Original Article

Efficacy and safety of low-dose liposomal amphotericin B in adult patients undergoing unrelated cord blood transplantation

Running title: Low-dose liposomal amphotericin B in CBT

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Abstract

Liposomal amphotericin B (L-AMB) is widely used for empirical or preemptive therapy and treatment of invasive fungal infection after cord blood transplantation (CBT). We retrospectively examined the efficacy and safety of low-dose L-AMB in 48 adult patients of CBT from between 2006 and 2017 in our institute. Among the entire cohort, 42 (88%) patients received L-AMB as an empirical or preemptive therapy. The median daily dose of L-AMB and the median cumulative dose of L-AMB were 1.20 mg/kg/day (range, 0.62–2.60 mg/kg/day) and 30.6 mg/kg (range, 0.7–241.5 mg/kg), respectively. The median duration of L-AMB administration was 21.5 days (range, 1–313 days). Documented breakthrough fungal infection occurred in 1 patient during L-AMB treatment, and 43 (90%) patients survived for at least 7 days after the end of L-AMB treatment. Grade 3 or higher hypokalemia and hepatotoxicity were frequently observed during L-AMB treatment. However, no patients developed an increase in serum creatinine of grade 3 or higher. In the univariate analysis using a logistic regression model, a duration of L-AMB treatment of more than 21 days and a cumulative dose of L-AMB more than 30 mg/kg were significantly associated with nephrotoxicity and grade 3 hypokalemia. These data suggest that low-dose L-AMB may be safe and effective in adult patients undergoing CBT.

Keywords: liposomal amphotericin B, allogeneic hematopoietic cell transplantation, cord blood transplantation, invasive fungal infection, nephrotoxicity, hepatotoxicity

Introduction

Invasive fungal infections (IFIs) are a major cause of mortality and morbidity in recipients of allogeneic hematopoietic cell transplantation (HCT). Although the introduction of routine prophylactic use of antifungal drugs has decreased the incidence of IFI and improved survival after allogeneic HCT [1], it caused changes in the epidemiology of IFIs and the emergence of resistance against triazoles and echinocandins [2]. Liposomal amphotericin B (L-AMB) has a wide spectrum of activity against yeasts and molds including *Aspergillus* species and Mucormycosis [3,4]. Previous prospective studies have demonstrated that L-AMB is an effective empirical therapy for patients with persistent febrile neutropenia despite the use of prophylactic antifungal agents and targeted treatments of IFIs [5–8]. Although L-AMB has a significantly improved toxicity profile compared with conventional amphotericin B deoxycholate (D-AMB), adverse events such as hepatotoxicity, nephrotoxicity, electrolyte abnormalities, and infusion-related reactions also frequently occurred for a recommended-daily dose ranging between 3 and 6 mg/kg of L-AMB [3,4]. However, recent studies have suggested that a low-dose of L-AMB (approximately less than 2 mg/kg/day) is effective and has a lower toxicity for patients with hematological malignancies [9–14]. Therefore, the optimal daily dosage of L-AMB is controvertible.

Cord blood transplantation (CBT) is an acceptable alternative to unrelated allogeneic HCT for adult patients without human leukocyte antigen (HLA)-matched related or unrelated donors [15–18]. However, the incidence of

infection is higher in CBT than HCT from other graft sources [19,20]. This is probably due to prolonged neutropenia and delayed immune reconstitution, which remain the disadvantages of CBT. Indeed, an Italian study demonstrated that CBT was an independent risk factor for the development of IFI during the early and mid-phases of HCT [2]. Moreover, potentially nephrotoxic drugs, such as aminoglycosides, vancomycin, foscarnet, and calcineurin inhibitors, are commonly used in CBT recipients and could induce nephrotoxicity and electrolyte abnormalities along with the use of L-AMB. Based on these backgrounds, L-AMB was intravenously administered at a dosage of less than 3 mg/kg/day in adult patients undergoing CBT in our institute. Therefore, we retrospectively analyzed the efficacy and safety of low-dose L-AMB in patients undergoing CBT.

Patients and methods

Study patients and data collection

This retrospective study included 48 adult patients who received low-dose L-AMB during admission for single-unit unrelated CBT for hematological malignancies at the Institute of Medical Science, The University of Tokyo between January 2006 and December 2017. The indication of L-AMB for empirical or preemptive therapy, the treatment of IFI, and the daily dose of L-AMB were determined by the physicians. For patients who received a second or more cycles of L-AMB treatment, only the first cycle of L-AMB treatment was

included in this study. The clinical data, which included the characteristics of patients and transplantation, L-AMB treatment, laboratory parameters of renal and hepatic function, electrolyte abnormalities during treatment, and concomitant use of potential hepatotoxic and nephrotoxic drugs, were collected from the medical records. The institutional review board of the Institute of Medical Science, The University of Tokyo has approved this retrospective study.

Endpoints and definitions

The primary endpoints of this retrospective study were efficacy, safety, and toxicity of low-dose L-AMB treatment during CBT.

Efficacy was assessed according to the five endpoints used in previous reports [5–7]: (1) absence of any breakthrough fungal infection during therapy or within seven days after the end of therapy; (2) successful treatment of any baseline fungal infection; (3) survival for seven days after the end of therapy; (4) no premature discontinuation of L-AMB treatment because of drug-related toxicity or lack of efficacy; (5) resolution of fever, which was defined as a body temperature below 38°C for at least 48 h, during neutropenia. Diagnosis of IFI was based on the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) [21].

Safety and toxicity were assessed according to the adverse events, which were evaluated according to the National Cancer Institute-Common

Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0. Nephrotoxicity was defined as an increase in serum creatinine (Cre) of grade 2 or higher (i.e. an increase of 50% from baseline [22,23]). Pre-existing renal dysfunction was defined as an estimated glomerular filtration rate less than 60 ml/min at the baseline within 48 h of the first L-AMB treatment.

Statistical analysis

Continuous variables were compared with the Kruskal–Wallis and Steel–Dwass tests were used for multiple comparisons in the case of three groups. To identify variables affecting each adverse event, a univariate analysis was performed using a logistic regression model. The following variables were taken into consideration: age (≥50 vs. <50 years), sex (male vs. female), pre-existing renal dysfunction (yes vs. no), duration of L-AMB treatment (≥21 vs. <21 days), median daily dose of L-AMB based on actual body weight (≥1.2 vs. <1.2 mg/kg/day), cumulative dose of L-AMB based on actual body weight (≥30 vs. <30 mg/kg), cyclophosphamide in conditioning regimen (yes vs. no), busulfan in conditioning regimen (yes vs. no), methotrexate in graft-versus-host disease (GVHD) prophylaxis (yes vs. no), concomitant use of each drug, such as aminoglycosides, vancomycin, foscarnet, calcineurin inhibitors, and glucocorticoids (yes vs. no), and the number of potentially nephrotoxic drugs in concomitant use (≥2 vs. <2). For the univariate analysis to identify variables affecting nephrotoxicity, 2 patients were excluded who developed an increase in

Cre probably due to multiple organ failure within 10 days before death. All statistical analyses were performed in EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) [24]. All *P*-values were two-sided, and *P*-values<0.05 were considered as significant.

Results

Characteristics of patients and transplantations

The characteristics of patients and transplant procedures are summarized in Table 1. The median age was 48 years (range, 17–68 years). 36 (75%) of the patients were male, and the median body weight was 57.0 kg (range, 37.0–105.5 kg). The most common disease type was acute myeloid leukemia (40%). The myeloablative conditioning (MAC) regimen (88%) and methotrexate-based GVHD prophylaxis (65%) were more commonly performed in this study group. Among the MAC regimens, the most common conditioning regimen was total body irradiation 12 Gy, cyclophosphamide, and cytosine arabinoside with or without granulocyte colony-stimulating factor in patients with myeloid or lymphoid malignancies, respectively [25–27].

Treatment of L-AMB

The characteristics of L-AMB treatment are summarized in Table 2. All patients

had received systemic antifungal drugs as prophylaxis with either voriconazole (n=34), fluconazole (n=2), or micafungin (n=12) before switching to L-AMB. Among the entire cohort, 42 (88%) patients received L-AMB for empirical or preemptive therapy, and 6 (12%) patients received L-AMB for treatment of probable or proven IFI. The median daily dose of L-AMB and the mean daily dose of L-AMB based on the actual body weight were 1.20 mg/kg/day (range, 0.62–2.60 mg/kg/day) and 1.23 mg/kg/day (range, 0.62–2.47 mg/kg/day), respectively. The median duration of L-AMB administration was 21.5 days (range, 1–313 days). The median cumulative dose of L-AMB based on the actual body weight was 30.6 mg/kg (range, 0.7–241.5 mg/kg). Twenty-three patients started to receive L-AMB during neutropenia, which was defined as an absolute neutrophil count of less than 0.5×10^9/L. Among them, 15 patients received L-AMB as an empirical therapy for febrile neutropenia. During L-AMB treatment, aminoglycosides, vancomycin, foscarnet, calcineurin inhibitors, and glucocorticoids were concomitantly used in 9 (21%), 25 (58%), 14 (33%), 30 (70%), and 19 (44%) patients, respectively.

Efficacy of L-AMB treatment

The efficacies of L-AMB treatment after CBT are summarized in Table 3. Documented breakthrough fungal infection occurred in 1 patient during L-AMB treatment (fungemia due to Fusarium), indicating that the absence of breakthrough fungal infection was observed in 47 (98%) of the 48 patients. Among 6 evaluable patients who had a baseline fungal infection (proven

candidemia, n=2 and probable pulmonary aspergillosis, n=4), 4 (67%) patients achieved successful treatment of IFI. Finally, 43 (90%) patients survived for at least 7 days after the end of L-AMB treatment. Among the remaining 5 patients who died, the causes of death were as follows: Fusarium fungemia (n=1), candidemia (n=1), Pneumocystis pneumonia (n=1), multiple organ failure (n=1), and relapse (n=1). Among the 43 patients survived for at least 7 days after the end of L-AMB treatment, 17 (40%) patients prematurely discontinued L-AMB treatment because of drug-related toxicity (n=14) or lack of efficacy (n=3). Among 15 patients receiving L-AMB as an empirical therapy for febrile neutropenia, 9 (60%) patients had resolution of fever during neutropenia.

Safety and toxicity during L-AMB treatment

The most common toxicities observed during L-AMB treatment were infusion-related reactions, hepatotoxicity, nephrotoxicity, and electrolyte abnormalities (Table 4).

Infusion-related reactions including skin eruption, nausea, dyspnea, wheezing, fever, and muscle pain occurred immediately after the start of the first infusion of L-AMB in 4 (8%) patients, which results in the discontinuation of L-AMB.

As for hepatotoxicity, elevations of serum levels of aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), and total-bilirubin (T-bil) during L-AMB treatment were frequently observed, and grade 3 or higher elevations of AST, ALT, ALP, and T-bil were

observed in 4 (8%), 7 (15%), 2 (7%), and 13 (27%) patients, respectively (Table 3). Although there were significant differences of each level between AST, ALT, ALP, and T-bil at baseline and those at the time of maximum elevation during L-AMB treatment, serum levels of AST and T-bil at baseline were not significantly different from each level at the end of L-AMB treatment (Figure 1). In the univariate analysis using a logistic regression model, a duration of L-AMB treatment of more than 21 days was significantly associated with grade 2 or higher elevations of AST (hazard ratio [HR], 4.44; *P*=0.04) and ALP (HR, 4.44; *P*=0.04).

Nephrotoxicity, which was defined as an increase in Cre of grade 2 or higher, was observed in 12 (26%) patients. The median duration to development of nephrotoxicity was 24.5 days (range, 2–127 days). Grade 3 hypokalemia was observed in 11 (23%) patients. The median duration to development of hypokalemia was 19 days (range, 3–83 days). Although grade 1 hypomagnesemia was observed in 23 (48%) patients, grade 2 or higher hypomagnesemia was never observed during L-AMB treatment. Although there were significant differences at each level between blood urea nitrogen (BUN), Cre, potassium, and magnesium at baseline and at the time of maximum elevation or minimum reduction during L-AMB treatment, serum levels of potassium and magnesium at baseline were not significantly different from each level at the end of L-AMB treatment (Figure 1). In the univariate analysis using a logistic regression model, a duration of L-AMB treatment of more than 21 days (HR, 20.20; *P*=0.006) and a cumulative dose of L-AMB of more than 30 mg/kg (HR, 20.20; *P*=0.006) were significantly associated with nephrotoxicity. In

addition, a duration of L-AMB treatment of more than 21 days (HR, 5.91; *P*=0.03) and a cumulative dose of L-AMB of more than 30 mg/kg (HR, 5.91; *P*=0.03) were significantly associated with grade 3 hypokalemia.

Survival after cessation of L-AMB treatment

At 30 days after cessation of L-AMB treatment, 9 (19%) patients had died. The primary causes of death were GVHD with or without organ failure in 5 patients, fungemia (due to *Candida*, or *Fusarium*) in 2 patients, *Pneumocystis* pneumonia in one patient, and underlying hematologic disease in one patient.

Discussion

The purpose of this single-center retrospective study was to evaluate the efficacy and safety of low-dose L-AMB in adult patients undergoing CBT. Although the majority of patients received L-AMB for empirical or preemptive therapy during CBT, and the median daily dose of L-AMB was 1.20 mg/kg/day, only one patient developed breakthrough fungal infection. In addition, grade 3 or higher hypokalemia and hepatotoxicity, which were defined according to the criteria of the NCI-CTCAE, were frequently observed. However, no patients developed an increase in Cre of grade 3 or higher. These data indicated that low-dose L-AMB may be safe and effective in adult patients undergoing CBT.

The optimal daily dose of L-AMB seems to be dependent on the types of fungus infection and the strategy for L-AMB therapy. Daily doses ranging

between 3 mg/kg and 5 mg/kg of L-AMB have been recommended for the treatment of invasive aspergillosis and candidiasis [8,28,29], whereas higher doses of at least 5mg/kg of L-AMB could be more effective for the treatment of Cryptococcus meningitis [30] and Mucormycosis [31,32]. However, Ellis et al. reported a prospective randomized trial that compared the efficacy of two dosages of L-AMB for treatment of invasive aspergillosis and found that a daily dose of 1 mg/kg was as effective as that of 4 mg/kg [10]. Moreover, although the optimal dose of L-AMB for empirical or preemptive therapy during neutropenia remains unclear, several prospective and retrospective studies have suggested that a daily dose as low as 1 mg/kg of L-AMB is effective for empirical therapy and for prevention of IFI during neutropenia [9–14]. Among these studies, Prentice et al. reported a prospective randomized trial that compared the efficacy of two dosages of L-AMB and conventional D-AMB for empirical therapy during neutropenia and found that a daily dose of 1 mg/kg of L-AMB could provide an efficacy similar to that of 3 mg/kg of L-AMB [9]. Indeed, our study showed that a median daily dose of 1.20 mg/kg of L-AMB provided favorable results for the majority of empirical or preemptive antifungal therapies because of the absence of breakthrough fungal infection and survival for at least 7 days after the end of L-AMB treatment in adult patients undergoing CBT. These were consistent with previous, larger studies with a daily dose of 3 mg/kg of L-AMB for empirical therapy during neutropenia [5–7]. Moreover, the successful treatment of base-line fungal infection in our study was also comparable with those in previous studies [5,6], although only 6 patients were diagnosed with proven (n=2) or probable (n=4) baseline IFI. Therefore, these data suggested that

low-dose L-AMB could be effective for empirical antifungal therapy, but further studies are required to clarify the optimal dose of L-AMB for treatment of IFI in adult patients undergoing CBT.

In our study, a higher incidence of hepatotoxicity was observed compared with those in previous studies [5–7,22,33], which might be related to differences in the definition of hepatotoxicity in each study. Nevertheless, the cause of hepatotoxicity is likely to be multifactorial in allogeneic HCT. Therefore, it was difficult to determine whether hepatotoxicity was due to the toxicity of L-AMB or other complications following CBT, such as hepatic sinusoidal obstruction syndrome, GVHD, infections, or concomitant use of other potentially hepatotoxic drugs [34]. Moreover, our study demonstrated that hepatotoxicity was not associated with a cumulative dose of L-AMB, which is consistent with a previous study [22]. By contrast, although several potentially nephrotoxic drugs, such as aminoglycosides, vancomycin, foscarnet, and calcineurin inhibitors, are commonly used in patients undergoing CBT, our study showed that nephrotoxicity was infrequently observed compared with previous studies [5,6,22,35]. This lower nephrotoxicity in our study was the biggest advantage of low-dose L-AMB treatment, although it might have been partly due to the fact that several patients stopped L-AMB before the onset of nephrotoxicity. Interestingly, our study demonstrated that nephrotoxicity was associated with a cumulative dose of L-AMB, which is consistent with a previous study [22]. Based on these data, hepatotoxicity appears to be dose-independent on the toxicity of L-AMB, whereas nephrotoxicity appears to be dose-dependent on the toxicity of L-AMB. These data suggested that the low-dose L-AMB contributed to the lower

rate of nephrotoxicity but not hepatotoxicity even after long-term use of L-AMB.

Our study had several limitations. First, this study was a retrospective single-institute analysis. Therefore, the optimal daily dose of L-AMB for empirical therapy and treatment of IFI after CBT requires further confirmation using prospective, multicenter studies in the future. Second, all adverse events possibly related to L-AMB are likely to be multifactorial after CBT, and a small number of patients received low-dose L-AMB after CBT. Therefore, our results might have been affected by a lower statistical power to detect the true effects. Third, we were not able to use several novel triazole antifungal drugs, such as posaconazole and isavuconazole, for prophylaxis and treatment of IFIs in this study, because these drugs were not commercially available in Japan during this study period. Although these novel drugs have an extended-spectrum activity against yeasts and molds including not only *Aspergillus* species but also Mucormycosis, the effect of these drugs on liver and renal function is usually mild in the HCT setting [36,37]. Therefore, these novel triazole antifungal drugs might be safe and effective in patients receiving CBT. Further studies are required to optimize the antifungal strategy for patients receiving CBT.

In conclusion, our data demonstrated that low-dose L-AMB had no inferior efficacy and a lower rate of nephrotoxicity for adult patients undergoing CBT. Therefore, the relationship between the optimal daily dose of L-AMB and efficacy or safety in CBT recipients should be investigated in future studies.

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Conflict of Interest

The authors declare no competing financial interests.

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Figure 1. Kinetics of hepatic and renal function parameters and electrolytes at baseline, the maximum or minimum values during L-AMB treatment, and the values at the end of therapy.

The line inside the box, the lower and upper box ends, and the lower and upper

whiskers represent the median value, the 25th and 75th percentiles, and the 10th and 90th percentiles of each value, respectively. The statistical differences between each of the two groups among baseline, maximum or minimum values during L-AMB, and at the end of therapy were analyzed by the Kruskal–Wallis and Steel–Dwass tests.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS; not significant

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; T-bil, total-bilirubin; BUN, blood urea nitrogen; Cre, creatinine; K, potassium; Mg, magnesium.

Table 1. Characteristics of the patients, cord blood units, and transplantations.

Characteristic	Value
Number of patients	48
Age at CBT, median (range), years	48 (17–68)
Body weight, median (range), kg	57.0 (37.0–105.5)
Sex	
Male	36 (75%)
Female	12 (25%)
Disease type	
AML	19 (40%)
ALL	8 (17%)
MDS	12 (25%)
CML	4 (8%)
NHL/ATL	4 (8%)
CAEBV	1 (2%)
Conditioning regimen	
MAC	42 (88%)
RIC	6 (13%)
Cyclophosphamide in conditioning regimen	33 (69%)
Busulfan in conditioning regimen	11 (23%)
GVHD prophylaxis	
CSP+MTX	31 (65%)
CSP+MMF	11 (23%)
CSP+PSL	1 (2%)
CSP	4 (8%)
TAC	1 (2%)

CBT, cord blood transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia-lymphoma; CAEBV, chronic active Epstein–Barr virus infection; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; CSP, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; PSL, predonisolone; TAC, tacrolimus.

Table 2. Characteristics of liposomal amphotericin B treatment.

Characteristic	Value
Indication of L-AMB	
Empirical or preemptive therapy	42 (88%)
Treatment of probable or proven IFI	6 (12%)
Daily dose of L-AMB, mg/kg/day, median (range)	1.20 (0.62–2.60)
Duration of L-AMB treatment, days, median (range)	21.5 (1-313)
Cumulative dose of L-AMB, mg/kg, median (range)	30.6 (0.7–241.5)
Concomitant use of other drugs during L-AMB treatment	
Aminoglycoside	10 (21%)
Vancomycin	27 (56%)
Foscarnet	16 (33%)
Calcineurin inhibitors	33 (69%)
Glucocorticoids	21 (44%)
Number of nephrotoxic drugs in concomitant use	
0	4 (8%)
1	10 (21%)
2	29 (60%)
3	2 (4%)
4	3 (6%)

Table 3. Efficacy of low-dose L-AMB after CBT.

Response indicator	Number of patients
No breakthrough fungal infection	47/48 (98%)
Successful treatment of base-line fungal infection	4/6 (67%)
Survival for at least 7 days after the end of treatment	43/48 (90%)
No discontinuation due to toxicity or lack of efficacy	26/43 (60%)
Resolution of fever during neutropenia	9/15 (60%)

Table 4. Toxicity of low-dose L-AMB after CBT.

	Grade 1	Grade 2	Grade 3	Grade 4	Total
AST	20 (42%)	5 (10%)	4 (8%)	0	29 (60%)
ALT	20 (42%)	5 (10%)	7 (15%)	0	32 (67%)
ALP	23 (48%)	11 (23%)	2 (4%)	0	36 (75%)
T-bilirubin	10 (21%)	4 (8%)	7 (15%)	4 (8%)	25 (52%)
Serum creatinine	27 (56%)	12 (25%)	0	0	39 (81%)
Hypokalemia	4 (8%)	19 (40%)	11 (23%)	0	34 (71%)
Hypomagnesemia	23 (48%)	0	0	0	23 (48%)

Toxicities were evaluated according to NCI-CTCAE version 4.0.

Figure1