Systemic mycoses during prophylactical use of liposomal amphotericin B (Ambisome®) after liver transplantation

Systemische Mykosen unter liposomaler Amphotericin B-Prophylaxe (Ambisome®) nach Lebertransplantation

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Key words. Mycoses, systemic infection, liver transplantation, prophylaxis, liposomal amphotericin B, Ambisome®


Summary. We investigated the prophylactical administration of liposomal amphotericin B (Ambisome®) in the early phase after liver transplantation (LTx). Fifty-eight patients received Ambisome® prophylactically after LTx. Ambisome® (1 mg kg⁻¹ day⁻¹) was given intravenously for 7 days after LTx. Immunosuppressive prophylaxis was cyclosporin A (CsA) based in 11 patients. Forty-seven patients had a tacrolimus-based immunosuppressive regimen. CsA and tacrolimus dosages were adjusted to trough levels of 150–250 ng ml⁻¹ (EMIT) and 5–15 ng ml⁻¹ (MEIA II) respectively. Three patients died from sepsis due to Aspergillus fumigatus infection. Reasons for a fatal outcome were foudroyant Aspergillus pneumonia in a patient transplanted for fulminant hepatic failure on post-operative day (pod) 8; Aspergillus sepsis with severe endocarditis in a patient with two retransplantations for graft non-/dysfunction on pod 24; and disseminated aspergillosis due to Aspergillus fumigatus in a patient retransplanted for primary non-function (pod 19). All three patients underwent haemofiltration for renal failure. One patient with Candida albicans sepsis (pod 4) recovered under increased dosage of Ambisome® (5 mg kg⁻¹ per day). Ambisome® (1 mg kg⁻¹ per day) seems to be beneficial against systemic Candida infections. However, the onset of systemic Aspergillus infections could not be prevented. Obviously, higher Ambisome® doses appear to be necessary against Aspergillus. We recommend the use of Ambisome® (3 mg kg⁻¹ per day) for patients with risk factors such as graft dys-/non-function, retransplantation, haemofiltration and complicated acute liver failure to prevent invasive aspergillosis.

Candida albicans


Introduction

Systemic mycotic infections are life-threatening complications in immunosuppressed solid organ transplant recipients. Invasive fungal infections occur in 5–45% of patients and are a major cause of morbidity and mortality [1]. Diagnosis of most fungal infections in solid organ transplantation is usually made in the first 2 months after transplantation [2]. Invasive fungal infections occur more frequently in liver graft recipients than in other type of solid organ transplant recipient [3]. The most common pathogens in the majority of solid organ transplant recipients are Candida species, followed by Aspergillus species [1]. Aspergillus infections are rare but devastating complications after solid organ transplantation, with mortality rates approaching 78–100% [4, 5].

Difficulties in establishing an early diagnosis, lack of effective therapy in many situations, difficult management of certain antifungal drugs and limited data on effective regimens for antifungal prophylaxis represent major problems in the management of fungal infections after liver transplantation [6].

Of the currently available antimycotics, amphotericin B is the drug of choice for life-threatening mycotic infections. The triazoles fluconazole and itraconazole may be effective alternatives for less serious infections due to susceptible organisms [1]. The use of antifungal drugs is limited by their toxicity. Furthermore, amphotericin B could aggravate nephrotoxicity of the immunosuppressants cyclosporin A (CsA) and tacrolimus [7]. Moreover, fluconazole can inhibit CYP3A metabolism of CsA and tacrolimus resulting in overimmunosuppression [8, 9].

There is only little experience in antifungal prophylaxis after liver transplantation. Ambisome®, a small unilamellar liposome preparation (45–80 nm) containing amphotericin B in the bilayer, shows reduced toxicity and elevated peak plasma level compared with conventional amphotericin B without loss of the broad-spectrum antifungal activity of amphotericin B [10]. Therefore, we investigated the effectiveness of prophylactical liposomal amphotericin B (Ambisome®) administration in the early phase after LTx.

Patients and methods

Sixty-four liver transplantations were performed in 58 patients [30 men, 28 women; median (range) age 53 (6–70) years]. Indications for liver transplantation were post-hepatic cirrhosis due to hepatitis C virus infection [18], post-alcoholic cirrhosis [10], fulminant hepatic failure [7], post-hepatic cirrhosis due to hepatitis B virus infection [6], graft non-/dysfunction [6], zytopenic cirrhosis [4], primary biliary cirrhosis [3], adult polycystic liver disease [2], hepatic metastasis of extrahepatic resected carcinoma [2], haemochromatosis [1], Budd–Chiari syndrome after bone marrow transplantation for veno-occlusive disease [1], autoimmune hepatitis [1], proximal bile duct carcinoma [1], post-hepatic cirrhosis due to hepatitis A–D virus infection [1] and haemochromatosis [1]. Initial immunosuppressive prophylaxis consisted of cyclosporin A 1–2 mg kg⁻¹ per day i.v. (Sandimmum®, Sandoz, Basle, Switzerland), antilymphocyte globulin [9] 3 mg kg⁻¹ per day i.v. (ATG-Fresenius®, Bad Homburg, Germany) or RT563 (2) 10 mg kg⁻¹ per day (Biotest, Dreieich, Germany) and prednisolone 1 mg kg⁻¹ per day i.v. (Solu-Decortin H®, Merck, Darmstadt, Germany) in 11 patients. Forty-seven patients received tacrolimus 0.1 mg kg⁻¹ per day p.o. (Prograf®, Fujisawa Pharmaceuticals, Osaka, Japan) primarily in combination with prednisolone p.o. (26) or mycophenolate mofetil (MMF) 10–40 mg kg⁻¹ per day p.o. (Cell Cept®, Hoffmann-La-Roche, Grenzach-Whylen, Germany), MMF only (11) or prednisolone and MMF [10]. All patients received a steroid bolus of 500 mg of methylprednisolone i.v. (Urbason®, Hoechst, Frankfurt am Main, Germany) intraoperatively. During the first 2 months cyclosporin A (CsA) and tacrolimus dosages were adjusted to trough levels in the range of 150–250 μg l⁻¹ for CsA using the enzyme-multiplied immunoassay (EMIT) (EMIT®, Behring, Marburg, Germany) and 5–15 μg l⁻¹ for tacrolimus using an enzyme-linked immunosorbent assay (ELISA) (Protrac II®, Inestar, Stillwater, MN, USA) or microparticle enzyme immunoassay (MEIA) (Tacrolimus-MEIA II®, Abbott, Chicago, IL, USA).
All 58 patients received liposomal amphotericin B Ambisome® Vestar, San Dimas, CA, USA prophylactically after LTx. Ambisome® (1 mg kg\(^{-1}\) per day) was given i.v. for 7 days after LTx. Cefotaxime 3 × 2 g per day i.v. (Cloran® Hoechst, Frankfurt am Main, Germany) was given perioperatively for antibacterial prophylaxis. Until discharge from hospital, selective bowel decontamination ‘CAT’ colistin sulphate 2.5 g, amphotericin to 100.0, tobramycin 2.0 g 4 × 2 ml per day was administrated p.o. to all patients.

Screening for mycotic infections included serological tests, cultures and smears of all central lines, drainages, catheters, wound, biopsies, mouth, nose, anogenital region, fluids (blood, bile, urine, stool, saliva) drawn at least once a week.

Testing of Candida species included corn meal agar cultures for detection of Candida albicans and API-AUX® (BioMerieux, Nürtingen, Germany) to differentiate other Candida species. Serological screening included latex-agglutination test for Candida thermostable antigen (Ramco, Houston, TX, USA) and Candida-specific in-house ELISAs for IgG and IgM.

Aspergillus species were detected by growth on solid media including malt agar, micromorphology and growth at 45 °C (Aspergillus fumigatus). In addition, a specific serological Aspergillus antigen latex agglutination for the galactomannan antigen test (Pastorex, Sanofi-Pasteur, Marnes la Coquette, France) was performed.

Deep fungal infection was defined, according to Castaldo et al. [2] as (1) histological evidence of tissue invasion on biopsy or autopsy; (2) positive culture from a deep tissue compartment (e.g. blood, cerebrospinal fluid, peritoneal fluid) or biopsy specimen; (3) positive cultures from multiple sites (three or more), such as urine, wound and other sites; or (4) presence of budding yeast, hyphae or positive culture from a bronchoalveolar lavage (BAL) specimen with clinical and/or radiological evidence of pneumonitis [2].

Results

Four of 58 patients developed systemic mycoses after liver transplantation. Three of these patients died from invasive aspergillosis. Reasons for a fatal outcome were foudroyant Aspergillus pneumonia, invasive aspergillosis with fungal endocarditis and disseminated aspergillosis. All three patients underwent haemofiltration for renal failure. One patient with Candida albicans sepsis (post-operative day [pod] 4) recovered under increased dosage of Ambisome®. All of the patients had received antimycotic prophylaxis using Ambisome® (1 mg kg\(^{-1}\) per day) for 7 days after transplantation.

Case 1 (M.C.)

The first patient transplanted at our institution was a 42-year-old man (Child C, clinical status 5) [11], who underwent emergency liver transplantation for un treatable oesophageal bleeding because of decompensated post-alcoholic cirrhosis, who had been treated previously by repeated sclerotherapy, intermittent balloon tamponade and six transjugular intrahepatic portosystemic shunts (TIPS). Portal vein thrombosis due to a dislocated TIPS was found to be the reason for life-threatening bleeding [12]. Transplantation was performed orthotopically using the standard technique and the portal vein was desobliterated. The graft showed good primary function. Initial immunosuppression consisted of CsA, ATG and prednisolone. Mean (range) CsA blood levels and screening included latex-agglutination test for Candida thermostable antigen (Ramco, Houston, TX, USA) and Candida-specific in-house ELISAs for IgG and IgM.

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<3 μg l⁻¹ on pod 1 and the arterial ketone body ratio was 0.54–0.62 within 36 h after LTx. Tacrolimus was discontinued on pod 2 because of high tacrolimus blood levels (33.0–43.0 μg l⁻¹), reflecting impaired liver function. The graft function seemed to recover on pod 6 without the need for retransplantation. Haemofiltration was necessary during the whole post-operative course. Pulmonary gas exchange deteriorated on pod 7. Therefore, a bronchoscopy was performed, showing signs of pneumonia, and BAL was carried out. On pod 8 the pulmonary situation deteriorated dramatically, and repeated bronchoscopy showed pulmonary bleeding and severe pneumonia. The patient died on the next day because of pulmonary failure, and on the same day Aspergillus fumigatus was detected in the BAL taken on pod 7.

Case 3 (G.Z.)
A 22-year-old man (Child C, clinical status 3) [11] underwent liver transplantation for end-stage cirrhosis due to viral hepatitis B and D complicated by a partial portal vein thrombosis. Two retransplantations were required for primary non-function on pod 3 and graft dysfunction on pod 22. The third graft showed excellent primary function and the patient received Ambisome® 1 mg kg⁻¹, vancomycin and meropeneme for fever of unidentified origin (pods 22–24). Haemofiltration became necessary on pod 2 for renal failure and was continued until pod 17. Mean (range) tacrolimus blood levels were 12.05 (2.0–21.4) μg l⁻¹ under a dose of 0.04 (0.01–0.09) mg kg⁻¹ per day p.o. The patient died because of cardiopulmonary failure on pod 24. Severe endocarditis and disseminated aspergillosis was found post mortem. There was no evidence for invasive mycotic infection from screening until death. Retrospectively, the first positive Aspergillus antigen test in serum was on pod 20.

Case 4 (M.F.)
A 60-year-old male (Child B, clinical status 3) [11] received a liver graft for end-stage cirrhosis because of viral hepatitis C. Transaminases peak was 6356 U l⁻¹ for AST and 3438 U l⁻¹ for ALT. The graft showed no bile secretion; the coagulation factor V was 31%, and the patient required substitution of fresh frozen plasma. Retransplantation was performed for primary non-function on pod 5. Renal failure occurred on pod 1 and the patient underwent haemofiltration. The second graft revealed good liver function. Tacrolimus, MMF and prednisolone were administered for initial immunosuppression with discontinuation of tacrolimus before reLTx. The post-operative course was complicated by brain oedema, continuation of haemofiltration (pods 1–19), mechanical ventilation (pods 1–19), leucopenia 2100 μl⁻¹, and onset of infection of unknown origin. Antibiotic therapy with piperacilline and tazobactam (Tazobac, Lederle) was started after retransplantation and was switched to meropenene (Meronem, Zeneca) on pod 12. Methylprednisolone was given 3 × 500 mg i.v. pods 14–16, because of elevated liver enzymes (AST from 16 U l⁻¹ to 490 U l⁻¹). No signs of rejection were seen in liver biopsy. The patient developed fever 39.7 °C on pod 16. The patient had multiple ischaemic areas, compression of the IVth liquor ventricle and small areas of haemorrhages in a computerized tomography scan. Screening for systemic mycoses was negative until the patient died on pod 19. Disseminated aspergillosis was found histologically in the endocardium, lung and brain. Retrospectively, Aspergillus antigen in serum became positive on pod 15.

Discussion
Invasive fungal infections are an increasing problem in patients with immunosuppression after solid organ transplantation, bone marrow transplantation, acute leukaemia or acquired immunodeficiency syndrome [13]. In particular, invasive aspergillosis seems to have increased in immuno-compromised patients during recent years. The high mortality of aspergillosis in liver transplant recipients is a particularly serious problem in the early post-operative period [14], and the first prophylactic regimens have been investigated to curb this problem (Table 1). Tollemar et al. [15] first reported on such antimycotic prophylaxis using liposomal amphotericin B (Ambisome®) in a prospectively randomized double-blind, placebo-controlled study 77 patients received 5 days of prophylaxis starting during the transplantation with either liposomal amphotericin B (Ambisome®) 1 mg kg⁻¹ per day (n = 40) or placebo (n = 37). The incidence of invasive fungal infections was remarkably low 0/40 (0%) in the Ambisome® group vs. 6/37 (16%) in the placebo group (P < 0.01). However, patient survival at 30 days was 92% vs. 94% for Ambisome® and placebo-treated patients respectively. Prophylaxis with Ambisome® was $5000 less expensive than reLTx. The post-operative course was complicated by brain oedema, continuation of haemofiltration (pods 1–19), mechanical ventilation (pods 1–19), leucopenia 2100 μl⁻¹, and onset of infection of unknown origin. Antibiotic therapy with piperacilline and tazobactam (Tazobac, Lederle) was started after retransplantation and was switched to meropenene (Meronem, Zeneca) on pod 12. Methylprednisolone was given 3 × 500 mg i.v. pods 14–16, because of elevated liver enzymes (AST from 16 U l⁻¹ to 490 U l⁻¹). No signs of rejection were seen in liver biopsy. The patient developed fever 39.7 °C on pod 16. The patient had multiple ischaemic areas, compression of the IVth liquor ventricle and small areas of haemorrhages in a computerized tomography scan. Screening for systemic mycoses was negative until the patient died on pod 19. Disseminated aspergillosis was found histologically in the endocardium, lung and brain. Retrospectively, Aspergillus antigen in serum became positive on pod 15.
Ambisome® prophylaxis after liver transplantation

Table 1. Overview of studies using antimycotic prophylaxis after liver transplantation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transplantations (patients)</th>
<th>Systemic mycotic infections incidence (mortality)</th>
<th>Antimycotic drug (route of administration); dose (P/T); duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kusne [4]</td>
<td>101 LTx</td>
<td>14.0% (82.0%)</td>
<td>Amphotericin B (i.v.); 10 mg day⁻¹ (P); 10–14 days</td>
</tr>
<tr>
<td>Mora [35]</td>
<td>180 LTx</td>
<td>7.5% (1.3%)</td>
<td>Nystatin (p.o.); 500,000 units (P)</td>
</tr>
<tr>
<td>Hadley [1]</td>
<td>124 LTx</td>
<td>15.0% (NA)</td>
<td>Ambisome 1 mg kg⁻¹ (P); 5 days</td>
</tr>
<tr>
<td>Tollemar [15]</td>
<td>40 LTx</td>
<td>0.0% (0.0%)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Huddinge, Sweden</td>
<td>37 LTx</td>
<td>16.0% (16.6%)</td>
<td>Ambisome 1 mg kg⁻¹ (P); 7 days</td>
</tr>
<tr>
<td>Leif (1998) Göttingen, Germany</td>
<td>64 (58) LTx</td>
<td>6.8% (75.0%)</td>
<td></td>
</tr>
</tbody>
</table>

1 mg kg⁻¹ per day of conventional amphotericin B is recommended for treatment of aspergillosis [16]. Amphotericin B 0.5 mg kg⁻¹ per day is probably ineffective as antifungal prophylaxis after liver transplantation, resulting in 3/55 invasive aspergilloses [17].

From our results, Ambisome® (1 mg kg⁻¹ per day) seems to be beneficial against systemic Candida infections. However, the onset of systemic Aspergillus infections could not be prevented.

Obviously, higher Ambisome® doses appear to be necessary against Aspergillus species. Reflecting the patients’ preoperative status, all patients with invasive fungal infection had an advanced stage of end-stage liver disease. The following risk factors were found in the four patients with systemic mycoses: primary non-/dysfunction, complicated late-stage acute liver failure, retransplantation or haemofiltration/-dialysis for renal failure, long-time mechanical ventilation, antibiotic therapy for infection of unknown origin or pretransplant hospitalization. These findings were previously confirmed by Patel et al. [6] Patel & Paya [14] and Collins et al. [18]. Furthermore, separate risk factors have been analysed for Candida (intra-abdominal bleeding), Aspergillus (fulminant hepatitis) and cryptococcal (symptomatic cytomegalovirus infection) infections [6]. Grauhan et al. [19] reported 13 patients with fungal infections after liver transplantation. They found no correlation between fungal infections and potential risk factors, such as rejection treatment, dialysis, mechanical ventilation, graft failure, long operation time, second transplant, serious non-fungal infection. No significant difference for deep fungal infections was seen between CsA and tacrolimus in a study comparing major infectious complications and outcomes after liver transplantation. Deep fungal infections occurred in 17% (17/102 patients) in the CsA group compared with 9% (2/22) in the tacrolimus group [20]. However, a comparison of four study periods (1988–94) demonstrated a major shift in the microbial aetiologies of infections, particularly pneumonitis, after liver transplantation. Although the overall incidence of pneumonia decreased from 34% to 15%, the incidence of fungal pneumonia increased from 9% to 37%. This changing pattern of microbial aetiologies is suggested to be caused by the era of modern immunosuppressive agents [21].

The early detection of invasive Aspergillus infection poses a serious problem. All three diagnoses of aspergillosis were only confirmed post mortem.

The therapeutic options include the current antifungal drugs available: conventional amphotericin B, liposomal amphotericin B (Ambisome®), fluconazole, itraconazole and fluconazole. These drugs have a wide spectrum of adverse events.
References


