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Treatment of invasive fungal infections in high risk hematological patients. The outcome with liposomal amphotericin B is not negatively affected by prior administration of mold-active azoles

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ABSTRACT

There are concerns of a reduced effect of liposomal amphotericin B (L-AmB) given sequentially after mold-active azoles due to a possible antagonism in their antifungal mechanism. To investigate this possible effect in the clinic, we retrospectively studied 182 high risk hematologic patients with invasive fungal infections (IFI) who were treated with L-AmB. Overall, 96 patients (52.7%) had possible, 52 (28.6%) probable and 34 (18.7%) proven IFI according to EORTC classification. Most had suspected or proven invasive aspergillosis. We compared patients with prior exposure to mold-active azoles (n=100) to those having not (n=82). The group with prior mold-active azoles included more patients with poor risk features for IFI as acute myeloid leukemia (p<0.05) and prolonged neutropenia (p<0.05). A favorable response in the IFI, defined as a complete or partial response, was achieved in 75% and 74.4% of patients in the whole cohort, and in 66% and 74.4% of patients with probable or proven IFI in the two groups. None of these differences were significant. Multivariate analysis showed that refractory baseline disease and renal dysfunction were adverse factors for response in the IFI (p<0.05). Survival was poorer for patients with prior broad spectrum azoles (p<0.05), and for those who did not recover from neutropenia (p<0.05). In conclusion, the effectiveness of treatment of breakthrough fungal infection with L-AmB is not likely to be affected by prior exposure to mold-active azoles prophylaxis, but survival largely depends on host and disease factors.

KEY WORDS: Acute myeloid leukemia, Allogeneic transplantation, Aspergillosis, Breakthrough fungal infection, mold active azole prophylaxis

Tratamiento de infecciones fúngicas en pacientes hematológicos de alto riesgo. La respuesta de AmBisome no se modifica con el uso previo de azoles de amplio espectro

RESUMEN

Existe cierta inquietud sobre una reducción del efecto de la anfotericina B liposomal (L-AmB) administrada secuencialmente después de la administración de azoles activos frente a mohos debido a un posible antagonismo en su mecanismo antifúngico. Para investigar este posible efecto en la práctica clínica, hemos estudiado retrospectivamente 182 pacientes hematológicos con infecciones fúngicas invasivas (IFI) de alto riesgo que fueron tratados con L-AmB. En total, 96 pacientes (52,7%) tenían IFI posible, 52 (28,6%) probable y 34 (18,7%) probada de acuerdo con la clasificación de la EORTC. La mayoría presentaban aspergilosis invasiva. Comparamos los pacientes con exposición previa a azoles activos frente a mohos (n=100) con aquellos no expuestos (n=82). El grupo con exposición previa a azoles activos frente a mohos incluía más pacientes con características de alto riesgo de IFI, como leucemia mieloide aguda (p<0,05) y neutropenia prolongada (p<0,05). Se alcanzó una respuesta favorable en la IFI, definida como una respuesta completa o parcial, en 75,0% y 74,4% de los pacientes de la cohorte completa y en 66,0% y 74,4% de los pacientes con IFI probable o probada en los dos grupos. Ninguna de estas diferencias fue significativa. El análisis multivariante mostró que la enfermedad basal y la disfunción renal eran factores adversos para la respuesta en la IFI (p<0,05). La supervivencia fue peor en los pacientes tratados con azoles de amplio espectro (p<0,05) y en aquellos en los que no se resolvió la neutropenia (p<0,05). En conclusión, la eficacia del tratamiento con L-AmB de una infección fúngica de brecha probablemente no se vea afectada por la exposición previa a un tratamiento profiláctico con azoles activos frente a mohos, dependiendo la supervivencia más bien de los factores del huésped y de la enfermedad de base.

Palabras clave: Leucemia mieloide aguda, trasplante alogénico, aspergilosis, infecciones fúngicas de brecha, profilaxis con azoles activos frente a hongos filamentosos

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INTRODUCTION

Patients with hematologic malignancies undergoing intensive chemotherapy or allogeneic hematopoietic stem cell transplantation are at high risk of invasive fungal infections (IFI) due to poor immune function, neutropenia, break of mucosal barriers and other predisposing factors. In these patients IFI is a major cause of mortality, morbidity and unacceptable delays in scheduled sequential therapy needed for the control and cure of the underlying diseases¹.

Mold fungal infections, mainly represented by Invasive Aspergillosis, are the most frequent so far. However, yeasts resistant to azoles and emergent fungi are increasing in frequency in part due to mold active azole prophylaxis^{2,3}. Liposomal amphotericin B (AmBisome, L- AmB) is a polyene antifungal agent indicated for empiric and targeted therapy of IFI because of its wide spectrum and efficacy. For IFI caused by *Aspergillus*, the mold-active azole voriconazole is usually recommended first and L-AmB is used as an alternative^{4,5}. Even with such active antifungal agents available, the outcome is generally poor if therapy of the IFI is delayed. Early instauration of treatment for a suspected IFI is considered a standard of practice in hematological patients with neutropenia in order to obtain a rapid control of the IFI and improve the outcome⁶. Although CT scans and serum *Aspergillus* antigen detection (Platelia[®]) are available, low sensitivity of galactomanan in some circumstances and low specificity of radiological tools has made preemptive therapy a difficult daily routine approach in febrile neutropenic patients⁷.

Broad spectrum azole prophylaxis is an effective approach to reduce the incidence of moulds in high risk hematologic patients. Azoles such as, itraconazole and posaconazole are effective both in the acute myelogenous leukemia and allogeneic HSCT settings. Voriconazole has also been used with some success in prophylaxis⁸⁻¹⁰. However, antifungal prophylaxis has neither eliminated the risk of IFI, nor the need for antifungal treatment in the uncontrolled febrile patient with neutropenia. Now there are several options for the treatment of IFI, but concerns have been raised for the use of them in some sequence for mold infections. In particular, the use of polyenes in patients who have been receiving mold-active azoles, as a consequence of a potential inhibition of the antifungal effect¹¹ which could be due to the inhibition by azoles of the synthesis of ergosterol, a component of the fungal membrane which is utilized by polyenes to exert their antifungal effect. In a retrospective study Steinbach et al.¹² did not find this effect in a large series of patients with combined or sequential antifungal therapy. In addition, Cornely et al. addressed this issue in a *post hoc* analysis of the Ambload trial¹³, in which two different dose levels of Liposomal AmB (L-AmB) were compared for the initial treatment of IFI and concluded that patients who received prior azoles had the same response as patients without azoles exposure¹⁴. In the present retrospective real-life data study we aimed to confirm the efficacy of the use of L-AmB for the treatment of breakthrough IFI in high risk hematological patients, who were previously receiving mold-active azoles.

PATIENTS AND METHODS

This study was conducted and approved by the Ethics Committee at 25 Spanish institutions and performed in accordance with the Helsinki Declaration principles. Eligible subjects were patients with hematological malignancies under treatment with chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT) who had a diagnosis of invasive fungal infection (IFI) treated with liposomal AmB (L-AmB). Definitions of IFI were based on the EORTC/MSG 2002 criteria¹⁵ instead of the updated 2008 criteria¹⁶ since enrollment of patients was started in 2007. Briefly, possible IFI required at least two host factors criteria plus one microbiological factor, or one major clinical (or two minor) criteria. Probable IFI required at least two host factors plus both one microbiological and one major (or two minor) clinical criteria. Proven IFI required a histopathological demonstration of invasive mycosis or a positive culture from an appropriate biological sample. For an accurate diagnosis of IFI clinical and radiological (CT scan) data, serum *Aspergillus* galactomannan, Platelia[®], (GM) results and microbiological results from bronchoalveolar lavage (BAL) or biopsy specimens were collected by their attending physicians.

Patients were distributed in two groups according to prior exposure with itraconazole, voriconazole or posaconazole (group A); or with fluconazole or no azole (group B) before the start of treatment with L-AmB.

Table 1 Patient characteristics

	Group A	Group B
	Previous azoles	No mold-active azoles
N = 182	100	82
Age Median (Range) years	47 (1-75)	50 (2-77)
Baseline Disease Type		
Acute myeloid leukemia (%)	52 (52.0)*	29 (35.4)*
Acute lymphoblastic leukemia (%)	22 (22.0)	17 (20.7)
Myelodysplastic syndrome (%)	6 (6.0)	3 (3.7)
Lymphoid malignancy (%)	20 (20.0)*	33 (40.2)*
Baseline Disease Treatment Phase		
Remission Induction therapy (%)	23 (23.5)	19 (23.8)
Post-remission therapy (%)	41 (41.8)	39 (48.8)
Relapse or refractory disease therapy (%)	34 (34.6)	22 (22.7)
Neutropenia severe, < 0.5 10e9/L (%)	84 (84.0)*	60 (73.2)*
Neutropenia severe lasting > 10 days (%)	71 (71.0)	41 (68.3)
Any previous IFI before this episode (%)	18 (18.0)	9 (11.0)
Allogeneic HSCT (%)	44 (44.0)	29 (35.4)
Treatment with high dose steroids (%) ¹	18 (18.0)*	28 (34.1)*

* p<0.05

¹ prednisone 1 mg/kg or equivalent, for more than 14 days

Table 2 | Microbiological criteria in patients with probable and proven IFI

Group A Prior mold-active azoles N=100		Group B No mold-active azoles N=82	
Possible IFI N=53	Probable or proven IFI N=47	Possible IFI N=43	Probable or proven IFI N=39
	27 5 15		13 6 20
		Serum GM BAL GM Fungal isolation	
N=15	Fungal species	N=20	
3	<i>Aspergillus sp</i>	8	
4	<i>Aspergillus fumigatus</i>	8	
	<i>Aspergillus flavus</i>	1	
2	<i>Aspergillus niger</i>		
1	<i>Aspergillus terreus</i>		
	<i>Aspergillus versicolor</i>	1	
1	<i>Mucor sp</i>		
1	<i>Scedosporium apiospermum</i>		
	<i>Fusarium</i>	1	
1	<i>Candida albicans</i>		
1	<i>Candida parapsilosis</i>	1	
1	<i>Rhodotorula</i>		

The primary endpoint was the achievement of a favorable response, consisting in a complete or partial response recorded at the end of treatment. Complete response required the resolution of all signs of IFI; partial response required at least clinical and radiological improvement. Secondary endpoints included safety issues and survival at 12 weeks.

Patients' characteristics were compared by Chi-square, Fischer exact test, Student's t-test or Mann-Whitney U-test as appropriate. Logistic regression analysis with the Cox method was used to identify variables for response and survival. A p value of less than 0.05 was considered significant. Statistical analyses were performed using SPSS, version 18.

RESULTS

The characteristics of 182 evaluable patients are shown in table 1. Group A included 100 patients (54.9%) with prior

mold-active azoles, consisting in 39 patients with itraconazole, 35 with voriconazole and 26 with posaconazole. Group B consisted in 82 patients (45.1%), 40 with prior fluconazole and 42 with no prior azoles. The two groups were different in key clinical characteristics. Group A included more patients with acute myelogenous leukemia (AML) and with severe neutropenia lasting longer than 10 days. Conversely, more patients with lymphoid malignancies and treated with high dose corticosteroids were in group B. These differences are relevant for the incidence and outcome of an IFI, and are explained by the current practice and the licensed use of mold-active prophylaxis in neutropenic AML patients.

The median duration of prior treatment with azoles was 25 days for voriconazole, 19 days for itraconazole, 16 days for posaconazole and 15 days for fluconazole.

By EORTC/MSG 2002 criteria ninety six patients (52.7%) had a possible, 52 (28.6%) a probable and 34 (18.7%) a proven

Table 3 Responses to treatment with L-AmB

	Group A	Group B	p value*
	100	82	
	N (%)	N (%)	
Favorable response (overall)			
Favorable response	75 (75.0)	61 (74.4)	ns
In Possible IFI	44 (83.0)	32 (74.4)	ns
In Proven or Probable IFI	31 (66.0)	29 (74.4)	ns
Response at end of treatment			
Favorable response	73 (73.0)	55 (67.1)	ns
Complete response	50 (50)	44 (53.7)	ns
Partial response	23 (23)	11 (13.4)	ns
Response at 4 weeks			
Favorable response	57 (57.0)	50 (61.0)	ns
Complete response	46 (46)	41 (50.0)	ns
Partial response	11 (11)	9 (11.0)	ns
Response at 12 weeks			
Favorable response	41 (41.0)	41 (50.0)	ns
Complete response	37 (37)	39 (47.6)	ns
Partial response	4 (4)	2 (2.4)	ns

IFI. For the statistical analysis, patients with probable or proven IFI were pooled in one group with 86 patients (47.3%). This group included 47 (47%) of the 100 patients from group A and 39 (47.6%) of the 82 patients from group B. Seventy-nine out of these 86 patients (88.4%) were diagnosed of invasive aspergillosis (IA). In group A they were 27 patients with a positive serum GM test as a microbiological criterion, 5 with a positive BAL GM test and 15 patients with fungal demonstration by stain or culture. In group B there were 13 patients with a positive serum GM result, 6 patients in a positive BAL GM and 20 patients with fungal demonstration. The types of identified mycoses are detailed in table 2.

Treatment with L-AmB was given at a median dose of 3 mg/kg for a median of 18 days in group A, and 15 days in group B. Eight patients (4.4%) had to discontinue L-AmB due to side effects.

A favorable response (complete or partial response at the end of treatment, 4 weeks or 12 weeks) was achieved in 75% (95% CI: 66.5%–83.5%) in group A and 74.4% (95% CI: 64.9%–83.8%) in group B (p=0.925). In the subset of patients with probable or proven IFI, the response was 66% in group A and 74.4% in group B (p=0.398) as is shown in table 3. The Cox model run for the most common clinical variables showed that renal dysfunction (OR 0.207; 95% CI: 0.044-0.973; p<0.05) and refractory hematological disease (OR 0.088; 95% CI: 0.017-0.438; p<0.05) reduced the probability of achieving a favorable response. The survival at 12 weeks was lower for mold-active azole-exposed patients (group A) than for non-exposed (group B): 42% vs. 59.8%; p= 0.017 (table 4). There were major differences in subsets of patients in group A. The survival was only 29.8% for probable or proven IFI, compared to 52.8% for possible IFI. The survival in the same subsets in group B was not different (59% and 60.4%, respectively). When we performed the analysis restricted to the patients with probable or proven IFI, the log-rank test showed different survival probabilities: median 3.7 months (95% CI: 2.0-5.4) for group A, and 13.9 months (95% CI: 5.9-21.9) for group B (p=0.008). The Cox analysis identified prior exposure to mold-active azoles as a risk factor for mortality (OR 3.617; 95% CI 1.638-7.986; p<0.05). Achievement of a favorable response in the IFI (OR 0.010; 95% CI 0.001-0.079; p<0.05) and recovery of neutropenia (OR 0.380; 95% CI 0.149-0.967; p<0.05) were independent factors for survival.

Table 4 Survival after treatment with L-AmB

	Group A		Group B	
	100		82	
	N (%)		N (%)	
Survival				
At end of therapy	83 (83.0)		70 (85.4)	
At 4 weeks	62 (62.2)*		64 (78.0)*	
At 12 weeks	42 (42.0)**		49 (59.8)**	
	Possible IFI	Probable Proven IFI	Possible IFI	Probable Proven IFI
	53 (%)	47 (%)	43 (%)	39 (%)
At end of therapy	43 (81.1)	40 (85.1)	34 (79.1)	36 (92.3)
At 4 weeks	34 (64.2)	28 (59.6)	31 (72.1)	30 (76.9)
At 12 weeks	28 (52.8)	14 (29.8)*	26 (60.4)	23 (59.0)*

* p=0.02
** p= 0.017

DISCUSSION

In this retrospective study we collected data from 182 high risk hematologic patients with different myeloid and lymphoid malignancies who were treated with L-AmB for a possible, probable or proven IFI. We divided them in two groups according to prior mold-active azoles exposure or not. These two groups were not balanced, since there were more patients with AML and presenting long-lasting neutropenia receiving mold-active azoles.

These differences are according to the current practice of utilize mold-active prophylaxis in AML patients with chemotherapy induced neutropenia or allogeneic HSCT and graft versus host disease. In the group without prior mold-active azoles and L-AmB treated IFI there were many patients with lymphoid malignancies. This is interesting since lymphoid malignancies are now recognized as an emerging group at risk of IFI¹⁷, which is probably due to the use of more aggressive therapies for this population. The majority of cases of IFI in our study were invasive aspergillosis, where the treatment that we used primarily with L-AmB is considered a good alternative to the preferred drug voriconazole^{2,3,5,13}. We found that the effectiveness of L-AmB was virtually identical in patients who received prior mold-active azoles than in those who did not, with an overall favorable response rate near to 75%. The responses in probable or proven IFD (66% vs. 74.4%), and the rate of complete responses at 12 weeks, 37% and 47.6% in the two subsets were not significantly different, indicating that prior mold-active azoles does not impair or limit the therapeutic effect of L-AmB. Our results are coincident with the study of Cornely et al.¹⁴ that reported similar responses, 49.1% with and 45.9% without prior mold-active azoles. Our multivariate analysis showed that renal insufficiency and refractory hematological disease were factors associated with poor response in the IFI ($p < 0.05$) highlighting the importance of patients' and underlying disease condition in the resolution of the IFI. Also, we found that prior mold-active azoles exposure was an independent risk factor for mortality, but this has to be taken with caution since the groups were not balanced by risk factors and we did not find a reduced rate of success in the IFI in this group. The survival in the group of prior azoles was somehow different in patients with possible IFI (52.8%) with respect to probable or proven IFI (29.8%) which is conceivable, since previous studies have suggested that the efficacy of L-AmB may be greater when the fungal infection is in an earlier phase^{18,19}.

The mortality at 12 weeks in this series was high, and this poor outcome probably reflects the high risk features of the study population. Similar mortality result were observed in another real-life data study in high-risk patients receiving antifungal prophylaxis with posaconazole where 59% patients with breakthrough IFI died and in patients with proven disease ($n=11$), 73% died, where as only 1 death was attributed to fungal disease (6%)². In our study the only predictive factors for survival we found were the recovery of neutropenia and the achievement of a response in the IFI, reflecting the importance of the host factors as well as an early initiation of antifungal therapy. In conclusion, this study shows that the effectiveness of L-AmB is not reduced by prior mold-active azoles and confirms L-AmB as an attractive option for patients with breakthrough fungal infections after antifungal prophylaxis with broad-spectrum azoles.

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TRANSPARENCY DECLARATION

None to declare

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