, the patient was referred to the intensive care unit presenting with vomiting, bradycardia, tachypnea, blood pressure of 90/60 mmHg, and intracranial pressure

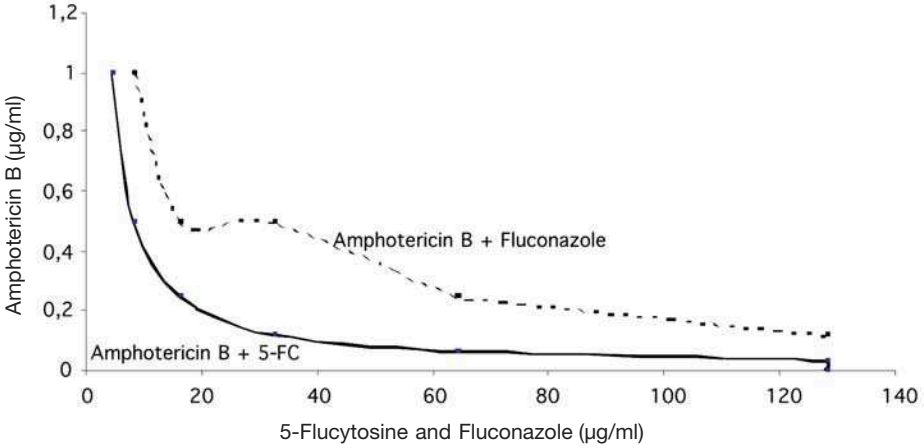

Figure 1. In vitro anticryptococcal activities of two pairs of drug combinations: Amphotericin B (AMB) plus fluconazole (FCZ) and AMB plus 5-flucytosine (5FC). Synergistic interaction was obtained for amphotericin B plus 5FC (an increasing in AMB concentration occurred concomitantlywith the reduction of 5FC levels and vice-versa in this combination) and additive interaction was obtained for AMB plus FCZ (no increasing of AMB concentration when in combination with FCZ, specifically when the concentration of this drug was increased from 16 to 32 µg/mL). Quality control organisms *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were included in each experiment to check the accuracy of the drug dilutions and the reproducibility of the results. The viability of these microorganisms was confirmed by subculturing onto Sabouraud dextrose agar.

> 60 mmHg. He was returned to the infirmary hemodynamically stable, but neurologically unstable, exhibiting high intracranial pressure. Due to high blood levels of urea and creatinine and high MIC values to triazoles, therapy with liposomal amphotericin B (2 mg/kg/day) was begun, and 28 days later the direct mycological exam was negative for the first time. Owing to persistent high intracranial pressure, resulting from insufficient ventricular drainage, the patient was submitted to surgery for introduction of a lumbar-peritoneal shunt. He was released 38 days after the operation, returning periodically for evaluation. Since then he has been asymptomatic and the encephalic magnetic resonance imaging has not shown any apparent alterations.

Currently, AMB alone, or in combination with 5-flucytosine, followed by fluconazole, remain the drugs of choice for cryptococcosis therapy [26]. In this case, AMB alone was used because co-treatment with 5-flucytosine is not available in local hospitals for routine usage. Due to development of renal insufficiency, fluconazole was then given. Resistance, however was suspected, and, subsequently confirmed by *in vitro* susceptibility testing.

Antifungal susceptibility tests are not routinely performed in laboratories of the Brazilian public services. In this case, the tests performed were important to select the appropriate antifungal therapy. Wide ranges for fluconazole MICs to *C. gattii* (up to ≥ 64 μg/ml) have been observed [28,32], and this could be secondary to therapy with antifungal drugs or a primary therapy [1,9,28,34]. In *C. neoformans,* resistance to fluconazole has been associated with increased expression of an ABC transporter-encoding gene (*CnAFR1*), revealing an active drug efflux mechanism [25]. *Cryptococcus neoformans* cross-resistance *in vitro* with itraconazole and ketoconazole has been demonstrated [10], but *C. gattii* resistance to itraconazole and cross-resistance with azoles is not commonly reported [1,20].

AMB is the most readily available formulation for patients at the public health services in Brazil, but liposomal amphotericin B was prescribed in this case, considering the renal insufficiency and the high MIC values to triazoles. Encapsulation of amphotericin B into liposomes appears to reduce its toxic effects and to improve its clinical efficacy, allowing for usage of higher doses [11,28]. Low MIC values for AMB correlated with more successful therapy adopted during treatment.

A possible strategy to enhance efficacy of treatment would be to combine antifungal agents with the goal of lowering the required dose, potentially reducing side effects [33]. Larsen et al. [16] demonstrated in a murine model that AMB combined with fluconazole would lead to more rapid sterilization of the central nervous system, because the increasing levels of fluconazole induced by its decreased clearance resulting from renal deterioration would allow for a decrease in AMB administration time. Thus, we tested the in vitro interaction of AMB and fluconazole. Results of this combination revealed a probable additive effect, indicating that it would have been an alternative to treatment. Since the interruption of fluconazole administration was immediately followed by administration of AMB, and considering that the patient developed renal insufficiency, we wondered whether the patient's clinical outcome could also have been a consequence of the presence of therapeutic concentrations of both drugs in the plasma due to decreased clearance. The combination between AMB and fluconazole was also studied in a murine model of cryptococosis by Barchiesi et al. [2]. These authors demonstrated that additive effect is commonly obtained for those drugs and that sequential therapy (fluconazole followed by AMB) can promote significant reductions of the fungal burdens in the CNS [2].

In this case, the initial treatment of persistent high intracranial pressure was insufficient and success was obtained only after surgery. Lumbar puncture and cerebrospinal fluid drain are the choice treatments of choice and they must be performed dialy to prevent patient sequelae and death [12,17].

Our report reinforces the necessity of *Cryptococcus* species identification and the utility of performing antifungal susceptibility testing to facilitate choosing the most suitable treatment for this type of fungal infection. It also suggests correlation between the patient's clinical outcome with the results of *in vitro* antifungal susceptibility testing.

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