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Short Communication

Very prolonged liposomal amphotericin B use leading to a lysosomal storage disease

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ABSTRACT

Amphotericin B is a powerful polyene antifungal drug used for treating systemic fungal infections and is usually administered for a short period. Side effects after prolonged use are unknown in humans. Here we report the case of a 28-year-old man suffering from chronic granulomatous disease (CGD), treated for invasive cerebral aspergillosis with liposomal amphotericin B (L-AmB) for a very long time (8 consecutive years). We describe the efficacy and safety of this treatment in the long term. Aspergillosis was kept under control as long as L-AmB therapy was maintained, but relapsed when the dose was reduced. No overt renal toxicity was noted. The patient gradually developed hepatosplenomegaly and pancytopenia. Abnormalities of bone marrow were similar to the sea-blue histiocyte syndrome. Liver biopsy showed images of nodular regenerative hyperplasia related to CGD as well as a histiocytic storage disease. We discuss the very prolonged use of L-AmB leading to the development of a lysosomal storage disease.

1. Introduction

Amphotericin B (AmB) is a powerful polyene antifungal drug used for treating systemic fungal infections and is usually administered for a short period. Side effects after prolonged use are unknown in humans. Here we report the case of a 28-year-old man suffering from chronic granulomatous disease (CGD), treated for invasive cerebral aspergillosis with liposomal amphotericin B (L-AmB) for a very long time (8 consecutive years).

2. Clinical history

Here we report a patient included in the French national registry of patients with primary immunodeficiency diseases held by the French National Reference Centre [1–3] (referred to by patient code b in Table 1 and supplemental appendix 2 of Blumental et al. [2]). In the neonatal period, the patient presented respiratory symptoms and fever leading to a diagnosis of *Aspergillus fumigatus* pulmonary infection, which was histologically and mycologically proven. The diagnosis of CGD was made by nitroblue tetrazolium test and was confirmed by molecular testing with an autosomal recessive inheritance pattern. The outcome was favourable with conventional AmB and flucytosine. Haematopoietic stem cell transplantation was not possible because of lack of a compatible donor. At the age of 4 years, aspergillosis infection recurred with liver, diaphragm and brain abscesses. Because of intracranial hypertension, a ventriculoperitoneal derivation was performed with a subcutaneous Ommaya

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reservoir connected to the brain ventricle. During this procedure, microbiological examinations failed to show any pathogen. The patient received conventional AmB infusion therapy. Because the fungal infection remained uncontrolled, intravenous (i.v.) AmB was associated with flucytosine and itraconazole. In addition, intrathecal instillation of amphotericin B deoxycholate was started using the Ommaya reservoir (0.2 mg once daily for 10 days). Surgical excision of the brain abscesses was not possible because of the large size and periventricular localisation of lesions. The outcome was favourable; nevertheless, a brain magnetic resonance imaging (MRI) scan performed 3 months later showed lesions as sequelae. Itraconazole maintenance therapy was then continued for the subsequent 5 years. At the age of 15 years he developed pulmonary *Phialophora richardsiae*, treated with L-AmB associated with flucytosine, with a total treatment duration of 1 year. At the age of 21 years, multiple cerebral abscesses and acute hydrocephalus recurred. A transcranial puncture of the brain lesion was performed. Hyphae were detected on microscopic examination, but culture remained negative. Antifungal treatment was then based on i.v. L-AmB (6 mg/kg/day). The outcome was favourable within 2 weeks and secondary prophylaxis with itraconazole was continued. However, immediately after cessation of L-AmB therapy multiple brain abscesses and acute hydrocephalus relapsed. Treatment with L-AmB was resumed with higher doses at 10 mg/kg/day. Itraconazole was changed to voriconazole, which was stopped due to drug-induced hepatitis. The role of voriconazole in the patient's hepatitis was documented by a re-introduction test and was related to a heterozygous mutation in the cytochrome *CYP2C19* gene. Treatment with L-AmB was then continued. The initial dose of 10 mg/kg/day for the first month was progressively reduced: 10 mg/kg three times a week for 3 months, then 5 mg/kg three times a week for 3 months and then 3 mg/kg three times a week. Several attempts for spacing injections (3 mg/kg twice a week) resulted in progression of brain lesions documented on MRI. Finally, treatment with i.v. L-AmB was continued at a maintenance dose of 3 mg/kg three times a week for 8 consecutive years. The total administered dose of L-AmB was estimated to 230 g (for a patient body weight of 36 kg). The patient gradually developed pancytopenia and hepatosplenomegaly.

3. Materials and methods

Biochemical blood and urine analysis were used to evaluate renal and hepatic function. Haematological analyses included a blood count and bone marrow aspiration with May-Grünwald–Giemsa (MGG) staining. Pathological examination of the liver biopsy was performed by venous transjugular catheterisation with hepatic pressure gradient measurement. The staining techniques used were haematoxylin–eosin stain, Periodic acid–Schiff (PAS) stain, Gordon and Sweet stain and MGG stain. Liver biopsies were analysed by pathologists and clinician experts in liver disease (CGue, MS and JCD).

4. Cytological and pathological results

After 8 years of exposure to AmB treatment, serum creatinine remained stable at 110 μmol/L (creatinine clearance estimated by the Cockcroft–Gault formula at 39 mL/min) and proteinuria was 0.8 g/day. Haematological parameters showed progressive pancytopenia (haemoglobin 8 g/dL, mean corpuscular volume of erythrocytes 85 fL, reticulocytes 40×10^9/L, leukocytes 1300/mm^3, platelets 25×10^9/L). Because of pancytopenia, bone marrow aspiration was performed showing an excess of histiocytic cells with foamy intralysosomal accumulation of lipid particles. MGG staining revealed blue-staining foamy macrophages and coarse dark blue cytoplasmic granules and foamy histiocytes containing numerous lipid particles. These data were consistent with the diagnosis of sea-blue histiocyte syndrome (Fig. 1). Furthermore, the patient progressively developed hepatomegaly and splenomegaly, with ascites and collateral venous circulation. Hepatic blood tests showed a bilirubin level of 16 μmol/L (normal value <17 μmol/L), γ-glutamyl transferase 613 international units (IU)/L (normal value <85 IU/L), alkaline phosphatase 698 IU/L (normal value <120 IU/L), aspartate aminotransferase 48 IU/L (normal value <35 IU/L), alanine aminotransferase 26 IU/L (normal value <43 IU/L), prothrombin time 66% (normal value 70–100%) and factor V 97% (normal value 70–120%). Hepatic ultrasound showed homogeneous hepatomegaly with signs of portal hypertension. To determine the cause of the hepatomegaly and to rule out an opportunistic infection, a liver biopsy was performed by venous transjugular catheterisation. The hepatic pressure gradient was measured at 8 mmHg. Liver pathological examination revealed lobules with intrasinusoidal macrophage accumulation associated with atrophy of hepatocellular plates (Fig. 2a). The atrophic plates alternated with roughly nodular areas of hyperplastic plates; PAS stain showed nodular regenerative hyperplasia (Fig. 2b). Intrasinusoidal macrophages contained a granular pigment stained by the Gordon and Sweet stain compatible with ceroid pigments and associated with haemosiderin (Fig. 2c). MGG staining demonstrated blue-staining foamy macrophages (Fig. 2d). The portal tracts were normal in size and contained pigmented macrophages. There was no epithelioid granuloma. In summary, liver biopsy showed features of nodular regenerative hyperplasia associated with a macrophage storage disease with intralysosomal lipid accumulation. In the absence of alternative therapy, treatment with AmB was maintained. In May 2012, because of a poor venous access, L-AmB treatment was discontinued and the patient died due to progression of brain abscess.

5. Discussion

Here we report the case of a patient with invasive fungal infection with cerebral abscess requiring extended L-AmB treatment because of uncontrolled fungal disease. The efficacy and safety of L-AmB over such an extended period is currently unknown. To our knowledge, the effects of such a high cumulative dose have never been reported. Interestingly, every attempt to reduce AmB doses led to a reactivation of brain lesions, suggesting that the L-AmB response was dose-dependent. We emphasise that despite a very prolonged exposure to AmB leading to possible selection pressure on fungal pathogens, the patient did not develop a drug-resistant mould. Renal function remained stable and at an acceptable level.

Invasive fungal infection remains a critical issue among patients with CGD, with a high fatality rate (up to 50% in some series) [4,5]. Fungi are responsible for >90% of brain abscesses in immunocompromised patients, and *Aspergillus* is the most common organism isolated [6]. In the case of brain involvement caused by *Aspergillus*, mortality is ca. 80% [7], and successful therapy is attributed to prolonged antifungal therapy [8]. Thanks to the availability of new antifungal drugs and the use of itraconazole prophylaxis, patient survival has improved, as reported in the recent French cohort [2]. The decision to discontinue antifungal therapy is all the more difficult because active infection and sequelae lesions could be difficult to differentiate [2].

The current patient progressively developed hepatosplenomegaly, mainly due to nodular regenerative hyperplasia, which is related to CGD [9]. Furthermore, bone marrow and liver pathological examination both showed features of lysosomal storage disease. The sea-blue histiocyte syndrome is related to