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ORIGINAL ARTICLE

Addition of aerosolized deoxycholate amphotericin B to systemic prophylaxis to prevent airways invasive fungal infections in allogeneic hematopoietic SCT: a single-center retrospective study

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Invasive fungal infections (IFIs) still pose major challenges in allogeneic hematopoietic SCT (HSCT), and effective antifungal prophylaxis remains a matter of debate. The aim of this retrospective study was to evaluate the toxicity and the impact of aerosolized deoxycholate amphotericin B (aero-d-AmB) on respiratory tract IFIs (airways IFIs) in a homogeneous cohort of allogeneic HSCT patients, transplanted at one institution. Since 1999, 102 consecutive patients were transplanted from matched related ($N=71$) or unrelated donor (MUD). Aero-d-AmB was administered for a median time of 16 days (range 2–45), in addition to systemic antifungal prophylaxis. Prolonged administration was neither associated with increased severe bacterial infections, nor with severe adverse events. In 16 patients in whom aero-d-AmB was delivered for less than 8 days, due to worsened clinical conditions or poor compliance, proven or probable airways IFIs were diagnosed in three cases (one mucormycosis and one fusariosis and one probable aspergillosis), whereas in 84 patients receiving aero-d-AmB for $\geqslant 8$ days, one possible and one probable aspergillosis were diagnosed. A shortened administration (<8 days) of aero-d-AmB was therefore associated with an increased risk of both total airways IFIs ($P=0.027$) and proven/probable IFIs ($P=0.012$). At multivariate analysis prolonged aero-d-AmB administration retained an independent protective effect on airways IFIs ($P=0.026$) whereas a MUD transplant was associated with a borderline increase of IFIs risk ($P=0.052$). Overall, 95.1% of patients did not experience airways IFIs and no patient died due to IFIs. In this cohort of patients, prolonged aero-d-AmB seems to have a role in preventing respiratory tract IFIs, but a randomized controlled trial is recommended to verify the impact of this prophylaxis in the setting of allogeneic HSCT.

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Introduction

Invasive fungal infections (IFIs) are a major complication after allogeneic hematopoietic SCT (HSCT) and their risk factors are well recognized.[1] Prevalence of IFIs is up to 20% and affects TRM in this setting.[2] New antifungal drugs are now available but the costs and the toxicity of these systemic treatments remain high. The systemic use of deoxycholate amphotericin B (d-AmB) in the allogeneic transplanted patient is limited due to its nephrotoxicity; itraconazole could prevent invasive aspergillosis but liver toxicity and gastrointestinal toxicity hamper its use. Recently, posaconazole, a broad-spectrum azole, was approved for IFIs prevention during prolonged neutropenia and GVHD.[3,4] Aerosolized d-AmB failed to reduce the incidence of IFIs in a large randomized study among patients with prolonged neutropenia, but no allogeneic transplanted patients were enrolled.[5]

In this retrospective study, we investigated the safety and tolerability of a local prophylaxis with aerosolized d-AmB, administered together with systemic antifungal prophylaxis in hematological patients undergoing allogeneic HSCT. As efficacy end point, the impact of prolonged aero-d-AmB on airways IFIs incidence at 120 days from transplant was investigated.

Patients and methods

Patient characteristics

After obtaining informed consent, aero-d-AmB was proposed as part of antifungal prophylaxis to 102 consecutive patients who underwent allogeneic HSCT in HEPA-filtered laminar-flow single room at our institution since 1999. Table 1 summarizes the hematological diagnoses and the distribution of major risk factors for IFIs

Table 1 Hematological diagnosis and distribution of major risk factors for IFIs

	N	*Advanced disease*	*Previous IFI*	*MUD*
Acute Leukemia/MDS	62	26	2	19
Chronic Leukemia	10	5	0	3
Lymphomas	14	14	0	3
Multiple myeloma	15	12	0	2
Aplastic anemia	1	1	0	1
Total	102	59	2	31

Abbreviations: IFI = invasive fungal infection; MDS = myelodysplastic syndrome; MUD = matched unrelated donor.

development among patients treated. In total 31 patients were transplanted with an unrelated donor, and only 43 patients were in CR at transplant. Median age at transplantation was 47 years (range 17–69). All patients underwent screening tests to exclude IFI at the time of the transplant (chest and sinuses radiographs or CT, nasal and pharyngeal swab). IFIs were defined as proven, probable or possible according to European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria.[6] IFI belonging to the airway tract (nose, paranasal sinuses, pharynx, larynx, trachea and lungs) was defined as 'airways IFI'. Diagnoses of IFIs were registered until day +120. Only two patients with AML had experienced pretransplant IFIs (one systemic candidiasis and one probable aspergillosis). Adverse events potentially related to treatment were monitored and reported during and after each administration of aero-d-AmB and classified according to World Health Organization's Common Toxicity Criteria until day +120.

Antifungal systemic prophylaxis and treatment

Antifungal systemic prophylaxis consisted of fluconazole in 74 (72.5%), itraconazole in 16 (15.7%), caspofungin in 6 (5.9%), liposomial-amphotericin B in 3 (2.9%), amphotericin-B deoxycholate in 1 (1%) or posaconazole in 2 (2%) of 102 patients. The choice of the systemic prophylaxis was based on clinical criteria, depending on tolerability, toxicity, previous diagnosis of IFI and the presence of concurrent risk factors (antithymocyte globulin, advanced disease or prolonged neutropenia). Empirical antifungal treatment was instituted if fever lasted more than 96 h and in presence of one of the following conditions: signs or symptoms suggestive of IFI, unrelated donor transplant, steroid treatment, advanced disease, prolonged neutropenia, age more than 40 years.

Administration of aero-d-AmB

Aerosolized d-AmB was administered from the beginning of conditioning twice daily at the dosage of 15 mg for 15 min in an aerosol mask at an oxygen flow regimen of 7 L/min (total daily dose 30 mg); 50 mg of d-AmB was diluted with 10 ml of bidistilled water: 3 ml (15 mg of d-AmB) of this solution was added to 2 ml of saline solution and aerosolized through the aerosol set (Airlife Misty-Neb Nebulizer with Aerosol Mask and 213 cm Oxygen Tubing; Cardinal Health Inc., McGaw Park, IL, USA); after the use, the masks were disinfected within Sekusept 2% for 6 h and reused for the same patient. The aerosol was administered every day until discharge, intolerance or refusal by the patient, start of systemic amphotericin B treatment, worsening of clinical conditions not allowing d-AmB inhalations. Because nasal swabs taken 8 days after the start of aerosolized d-AmB were negative in eight of the nine evaluable patients showing *Aspergillus* spp. colonization at nasal swab taken before the start of treatment, we defined aero-d-AmB therapy as adequate if more than 14 consecutive d-AmB administrations (7 days) could be delivered. Patients were subdivided in two subgroups according to the adequacy of aero-d-AmB received (Table 2):

- Group A: adequate aero-d-AmB, duration of inhalations >7 days
- Group B: inadequate aero-d-AmB, duration of inhalations ⩽7 days

Patients were considered evaluable for toxicity if at least one inhalation was delivered, and patients were considered evaluable for efficacy if they survived at least 15 days after transplant.

Table 2 Prevalence of airways IFIs according to the adequacy of aerosolized amphotericin B prophylaxis and to the type of systemic prophylaxis

	None	*Possible*	*Probable*	*Proven*	*Total*
Airways IFIs					
Group A	83	1	1	0	85
Group B	13	0	1	2	16
Airways IFIs and systemic prophylaxis					
Fluconazole	70	1	1	1	73
Anti-aspergillus drugs	26	0	1	1	28

Abbreviation: IFI = invasive fungal infection.
Group A, adequate aero-d-AmB: duration of inhalations >7 days.
Group B, inadequate aero-d-AmB: duration of inhalations ⩽7 days.

Statistical analysis

Data were analyzed in November 2009. Fisher's exact test and log-rank test were used to identify variables associated with the development of airways IFIs (proven/probable and proven) at 120 days after transplant at the univariate analysis. The following variables were included in the analysis: age, sex, disease status at transplant, type of donor, need for GVHD treatment, severe mucositis, antifungal systemic prophylaxis, adequacy of aero-d-AmB, life-threatening bacterial and viral infections. All the variables were included in the Cox regression model for the multivariate analysis on estimate cumulative incidence of airways IFIs at 120 days after transplant.

Statistical analysis was performed using the SPSS 13.0 package for Windows (Chicago, IL, USA).

Results

Dose, duration and toxicity of d-AmB, and causes of death

Aero-d-AmB was administered for a median time of 16 days (range 2–45). Median d-AmB exposure was 480 mg

per patient. In 16 patients, aero-d-AmB was delivered for less than 8 days, due to worsened clinical conditions (3 patients), refusal (5 patients), poor compliance (7 patients) or withdrawal because of adverse event (1 patient). Bad taste or mild cough at the start of therapy was the most frequently reported adverse events (89 and 85%, respectively, of the entire cohort); nausea was commonly associated with concomitant chemotherapy administration. Adequate administration was neither associated with increased life-threatening bacterial infections, nor severe adverse events; only one patient developed moderate bronchial spasm and inhalations were stopped after 5 days. None developed renal failure related to aero-d-AmB.

Overall, 22 patients died during the observation period (until day +120). Causes of death included relapse (12), acute GVHD (aGVHD) (2), viral infection (3) and bacterial infection (5). No fatal IFI was observed. There was no statistical difference in the mortality rate at 120 days between the two groups (18.75 vs 22.1%).

Efficacy

All patients, except one who died before day +15, were assessable for efficacy analysis, to evaluate the impact of aero-d-AmB on airways IFI incidence. As previously mentioned, eight of nine patients with a pretransplant nasal swab positive for *Aspergillus* spp. received aero-d-AmB and their subsequent surveillance swabs proved negative. The only patient with positive swab who did not receive adequate aero-d-AmB due to bronchial spasm developed a possible aspergillosis. Eighty-five patients received adequate aerosolized therapy (more than 14 inhalations, group A), whereas 16 patients (group B) received aero-d-AmB for less than 8 days. The two groups were comparable for the majority of risk factors (mucositis >2 World Health Organization's Common Toxicity Criteria, type of donor, diagnosis and status at transplant), but treatment for aGVHD was administered more frequently in patients from group B (56 vs 23%, $P=0.008$; Table 3). Overall, 95.1% of patients did not experience airways IFIs and none died due to IFIs. Proven and probable airways IFIs (one probable aspergillosis, one mucormycosis and one fusariosis) were diagnosed in 3 of 16 patients (18.8%) in group B whereas in group A two IFIs (one possible and one probable aspergillosis, 2.3%) were diagnosed in 85 evaluable patients (Table 2). A shortened administration (<8 days) of aero-d-AmB was therefore associated with a significantly increased risk of proven and probable airways IFIs (Fisher's exact test: $P=0.027$ for airways IFIs; $P=0.012$ for proven/probable airways IFIs). An unrelated donor transplant, disease status at transplantation, GVHD and mucositis were not associated with a significant risk of fungal infections at univariate analysis, although a matched unrelated donor (MUD) transplant was borderline associated with an increased risk of probable/proven airways IFIs ($P=0.077$). The type of systemic antifungal prophylaxis, which was used in 100% of patients, did not impact on the development of airways IFIs: among 73 patients receiving fluconazole, three airways IFIs developed compared to two among 28 treated with anti-aspergillus drugs (itraconazole, amphotericin B, posaconazole and caspofungin) (Table 2).

Table 3 Prevalence of airways IFIs according to the adequacy of aerosolized amphotericin B and to several risk factors for IFI development

	Airways IFIs				*Total*
	None	*Possible*	*Probable*	*Proven*	
Disease status at transplant					
CR					
Total	41	1	1	0	43
Group B	6	0	0	0	6
Group A	35	1	1	0	37
Advanced disease					
Total	39	0	0	2	41
Group B	5	0	0	2	7
Group A	34	0	0	0	34
Partial remission					
Total	17	0	1	0	18
Group B	2	0	1	0	3
Group A	15	0	0	0	15
Type of donor					
Matched related					
Total	69	1	1	0	71
Group B	11	0	1	0	12
Group A	58	1	0	0	59
Matched unrelated					
Total	28	0	1	2	31
Group B	2	0	0	2	4
Group A	26	0	1	0	27
GVHD					
Grade 0–1					
Total	66	1	1	1	69
Group B	6	0	0	1	7
Group A	60	1	1	0	62
Grade>1					
Total	26	0	1	1	28
Group B	7	0	1	1	9
Group A	19	0	0	0	19
Mucositis					
WHO grade 0–2					
Total	39	1	0	2	42
Group B	6	0	0	2	8
Group A	33	1	0	0	34
WHO grade >2					
Total	56	0	2	0	58
Group B	7	0	1	0	8
Group A	49	0	1	0	50

Abbreviations: IFI = invasive fungal infection; WHO = World Health Organization.
Group A, adequate aero-d-AmB: >7 days of inhalations.
Group B, inadequate aero-d-AmB: ⩽7 days of inhalations.

Mantel–Cox log-rank test. The estimated incidence of proven and probable airways IFIs at 120 days after transplant was 1.3% in the group B and 19% in the group A (Figure 1, $P=0.001$) whereas the estimated incidence of proven and probable airways IFIs at 120 days after transplant in the MUD group was 10.9% in comparison to 1.6% in the MRD group ($P=0.039$).

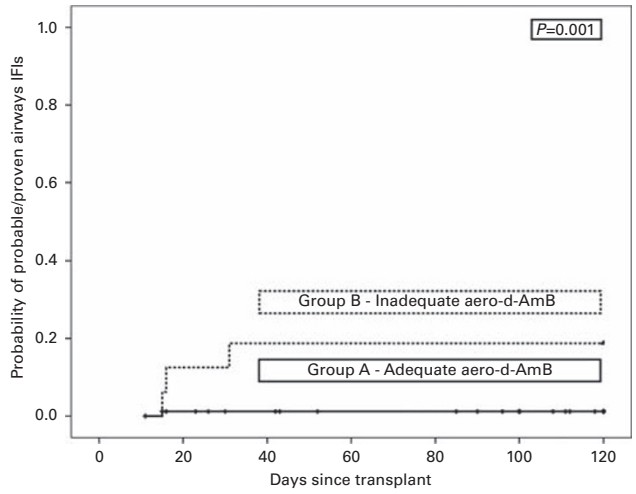

Figure 1 Estimate incidence of airways invasive fungal infections (IFIs) according to adequacy of aerosolized deoxycholate amphotericin B (aero-d-AmB). Group A: duration of inhalations >7 days (solid line). Group B: duration of inhalations $\leqslant 7$ days (dotted line).

Multivariate analysis. The Cox regression model identifies adequate aero-d-AmB administration as independent variable affecting incidence of airways IFIs at 120 days after transplant ($P=0.026$). A MUD transplant was of borderline statistical significance ($P=0.051$).

Discussion

Invasive fungal infections are a major challenge in the supportive care of highly immune-suppressed patients. Recent studies suggest that aerosolized lipidic formulations of amphotericin B could be effective in preventing airways IFIs in lung[7] and HSCT[8,9] and during prolonged neutropenia.[10] In lung transplant recipient, 50 mg of lipid complex amphotericin B was compared with 25 mg once daily of deoxycholate-amphotericin B inhalations:[7] in each arm full-dose treatment was delivered for 1 week followed by one inhalation weekly for 7 weeks. Both arms appeared to be associated with a low prevalence of fungal pneumonia in the early post transplant phase, but lipid complex formulation was associated with less adverse events. In 40 patients undergoing allogeneic HSCT, aerosolized lipid complex (50 mg once daily for 4 days, thereafter once weekly for 13 weeks) was well tolerated and only one proven IFI was reported during the study period.[8] In a large retrospective study[9] 10 mg of d-AmB was delivered twice daily during neutropenia in HSCT patients: in this cohort of patients a low cumulative incidence of IFIs was observed (3.6% at 120 days after transplant). In a more recent randomized controlled trial, 25 mg once daily of liposomal amphotericin B administered for 2 days weekly until neutrophil recovery was randomly compared with placebo in 271 patients during 407 episodes of prolonged neutropenia.[10] Invasive pulmonary aspergillosis was diagnosed more frequently in the placebo arm ($P=0.005$ at the intent-to-treat analysis, OR 0.26).

No clinical data are reported since 1999 among patients undergoing allogeneic HSCT and receiving aerosolized prophylaxis with d-AmB. In our patients, prolonged aero-d-AmB was not associated with severe adverse events. The only patient who developed moderate bronchial spasm after inhalation was subsequently treated with d-AmB for a possible aspergillosis without further problems.

The 15% dropout rate due to poor tolerance registered in our study is a significant drawback for a prophylactic strategy. However the problem of the low compliance to prophylactic aero-d-AmB regimen could be overcome by the adoption of a lipid formulation of AmB.

Indeed, the randomized controlled trial comparing aerosolized d-AmB with lipid complex formulation[7] showed more adverse events in the deoxycholate arm. However, no difference in the efficacy in preventing IFIs was shown. The number of patients reported to fail prophylaxis due to intolerance to d-AmB in that study was comparable to our setting (6 of 49 vs 16 of 102). Proven airways IFIs incidence was also similar (2 of 100 vs 2 of 102).

The results of the present comparative study confirm the potential efficacy of aero-d-AmB, when administered for a sufficiently long period, for the prevention of airways IFI in allogeneic transplant patients. In our patients, the efficacy of aero-d-AmB in sterilizing upper airways is further strengthened by the eight patients with a pretransplant positive nasal swab for aspergillus who subsequently proved negative after at least 1 week of inhalations. None of these eight patients developed airways IFIs. The results of the study should be interpreted with caution for two reasons—patients treated with steroids for aGVHD are more at risk to develop IFIs:[1] among 16 patients undertreated with amphotericin B inhalations, 9 were treated for aGVHD, whereas among 81 receiving adequate d-AmB inhalations 19 were treated for aGVHD. Although aGVHD was not significantly associated with an increased risk of airways IFIs at univariate and multivariate analyses, this high proportion of patients receiving high-dose methylprednisolone could represent a bias in the analysis. The second possible bias could be the impact of an unrelated donor on the prevalence of IFIs. In our series, only two patients developed proven airways IFIs. These patients received a matched unrelated transplant together with a shortened administration of aero-d-AmB: at multivariate Cox regression analysis an unrelated transplant represented a borderline risk factor for developing airways IFIs.

Nevertheless, even taking into account the relatively low number of proven fungal infections observed, the heterogeneity of conventional antifungal prophylaxis and the potential bias of this retrospective analysis, the results of this descriptive study suggest the potential efficacy in preventing airways IFIs in the setting of allogeneic transplanted patients treated with Aero-d-AmB.

This route of administration of d-AmB seems to have a role in preventing respiratory tract IFIs together with systemic azole oral prophylaxis but a well-designed randomized controlled trial is warranted to verify the impact of such prophylactic regimen in allogeneic transplant patients.

Conflict of interest

The authors declare no conflict of interest.

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