Liposomal amphotericin B (AmBisome) for fungal infections in immunocompromised adults and children

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Invasive fungal infections are rare but life-threatening infections, most often occurring in immunocompromised patients. For a long time, Amphotericin B has been the best choice for treatment, because it is fungicidal with a broad antifungal spectrum and minimal risk of resistance development. The therapeutic use of amphotericin B has, however, been limited by its toxicity—both acute as well as chronic. To counter this, amphotericin B has been encapsulated in liposomes, which reduces its toxicity and allows higher doses to be given. Ambisome is a true, spherical, small unilamellar liposome with a median size of 80 nm. The pharmacokinetic profile was changed, and the maximum concentration and AUC of amphotericin B after AmBisome treatment were greater than those found with the conventional drug. The highest tissue concentrations of Ambisome were found in the liver and spleen, and less than 1% of the administered dose was recovered in other organs. At Huddinge University Hospital, we were the first to use and report on the experience of AmBisome. We now have more than 12 years' experience in transplant recipients, with a good safety profile, improved rate of curing mycological proven infections and reduced mortality in fungal infections. In two placebo-controlled prophylactic trials, we found that AmBisome was effective for preventing fungal colonization and invasive fungal infections, respectively, in allogeneic stem cell and liver transplantation. In uncontrolled and, more recently, in randomized controlled studies at other centers, AmBisome has revealed lower toxicity and an efficacy equal or superior to that of the conventional drug in treating neutropenia-associated fever and proven invasive fungal infections in both adults as well as in children. Although investigators tend to increase the dose used, the optimal dose for probable or proven infection is still under debate. Based on our own experience in using AmBisome and the experience at other centers, we can conclude that AmBisome represents a major breakthrough in the treatment of invasive fungal infections, especially in immunocompromised patients.

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INTRODUCTION

Opportunistic fungal infections, mainly caused by Candida and Aspergillus spp., may be life-threatening in severely immunocompromised patients, such as organ and bone marrow transplant recipients. Amphotericin B has been the drug of choice. Amphotericin B is a macrocyclic polyene antibiotic derived from Streptomyces nodosus and is administered complexed with deoxycholate [1]. However, the therapeutic use of amphotericin B has been limited by its acute toxicity, including headache, chills, fever, nausea, vomiting, diarrhea, anorexia, malaise, muscle pain, phlebitis, hypocalcemia, anemia, bronchospasm, arrhythmias and, above all, nephrotoxicity [2,3]. Especially in transplant recipients treated with the immunosuppressive drug cyclosporin, therapy with amphotericin B causes a synergistic nephrotoxicity [4]. To reduce toxicity, amphotericin B has been encapsulated in liposomes, which allows higher doses [5,6]. In experimental animal studies as well as clinical trials, liposomal amphotericin B was shown to be effective against invasive fungi [7–10]. The incorporation of amphotericin B into liposomes, this alters the pharmacokinetic properties of the drug, which leads to changes in tissue distribution, antifungal activity and, most of all, tolerability. The first preparations of liposomal amphotericin B

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were prepared at the investigational centers shortly before therapy, because they could not be stored for any amount of time. However, more than 10 years ago, a lyophilized formulation consisting of liposomal amphotericin B incorporated into small unilamellar liposomes (mean diameter 45–80 nm) composed of hydrogenated soy phosphatidyl choline, cholest erol and distearoyl phosphatidylglycerol combined in a molar ratio of 2:1:0.8 was introduced. At Huddinge Hospital, we were the first to use and report the experience of liposomal amphotericin B (AmBisome) [11]. We have now experienced 12 years of use of AmBisome in transplant recipients.

PHARMACOKINETICS

Amphotericin B remains the drug of choice for treatment of a variety of invasive fungal infections. It is a poorly water-soluble polyene. The mechanism of action involves binding to membrane sterols of both fungal (ergosterol) and human (cholesterol) cells producing pores out of which cell contents leak, thus causing cell death. However, amphotericin B has a higher affinity to ergosterol than to cholesterol. Amphotericin B is fungicidal in vitro with a broad antifungal spectrum with minimal risk of development of resistance. It is highly lipophilic and administered as a complex with deoxycholate. Electrolytes in the intravenous solution lead to aggregation to colloid and so it is administered in dextrose [12]. At a dose of 1 mg/kg/day peak concentration achieved is 3.6 mg/L, and the area under the curve 34 mg/L/h. Amphotericin B is widely distributed in the body with a protein binding up to 95% [13]. In adults, CSF-concentration is only about 2–4% of that in serum. In children CSF-concentrations are much higher; up to 90% of concomitant serum concentration has been reported. The most important depots in the body are the liver, kidney and lungs. The means of elimination of the drug is not known and no metabolite has been identified. There has been a suggestion that tissue accumulation appears account for the majority of drug disposition and the drug has been detectable in liver, spleen and kidney for as long as one year after termination of therapy. Infusion of amphotericin B is poorly tolerated and toxicity is common. Acute toxicity includes fever, chills, nausea, vomiting, artralgia, myalgia, headache and anaphylaxis. Chronic or dose-dependent toxicity is renal toxicity, cardiotoxicity, anemia, potassium and magnesium wasting, among which the nephrotoxicity usually limits its therapeutic use [12].

Because of amphotericin B's toxicity, the drug has been incorporated into a variety of lipid formulations thereby reducing the toxic side events and increasing efficacy. Depending on the type of lipid incorporation, the pharmacokinetics of amphotericin B are altered, with the effect varying according to particle size and composition of the complex produced. Large structures are rapidly taken up by the reticuloendothelial system, whereas a small unilamellar liposome remains in the circulation for prolonged periods of time.

AmBisome is a true spherical small unilamellar liposome with a median size of 80 nm. Each liposome contains 10 mol.% amphotericin B. The exact mechanism behind the reduced toxicity of amphotericin B when given as a liposomal formulation is not known. Selected transfer of amphotericin B from this formulation to the target fungal cell wall, avoiding uptake to human cells, is believed to be the important mechanism [14]. If the drug is pharmacologically active when it is bound to the liposome or if it has to be released to reach its site of action is unknown. However, this complicates the interpretation of concentrations measured since most tests do not discriminate among free or lipid-complexed drug levels.

The maximum concentration of amphotericin B after 1 mg/kg/day of AmBisome was, in one analysis, up to three times that of the conventional drug - 12 vs. 3.6 mg/L of amphotericin B. The AUC was twice that of conventional amphotericin B, 60 vs. 34 mg/L/h. When the dose of AmBisome was increased to 5 mg/kg, the peak concentration increased to more than seven times (25–59 mg/L) and the AUC to more than 15 times (523 mg/L/h) compared with conventional drug [13]. Tissue concentrations of AmBisome were investigated and tissue samples taken in autopsy cases after a total dose of AmBisome ranged between 900 and 3428 mg. The highest concentrations were found in liver and spleen. Concentrations were highly variable but less than 1% of the administered dose was recovered in other organs such as kidney and heart [11].

PHARMACOKINETICS IN CHILDREN

The pharmacokinetic profile of AmBisome in children is not well studied, but the pharmacokinetics of amphotericin B are not the same in infants and children as in adults [15]. As compared with adults, children older than 3 months of age manifest more rapid clearance of amphotericin B. Premature neonates are characterized by extreme individual variation in the distribution and clearance of amphotericin B, and thus individual serum levels cannot be predicted with certainty. Some neonates continue to accumulate amphotericin B during the course of therapy, an observation suggesting that they require a dose interval of more than 24 h. Amphotericin B toxicity is less severe in infants and children than in adults, probably because of the more rapid clearance of the drug from children [15]. Studies of pharmacokinetics after administration of AmBisome in neonates and children are needed.
ARGUMENTS FOR THE USE OF LIPOSOMAL AMphotERICIN B

The arguments for the use of a lipid-associated form of amphotericin B are two-fold: first, that activity is dose dependent with the conventional drug, and that the ease of administration of AmBisome in particular allows greater doses to be administered; second, that toxicity is dose limiting but, with the liposomal formulations, there is a marked reduction of toxicity and, therefore, the possibility to increase doses. Thus, there is an increased therapeutic index with the use of the liposomal formulations of amphotericin B.

Until recently, we have had evaluation difficulties regarding lipid formulations of amphotericin B, because there are few well-designed trials and available data are based on heterogeneous groups of severely ill patients. Furthermore, treatment has been instituted more or less on a compassionate basis, because of treatment failures with other antifungal drugs, or because of toxicity or the risk of toxicity in patients. However, well-performed randomized trials comparing conventional drugs with liposomal amphotericin B have recently been performed. In the remainder of this article, we will discuss compassionate data — ours and those of others — randomized comparative data and, finally, treatment in children.

THE HUDDINGE EXPERIENCE OF AMBISOME

In the first study, four organ and four bone marrow transplant recipients were treated with AmBisome in a compassionate basis programme ranging from 9 to 48 days (median 15) with a median cumulative dose of 963 mg. Maximum dose ranged from 0.9 to 2.5 mg/kg. Six of the eight patients with proven or probable deep fungal infection recovered and were discharged from hospital. Two bone marrow transplant patients died during therapy. One was negative for fungi at autopsy; in the other patient, autopsy was not performed. Amphotericin B concentrations taken at autopsy ranged from 0.6 to 7.5 mg/kg tissue in bone marrow, liver and spleen, and from 0.6 to 8 in lung, heart, muscle and kidney. Two patients developed high alkaline phosphatase values during treatment, but no other acute side-effects were noted.

The first units to use AmBisome reported toxicity and efficacy [16,17]. These studies included 126 patients who were treated for 137 episodes of fungal infection at 43 investigational centres. There were 47 females and 79 males. The median age was 35 years (range 4–87). There were 72 patients with malignancies (among those 55 with leukemia), 17 organ transplant recipients, 12 with AIDS and 25 other. Among the patients, 25 were recipients of allogeneic or autologous bone marrow. AmBisome treatment was instituted after toxicity from previous amphotericin B treatment in 49 cases, renal insufficiency in 40 and failure of previous antifungal treatment in 41 instances. Doses of AmBisome of 3–5 mg/kg/day were administered. In the safety study, 133 episodes of therapy with AmBisome were evaluated [16]. Eleven of 71 patients with initially normal serum creatinine concentrations showed increased values after AmBisome therapy. However, 17 patients among 50 with initially high serum creatinine levels recovered normal renal function during AmBisome treatment. Hypocalcemia was the most common adverse event seen in 24 episodes. Nausea and vomiting were seen in three and arrhythmia in two. It was concluded that AmBisome appeared to be a safe alternative to conventional amphotericin B [16]. With conventional amphotericin B, acute toxicity and side-effects occur in a majority of the patients, even in those receiving as little as 0.3 mg/kg/day.

Some 108 episodes were clinically evaluable; among these 52 were caused by Candida spp. (although many had Candida in the respiratory tract only) and 34 by Aspergillus spp. Ninety-nine patients were treated for more than 1 week with a maximum dose of 0.7–5 mg/kg/day. Among 64 cases with proven invasive infections, 37 (58%) were cured, 12 (19%) improved and 15 (23%) failed to respond. The cure rate in the patients with Candida infections was 19/25 (76%), compared with 9/28 (32%) in Aspergillus infections (P < 0.01). Fungi were eradicated from a deep site in 35/54 (65%) of mycologically evaluable cases. The eradication rate was 83% for Candida species, compared with 41% for Aspergillus species. Fungi were eradicated in 14/22 patients with leukemia, 5/8 with immunodeficiencies, 9/10 with organ transplants, 1/3 with malignancies other than leukemia and 6/11 others. AmBisome tissue concentrations were studied in three autopsy cases. High concentrations of AmBisome were seen in liver and spleen. The lung showed variable concentrations and low concentrations were seen in heart and brain. In the kidney, concentrations were intermediate.

Efficacy evaluation of AmBisome is complicated by difficulties in the diagnosis of fungal infections, the poor condition of patients and patients' frequent concomitant infections. The cumulative dose of AmBisome and the length of treatment had no influence on the eradication rate of fungi. This may be because patients with poorer prognosis and those with a slower response rate were treated with high doses and for a longer period of time. Some 37% of the patients died within 4 weeks of AmBisome therapy, which emphasizes the poor prognosis in the patients included in this study. Furthermore, fungal infection was the cause or contributing cause of death in 25 patients. However, 16 of 20 patients in whom previous amphotericin B had failed were cured by or responded to AmBisome [18]. High-risk patients, such as bone marrow transplant recipients during the leukopenic phase, are often beyond therapy by the time the diagnosis of fungemia is made [19,20], so the experience of AmBisome was encouraging. It led to studies of prophylaxis and empirical therapy.
In our initial experience at Huddinge Hospital, 10 transplant patients receiving AmBisome were compared with 10 retrospective control patients given conventional amphotericin B [21]. Each group included eight bone marrow transplant recipients, one kidney transplant recipient and one liver transplant recipient. Among the patients treated with conventional amphotericin B, treatment was instituted as a result of nine Candida infections and one Aspergillus fumigatus infection. In the AmBisome group, treatment was instituted as a result of Candida infection in eight of 10 patients. In the amphotericin B group, the maximal daily dose ranged from 0.1 to 0.65 mg/kg and was given over 3–32 days. In the AmBisome group, maximal daily dose ranged from 0.9 to 2.3 mg/kg and was given over 8–28 days. All patients in the amphotericin B group experienced severe toxicity, especially nephrotoxicity, compared with only one patient with cholestasis in the AmBisome group. Only three out of 10 patients in the amphotericin B group responded to treatment, seven patients died and six patients still had evidence of invasive fungal infection at autopsy. In contrast, eight out of nine patients in the AmBisome group responded to treatment, and the patient who received prophylaxis had a successful course.

At Huddinge Hospital, we evaluated safety in 187 transplant recipients treated for 197 episodes given AmBisome for a median of 11 days [22,23]. This comprised our first 5 years' experience of AmBisome. The patients included 89 bone marrow transplant recipients, 64 liver transplant recipients, 20 renal transplant recipients, 10 recipients of combined kidney and pancreas transplants, two recipients of liver and pancreas transplants, one recipient of a single pancreas transplant and a recipient of a bone marrow transplant and a subsequent liver transplant. Median age was 36 years (range 0.5–72). AmBisome was given for a median of 11 days (range 1–112) with a maximum daily dose of 1.49 ± 0.7 mg/kg/day (mean ± SE). The total cumulative dose of AmBisome was 1.1 ± 1.78 g. Side-effects definitely attributed to AmBisome therapy included low potassium (n = 3), low back pain (3), dyspnœa (2), allergic rash (1), nausea and vomiting (1), confusion (1), rise in alkaline phosphatase (1) and cholecystitis (1), with an overall incidence of 13/197 (7%). AmBisome was discontinued due to side-effects in six (3%) of the cases. Side-effects possibly due to AmBisome therapy included low potassium (36%), increase in serum creatinine (31%), rise in alkaline phosphatases (26%) and fever (3%). The overall mean increase in serum creatinine was 20%. Other possible side-effects, such as headache, abdominal pain, rash, rise in bilirubin, cramps and pancreatitis, were seen in single patients. In these instances, AmBisome may have potentiated the toxicity of other drugs. All patients were treated with various potentially toxic drugs, including cyclosporin, some also with nephrotoxic antibiotics, diuretics and others. However, the side-effects of AmBisome were mild and manageable in most patients. None of the patients treated with AmBisome had an anaphylactic reaction. However, anaphylactic reactions caused by AmBisome have been reported [23]. From our study, it was concluded that AmBisome provided a great contrast to the situation with conventional amphotericin B, where acute toxicity and side-effects occurred in a majority of the patients. Although mild side-effects were seen in AmBisome-treated patients, these were rapidly reversible in most patients. As an example, 1 week after discontinuation of AmBisome, normalization or improvement was seen in the serum potassium and serum creatinine values of 66% of the patients.

Invasive fungal infection has been the cause of death in 11% of our patients during the amphotericin B era [20]. We therefore compared invasive fungal infections verified at autopsy during three different periods in our bone marrow transplant programme [24]. Before 1985, the patients were treated with monotherapy at prophylaxis against graft-vs.-host disease (GVHD). During this period, 30% of the patients developed moderate-to-severe GVHD and autopsy-proven invasive fungal infection was 15/129 (12%). Between October 1985 and December 1988, effective prophylaxis against GVHD combining methotrexate and cyclosporin, or giving T-cell depleted transplants was introduced [25–27]. With this more effective therapy, only 13% of the patients developed moderate-to-severe acute GVHD. However, the rate of autopsy-proven invasive fungal infection was still high (11/98, 11%). However, after the introduction of AmBisome in January 1989, the autopsy-proven invasive fungal infections decreased to 12/199 (6%), which was significantly decreased (P < 0.05), compared with the two previous periods. The frequency of autopsy in patients with a transplant was 73%, 69% and 62% in these three eras. As not all patients who died underwent autopsy, this finding has to be interpreted with some caution.

**AMBISOME PROPHYLAXIS**

Because of the difficulties in diagnosing invasive fungal infections, it was thought that prophylaxis with AmBisome would be the optimal strategy in bone marrow and liver transplant recipients, who are at high risk of invasive fungal infection. A randomized double-blind study of AmBisome prophylaxis was performed in bone marrow transplant recipients [28]. AmBisome was given when the neutrophil count had decreased to <0.5 × 10⁹/L and was continued until neutrophils recovered to this level, or an infection or a toxicity end-point was reached. Thirty-six patients received 1 mg/kg/day of AmBisome and 40 patients received placebo.
daily. Patient characteristics and clinical course were similar between the two groups. Fungal colonization decreased in the AmBisome group while it increased in the placebo group. By the end of prophylaxis, 8/24 (33%) of the patients receiving AmBisome were colonized, compared with 18/29 (62%) of the placebo patients \( (P = 0.05) \). Five and seven patients on AmBisome or placebo, respectively, were withdrawn due to a presumed fungal infection. Proven fungal infection occurred in one patient receiving AmBisome \( (Candida guilliermondii) \), compared with three patients receiving placebo \( (Candida guilliermondii II; Candida albicans I) \). AmBisome was well tolerated at a dose of 1 mg/kg/day and there was no statistical difference in side-effects between the two study groups. However, three patients in the AmBisome group experienced allergic reactions related to infusion of the drug. All three patients experienced flushing, erythema, breathing problems and headache. After discontinuation of the drug, the reactions resolved in all three patients. Four additional patients experienced adverse events, possibly related to AmBisome. One patient had confusion and fever. Another experienced nausea, one-third had vomiting and chills, and all these patients later received AmBisome for 16–21 days without new adverse events. The fourth patient had erythema and cholecystitis after 6 days of prophylaxis that resolved. In the placebo group, two patients had fever. Comparing pre-with post-treatment blood chemistry values, the AmBisome group had significant increases in blood urea nitrogen, 61% vs. 114% \( (P < 0.01) \) and creatinine 55% vs. 83% \( (P < 0.001) \). There was no difference in i.v. supplementation of potassium between AmBisome or placebo-treated patients during the study. The conclusion from this study was that AmBisome prophylaxis was well tolerated during the aplastic period in bone marrow transplant recipients. However, it was not completely effective in preventing \( Candida \) infection. Therefore, further studies are needed concerning aspergillosis and candidiasis before any recommendations can be made.

Among solid organ transplant recipients, orthotopic liver transplant recipients have had the highest reported incidence of invasive fungal infections ranging between 6% and 42% \[29,30\]. Most infections occur within the first postoperative month. The gastrointestinal tract is a major reservoir of \( Candida \), which explains the high incidence of invasive candidiasis in liver transplant recipients who receive extensive surgery, often involving the intestines. Therefore, a randomized double-blind study of AmBisome was performed \[31\]. Seventy-seven liver transplant recipients received 5 days of prophylaxis starting during the transplantation procedure with either AmBisome 1 mg/kg/day or placebo. Among 40 AmBisome-treated patients, no invasive \( Candida \) infection was seen during the first month, compared with five invasive \( Candida albicans \) infections among 37 control patients \( (P < 0.05) \). Furthermore, one placebo patient experienced \( Aspergillus niger \) pneumonia. Thus, the overall incidence of invasive fungal infection was 0/40 in the AmBisome group vs. 6/37 (16%) in the placebo group \( (P < 0.01) \). Patient survival did not differ between the two groups and at 30 days it was 92% vs. 94% for the AmBisome and the placebo-treated patients, respectively. Side-effects related to AmBisome included transient thrombocytopenia in two patients and low back pain in one patient. Regarding blood chemistry, alkaline phosphatase increased significantly in the AmBisome-treated patients who had 3.3 times the upper normal value at 30 days, as compared with 1.5 times in the placebo group \( (P < 0.01) \).

Other laboratory tests did not differ between the two groups. The costs for antifungal drugs in the AmBisome group was US$32,912 for prophylaxis and US$20,050 for treatment of suspected invasive fungal infection, a total of US$52,962. In the placebo group, the costs were US$37,943 for treatment of verified invasive fungal infections and US$15,033 for suspected invasive fungal infection with a total of US$52,976. However, the cost for prophylaxis in the AmBisome group was US$5,031 less than the cost for treatment of verified invasive fungal infection in the placebo group. This study showed that AmBisome prophylaxis was well tolerated and effective in preventing invasive fungal infection following liver transplantation.

This study was followed by a long-term follow-up where all patients who had received 5 days of prophylaxis were followed for at least 1 year \[32\]. Late invasive fungal infection occurred in four AmBisome-treated patients between 30 days to 1 year after transplantation with a median onset at day 81 (range 39–325). Three patients had \( Aspergillus \), all found at autopsy. One patient developed a \( Candida albicans \) cholangitis, which was successfully treated. In the placebo group, five patients developed late invasive fungal infections, four of which were candidiasis and one aspergillosis with a median onset at day 150 (range 41–365). All except one patient with \( Candida albicans \) peritonitis died. Thus, during the first year, significantly fewer patients \[4\] developed invasive fungal infection in the AmBisome group, compared with 11 in the placebo group \( (P < 0.05) \), resulting in a one-year probability of invasive fungal infection of 11% vs. 29% in the AmBisome and placebo group, respectively. Patient survival at 1 year was 80% in the AmBisome group, compared with 78% in the placebo group, respectively (ns). From our experience at Huddinge Hospital in bone marrow and organ transplant patients using AmBisome, we can conclude that AmBisome is safe and gives few side-effects. It is also effective and represent a major breakthrough in the treatment of invasive fungal infections. A concern is the high costs \[33\].

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OTHER CENTERS’ EXPERIENCE

During the last 10 years several studies from single centers with small patient numbers have shown AmBisome to be effective in infections other than those caused by Candida and Aspergillus spp. Nine of these noncomparative studies with immunocompromised patients treated for proven fungal infection were pooled together; of these three studies had mycological eradication as an endpoint [34]. For 121 cases of candidiasis, a median eradication rate of 79% (range 13–100%) was documented in 41 cases of aspergillosis, the median eradication rate was 67% (range 41–100%), and in 11 cases of other fungal infections, the eradication rate was 78%.

AmBisome was shown to be effective in AIDS patients suffering from cryptococcosis, in five small noncomparative trials. Doses ranged between 1 and 3 mg/kg/day for a median of 16–35 days [35]. The mycological cure rate ranged between 67 and 85% with a median of 71%. In one randomized trial of AmBisome 4 mg/kg vs. conventional amphotericin B 70.7 mg/kg for a median of 21 days, the mycological cure rate was significantly better for AmBisome, 79 vs. 38% for amphotericin B.

COMPARATIVE STUDIES

More recently, controlled studies in which AmBisome has been compared with conventional amphotericin B or Abelcet have been performed. There are four randomized studies in neutropenic patients in which AmBisome was used as empirical treatment and compared with conventional amphotericin B in two and to Abelcet in one and finally one study of fungal infections other than those caused by Aspergillus. During the last 10 years several studies from single centers with small patient numbers have shown AmBisome to be effective in infections other than those caused by Candida and Aspergillus spp. Nine of these noncomparative studies with immunocompromised patients treated for proven fungal infection were pooled together; of these three studies had mycological eradication as an endpoint [34]. For 121 cases of candidiasis, a median eradication rate of 79% (range 13–100%) was documented in 41 cases of aspergillosis, the median eradication rate was 67% (range 41–100%), and in 11 cases of other fungal infections, the eradication rate was 78%.

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There were significantly less toxicity with AmBisome as compared with the conventional drug. Fewer patients had infusion-related adverse events in the form of fever, 17% vs. 44%, chills or rigors, 18% vs. 54%, for AmBisome or the conventional drug, respectively. Nephrotoxicity, defined as two times upper normal limit value, was significantly less frequent among patients receiving AmBisome–19% compared with 34% for patients receiving amphotericin B (P < 0.001). With regard to efficacy, there were no differences between the study arms with regard to composite success rate (50% and 49%), survival rate (93% and 90%), or resolution of fever during neutropenia, 58% and 58% for AmBisome and amphotericin B, respectively. However, there were significantly fewer emerging fungal infections with AmBisome compared with amphotericin B, 5% vs. 9% (P = 0.021).

A study was performed in the Netherlands evaluating the hypothesis that there would be a better outcome of neutropenia-associated invasive fungal infections if higher doses of lipid formulations of amphotericin B were used [38]. AmBisome was compared with amphotericin B in a randomized multicenter study in the treatment of documented and suspected invasive fungal infections during neutropenia. AmBisome was given at a dose of 5 mg/kg/day compared with amphotericin B at a dose of 1 mg/kg/day. A total of 106 patients, out of which 66 were evaluable for efficacy, were included in the study. Of these, nine patients had documented fungemia, 17 patients had documented invasive mould infections and 40 patients had suspected pulmonary aspergillosis. Despite a dose of AmBisome of 5 mg/kg, toxicity was scarce and significantly fewer patients had kidney function deterioration compared with the conventional drug. Serum creatinine elevation at 14 days after treatment was 1.4 ± 5% for AmBisome, compared with 86 ± 9% with the conventional

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and discontinue of therapy was less frequent with AmBisome 3 day or Abelcet and not powered for efficacy, successful responses were seen in nephrotolcity was seen, 14.1% and 14.8% for AmBisome 3 AmBisome was less toxic and as effective or superior to AmBisome and Abelcet in 244 neutropenic patients with conventional drug in treating neutropenia-associated fever and fungal infection of unknown origin. However, the optimum dose for probable or established fungal infection is still under debate.

A study by the EORTC Invasive Fungal Infectious Cooperative Group comparing two doses of AmBisome for the treatment of invasive aspergillosis in neutropenic patients showed no benefit for a higher dose [39]. This study was performed in 120 patients out of whom 87 were eligible and evaluable. Doses compared were 1 vs. 4 mg/kg/day of AmBisome. Clinical responses were documented in 64% of the patients receiving 1 mg/kg/day and 48% of the patients receiving 4 mg/kg/day. With regard to radiological responses and the number of deaths due to invasive aspergillosis, there were no differences within the two study arms.

Although this study did not show any advantage of a higher dosage, a tendency among investigators today is to increase the dose used initially when treating patients with a suspected or proven fungal infection. Obviously, this will have a great economic impact, since AmBisome is much more expensive than the conventional drug, or any other antifungal drug available at present. However, efficacy and toxicity must be taken into consideration when costs are analyzed. This becomes evident in one study between two lipid formulations that have recently been presented. This was a US multicenter double-blind randomized head to head comparison of AmBisome and Abelcet in 244 neutropenic patients with unresolved fever after 3 days of antibiotic treatment [40]. The study was powered for safety and not for efficacy. Patients were randomized to receive either AmBisome 3 or 5 mg/kg/day or Abelcet 5 mg/kg/day. AmBisome had significantly less acute adverse events such as fever, chills and rigors compared with Abelcet. Furthermore, significantly lower rates of nephrotoxicity was seen, 14.1% and 14.8% for AmBisome 3 and 5 mg compared with 42.3% for Abelcet. Toxicity-related discontinuation of therapy was less frequent with AmBisome 3 and 5 mg, 12.9% and 12.3% vs. 32.1% for Abelcet. Although not powered for efficacy, successful responses were seen in 40% and 42% of AmBisome 3 and 5 mg/kg treated compared with 33.3% of Abelcet-treated patients.

**THERAPEUTIC USE IN THE MANAGEMENT OF FUNGAL INFECTIONS IN CHILDREN**

In most cases, intravenous amphotericin B is still the antifungal drug of choice in children. Owing to the manifest individual differences both in clinical and immunological status, there is no standard treatment. Dosage and duration of treatment are empirical. To avoid toxicity, AmBisome is indicated in patients who have failed to respond to conventional amphotericin B, patients who develop nephrotoxicity after receiving conventional amphotericin B, or patients in whom conventional amphotericin B is contraindicated because of renal impairment. Comparative trials are necessary to define the pharmacokinetics of AmBisome and optimal dosages in different kinds of immunocompromised children such as neonates, children with cancer and children receiving organ or bone marrow transplants.

**AMBISOME PROPHYLAXIS IN CHILDREN**

Tollemar and co-workers (1993, 1994) have, in two placebo-controlled, double-blind randomized trials, presented their experience with AmBisome prophylaxis in allogeneic bone marrow transplant and liver transplant recipients (both children and adults). The incidence of verified IFI was in the AmBisome groups 3% and 0%, compared with 8% and 16% in the placebo groups, respectively [28,32].

In a retrospective study, Ringdén and co-workers presented their experience with AmBisome prophylaxis and therapy for invasive fungal infections in children undergoing organ or allogeneic bone-marrow transplantation [41]. AmBisome was given as prophylaxis in 30 episodes in 25 children for a median of 14 days (3-46) in a dose of 1 mg/kg/day (range 0.6-4.3), with the total dose a median of 0.20 g [0.025-2.6]. Prophylaxis, 1 mg/kg/day was given during the neutropenic phase after BMT, when the polymorphonuclear count (PMN) was below 0.5 × 10⁹/L. In liver transplant patients, 1 mg/kg/day was given during the first 5 days. AmBisome was well tolerated as prophylaxis in transplanted children and few acute toxic side-effects were seen. The commonest probable side-effect of AmBisome in children given prophylaxis were, decreased serum potassium levels in 40%, renal toxicity in 37%, and increased alkaline phosphatase levels in 30%.

**AMBISOME TREATMENT IN CHILDREN**

AmBisome has been reported to be effective against systemic infection in children, including infants. This has been
demonstrated in several studies. In children with systemic mycoses who had failed to respond to or could not tolerate amphotericin B [42]; 11 children (median 7) received amphotericin B (6) or Ambisome (5). In both groups, three patients had highly suspected or proven fungal infection. Most of these were leukemic patients and seven had received bone marrow transplants. None of those treated with Ambisome died of fungal infection. The maximal dose of Ambisome used in this group was 6 mg/kg and the cumulative dose given was 122–311 mg/kg.

Two very low birth-weight infants treated with Ambisome recovered without sequelae. One of these cases had neonatal candidemia due to C. albicans, the other disseminated aspergillosis. The infants tolerated doses up to 5 mg/kg daily very well [43].

Nowoczyzn and co-workers reported, in 1992 Ambisome treatment in 10 neutropenic children with hematological malignancies and systemic fungal infection [44]. The median age of the children was 8.5 years (range 1.5–16.5 years). Six children were pretreated with conventional amphotericin-B plus flucytosine. The change to Ambisome in these children was due to toxicity in four and/or progression of pulmonary infiltrates in two children, respectively. Seven children suffered from candidiasis and three from aspergillosis. Seven of 10 children responded to Ambisome and survived.

The Ambisome dose was 1.5–2.5 mg/kg/day. Median duration of treatment was 15 days (4–35 days). Ambisome was well tolerated.

In 1994, Emminger et al. presented a study of 16 children, aged 3 months to 18 years of age (median 7.5 years), with cancer, who were treated with Ambisome [45]. Four had highly suspected or verified invasive fungal infection (C. albicans or C. glabrata), and 12 children received empirical treatment [45]. Fifteen of these children were severely granulocytopenic. Three of the four patients with highly suspected or verified infections were cured. The patient who failed had severe aplastic anemia with C. glabrata candidemia. Although large cumulative doses were given, no organ function abnormalities attributable to Ambisome were detected in any of 10 long-term survivors over a median observation time of 36 months (range 30–44 months).

Ringdén and colleagues, have included 14 immunocompromised children in a noncomparative study [46]. One child received treatment four times, and a total of 17 treatment episodes were studied. Median age was 14 years (range 9 months–16 years). Nine children had proven invasive fungal infection (IFI) — Candida in six cases and Aspergillus in three cases, and eight patients had suspected IFI. Among the eight cases with suspected IFI, six were clinically cured. Among the nine cases with proven IFI, seven were clinically cured (78%). Of the two failures, one boy had leukemia and an infection caused by C. krusei. The other had chronic granulomatous disease and Aspergillus spp. in lung and liver.

Hovi and colleagues reported fungal osteomyelitis due to Aspergillus flavus (2) and Saccharomycetes cerevisiae (1) in the jaws of three children on immunosuppressive chemotherapy [47]. One child with A. flavus received Ambisome 1 mg/kg and underwent surgery and survived.

Pasic and colleagues reported that the use of Ambisome was safe in bone marrow transplantation for primary immunodeficiency in 15 paediatric patients [48]. Ringdén and co-workers (1997), have in a retrospective study, presented their experience with Ambisome therapy for invasive fungal infections in children undergoing transplantation [41]. Ambisome was given as treatment for suspected invasive fungal infection (IFI) in 33 and for verified invasive fungal infection in 12 patients. Of 31 children with suspected IFI, fever disappeared in 21 (68%). In documented cases treated for 5 days or more, the clinical cure rate was 86%. Eradication of fungi from a deep site was verified in 8/10 and patient survival 1.5 years to more than 7 years post-treatment was 7/12 (58%). Ambisome was well tolerated as therapy in transplanted children.

**AMBISOME TREATMENT OF NEONATES**

Weitkamp and colleagues have reported the outcome and nephrotoxicity of treatment with Ambisome in 21 very low birth–weight (VLBW) infants [49]. The median gestational age was 25 weeks (range 23–31) with a median birth-weight of 730 g (range 450–1370). In 19 patients, a positive culture for Candida spp. was obtained and in two it was negative. Candida albicans was found in seven patients, C. parapsilosis in two and in one C. parapsilosis, C. tropicalis and C. krusei. All but one infant survived. All 19 patients with a verified infection had fungal eradication and clinical recovery. Antifungal therapy was started a median of 13 days (range 1–49) after birth. The median dose given was 2.6 mg/kg/day (range 1–5), and the median duration of the therapy was 28 days (range 11–79), corresponding to a median cumulative dose of 71 mg/kg (range 12–271). Hypokalemia was observed in 30% before, and 15% during Ambisome treatment. Median maximum daily potassium supplementation did not exceed doses usually recommended for VLBW infants. The median of the maximum creatinine levels before treatment was 121 μmol/L (range 71–221) and fell to 68 μmol/L (range 31–171) during treatment and 46 μmol/L (range 26–62) 21 days after termination of therapy.

Scarcella and co-workers report Ambisome treatment results for neonates with a verified deep fungal infection [49]. Some 40 preterm and four full-term newborn infants were treated. Thirty-one were infected with Candida albicans (70%) and others with Pichia carsonii, C. parapsilosis, C. sake, C.
The initial daily dose of AmBisome was 1 mg/kg of body weight; this was increased stepwise by 1 mg/kg to a maximal dosage of 5 mg/kg according to the patient's clinical condition. Administration of AmBisome was effective in 72.7% of patients (in all full-term and 28 preterm newborn infants). Five of six cases of meningitis recovered, and 63.6% of 33 VLBW infants survived. The initial AmBisome dose of 1 mg/kg/day eradicated the fungal infection in only two infants (5%). In the 12 unsuccessfully treated infants, diagnosis was made later than in surviving infants (24 ± 13 vs. 20 ± 11 days of life). The postmortem blood culture revealed C. albicans in eight cases, and P. carsonii, C. humicola, C. parapsilosis and C. guilliermondii in the other four cases. The duration of intravenous AmBisome therapy ranged from 7 to 49 days, the mean treatment duration was 22 ± 8 days. The initial daily dosage of AmBisome was 1 mg/kg of body weight; this was increased stepwise by 1 mg/kg to a maximal dosage of 5 mg/kg according to the patients' clinical condition. Hypokalemia was observed in 16 infants during treatment, but this condition was always transient and responded readily to potassium supplementation. No side-effects were observed.

AmBisome treatment of neonatal fungal infection is effective and safe. Randomized clinical trials are required to establish the most effective administration protocol for AmBisome, i.e. the starting dosage, the maximum effective dosage and the cumulative dosage, and to verify whether the preparation should be associated with another antifungal agent.

AMBISOME FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

AmBisome was shown to be effective in visceral leishmaniasis (kala azar) in six noncomparative trials in a total of 307 patients with doses ranging between 1 and 5 mg/kg/day over 7-21 days [34]. Responses in immunocompetent patients were 97-100% and few relapses were seen at 12 months follow-up. Response in AIDS patients were 82-88%, but the majority of patients relapsed between 2 and 22 months after treatment was discontinued.

AmBisome, administered in a variety of regimens over 7-21 days, is a generally effective treatment for visceral leishmaniasis in immunocompetent children, including those aged <2 years [34]. DiMartino and co-workers reported treatment of visceral leishmaniasis in 106 immunocompetent children with AmBisome [50]. The study group consisted of 55 boys and 51 girls, 3-months to 14-years old, with a mean age of 2.5 years. The purpose of the study was to optimize the balance between duration of treatment and total dose of drug capable of eradicating infection. Children were enrolled and assigned alternately to one of four dose groups.

The authors concluded that the optimal regimen in immunocompetent children with Leishmania infantum visceral leishmaniasis is a total dose of 18 mg/kg of AmBisome given as 3 mg/kg per day for 5 days, followed by 3 mg/kg administered as an outpatient regimen on day 10 [50]. Treatment was well tolerated and no adverse events were reported. In the children who failed or relapsed after only 15 mg/kg, all were cured by a total dose of 30 mg/kg.

ECONOMIC IMPACT OF AMBISOME THERAPY

In any discussion about antifungal treatment, the cost of liposomal amphotericin is brought up as the main objection to its use. Clearly, the acquisition cost of AmBisome is much greater than that of conventional amphotericin B. However, cost–benefit assessment of a more expensive antifungal agent should not be restricted only to acquisition costs. Instead, the potential impact on hospital cost savings by less toxicity and improved patient tolerability should be taken into account. However, so far, very few pharmacoeconomic studies have been performed with regard to antifungal treatment. The first with AmBisome was performed in 1992 at our center, where 58 organ and bone marrow transplant recipient were compared with regard to treatment with the conventional drug or AmBisome [51]. This study revealed improved survival from invasive fungal infections at discharge from hospital for kidney and pancreas recipients, and 51% involvement for bone marrow and liver transplant recipients with AmBisome compared with retrospective patients receiving conventional amphotericin B treatment. Improved survival was also seen at 2.5 years follow-up, in favor of AmBisome treatment. The calculated cost per life-year gained ranged in these patients between US$20 000 and 26 000; this is less than US$27 000, which, at that time, was the cost-effective threshold for a year of 'well life' gained.

A group from The Netherlands performed another economic evaluation, in the form of a decision analysis model, to evaluate outcome, cost and cost effectiveness of three different strategies according to data extracted from a fever of unknown origin study in the UK [52]. First-line therapy was given for 10 days and, if unsuccessful after 5 days, then it was complemented with second-line therapy also for 10 days. The therapies analyzed were conventional amphotericin B followed by AmBisome, i.e. 3 mg/kg/day, or AmBisome 1 mg/kg/day followed by 3 mg/kg/day or AmBisome 3 mg/kg/day followed by 5 mg/kg/day. With the efficacy figures found in UK data, AmBisome treatment in children at a dose of 1 mg/kg/day was less expensive than the use of conventional amphotericin B, i.e. US$10 134 vs. US$10 445. However, in adult patients, AmBisome at either dose of 1 and 3 mg/kg/day was more expensive than the conventional drug – US$15 509 and US$20 024 compared with US$13 674.
One problem with all these studies is that they only regard drug costs and disregard eventual extra costs caused by toxicity. More recently, some studies that take toxicity into account have been published. One study evaluated the economics of AmBisome as first-line empirical antifungal therapy using itemized hospital billing data from the randomized US study of AmBisome vs. amphotericin B [53]. A total of 414 patients from 19 centers were entered into this study, which covered costs from first dose of study medication until discharge from the hospital. Hospital costs were significantly higher for all patients who received AmBisome, i.e. US$48 962 vs. US$43 183 (P = 0.022). However, when the costs for study drugs were not included, the costs were lower for patients who had received AmBisome, i.e. US$39 648 vs. US$43 048. The authors conclude that this is mainly because of nephrotoxicity, which occurred more frequently in patients who had received the conventional drug, and this renders a longer hospital stay and incurs higher hospital costs. In their sensitivity analysis which reconciled differences in the rate of nephrotoxicity between cost sample and clinical study populations, the break-even cost for AmBisome was US$87/50 mg vial for all patients and US$112/50 mg vial for allogeneic stem cell transplant patients. Toxicity also has an impact on economics using the different lipid formulations of amphotericin B. An evaluation of the pharmacoeconomics of AmBisome vs. Abelcet was performed based on two US studies on empirical treatment in febrile neutropenic patients [53]. Abelcet at 5 mg/kg was associated with a higher incidence of nephrotoxicity compared with AmBisome at either dose of 3 or 5 mg/kg. Based on a decision analysis model using nephrotoxicity, dialysis and drug acquisition costs as primary outcomes, total hospital costs were lowest using AmBisome 3 mg/kg (US$47 747) compared with 5 mg/kg (US$53 033) and Abelcet at 5 mg/kg (US$52 133).

With the present pharmacoeconomic studies it becomes evident that additional studies are needed and that only looking at drug acquisition costs will be a narrow way of looking at antifungal treatment. So far, no study has evaluated the economic impact of patient suffering caused by toxicity (irrespective of the morbidity associated with this).

CONCLUSION

Conventional amphotericin B has been regarded as the standard of treatment for invasive fungal infections, despite the high mortality among immunocompromised patients. For the last decade, the lipidosomal formulation of amphotericin B, AmBisome, has been available and the picture has changed. Do we see a shift in treatment?

It is notoriously difficult to study antifungal treatment in a scientifically rigorous manner. This is evident with all early compassionate studies, pooled patient materials or small local patient series reported for safety and efficacy as well as for economy. Despite this, AmBisome has revealed without question an improved safety profile over conventional amphotericin B. This has enabled us to push dosing upwards with an improved therapeutic index. Outcomes in immunocompromised children and adults are certainly equivalent and often superior to conventional therapy in both uncontrolled as well as in the most recent controlled randomized studies. The improved outcome is, however, not without cost. Recent pharmacoeconomic studies clearly show that it is not enough to take into account only acquisition costs. In some patient categories, expensive treatment with AmBisome seems to be worthwhile. However, many questions remain, such as optimal dosing, total dose and when to both start and stop treatment. Thus, more well-controlled studies are greatly needed.

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