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Treatment outcome of invasive mould disease after sequential exposure to azoles and liposomal amphotericin B

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Objectives: To analyse the potential antagonism between azoles, which inhibit ergosterol synthesis, and polyenes, which bind directly to ergosterol in cell membranes, in patients receiving sequential azole–polyene treatment.

Methods: In an earlier randomized, double blind study of liposomal amphotericin as initial therapy for invasive filamentous fungal infection (IFFI), a 3 mg/kg/day dose had a favourable overall response rate of 50% and 12 week survival rate of 72%. No improved outcome was seen with 10 mg/kg/day for the first 14 days. The study population was further analysed for the effect of prior azole exposure on treatment responses to liposomal amphotericin B. The protocol allowed prior treatment with azoles for prophylaxis or empirical therapy, and for up to 4 days for the confirmed IFFI before starting liposomal amphotericin B. Outcomes were compared for subsets of patients based on receipt of any azole and receipt of voriconazole during the 30 day screening period prior to study treatment.

Results: Of 201 patients with data review board-confirmed IFFI, 116 (57.7%) received prior azoles and 36 (17.9%) received prior voriconazole. Favourable responses were achieved in 57 (49.1%) patients with prior azole exposure, in 39 (45.9%) without prior azole and in 13 (36.1%) with prior voriconazole. Numbers of patients alive at 12 weeks were 74 (63.8%) with any prior azole, 56 (65.9%) without prior azole and 26 (72.2%) after prior voriconazole. No differences were statistically significant.

Conclusions: Prior treatment with any azole or specifically with voriconazole did not seem to impact on overall response or survival in patients treated with liposomal amphotericin B for confirmed IFFI.

Keywords: aspergillosis, polyenes, antagonism

Introduction

Broad-spectrum azole compounds have recently been recommended for prophylaxis of fungal infections in leukaemia patients and allogeneic stem cell transplant recipients.[1–3] Once invasive fungal infection has been diagnosed polyenes are a therapeutic option with efficacy proven in large randomized trials.[4,5] Polyenes and azoles both target ergosterol for their antifungal activities.[6,7] Azoles inhibit ergosterol synthesis by inhibition of 14α-demethylase, which leads to depletion of ergosterol in the fungal cell membrane.[6] Polyenes bind directly to ergosterol in the fungal cell membrane, altering membrane permeability, which results in loss of intracellular contents and cell death.[7] Evidence for antagonism between polyenes and azoles has been found with simultaneous exposure *in vitro* and *in vivo*.[8] In a guinea pig model of invasive aspergillosis, concomitant as well as sequential exposure to voriconazole and liposomal amphotericin B was not associated with antagonism.[9] In light of these conflicting results sequential and concomitant exposure is a matter of concern, although there is no conclusive evidence that such an interaction has clinical relevance.[10]

Results from a randomized double blind trial of two dosing regimens of liposomal amphotericin B as initial therapy for invasive filamentous fungal disease (AmBiLoad trial) have recently been presented.[4] The standard dose of 3 mg/kg/day had a favourable overall response rate of 50% and a 12 week survival

rate of 72%. No improvements in outcomes were seen with a dose of 10 mg/kg/day for the first 14 days.

Further analysis of the study population was performed to assess the effect of prior azole exposure on treatment responses to liposomal amphotericin B. This trial for the first time offers the opportunity to study potential antagonism of sequential azole–liposomal amphotericin B therapy for invasive mould infections in a clinical setting.

Methods

In the AmBiLoad trial, patients with proven or probable invasive filamentous fungal disease by modified EORTC/MSG 2002 criteria were treated with liposomal amphotericin B and were randomly allocated to receive a daily dose of either 3 or 10 mg/kg for 14 days, followed by 3 mg/kg per day until investigator-defined end of study drug treatment.[4,11] The modification allowed for inclusion of patients with recent neutropenia or allogeneic stem cell transplantation on the basis of typical imaging results, i.e. halo or air crescent signs in chest CT. The protocol had been approved by the appropriate ethics committees and institutional review boards. Patients were entered into the study only after giving written informed consent. Per protocol prior treatment with azoles was allowed for prophylaxis or empirical therapy, and for up to 4 days for the confirmed invasive fungal disease before starting study drug treatment with liposomal amphotericin B. The information captured in the case report form of the AmBiLoad trial comprised the name of the prior antifungal and whether it was given within the last month before baseline. The indication for antifungals used prior to randomized treatment was not captured.

Proven and probable invasive filamentous fungal disease diagnoses and overall treatment responses at the end of study drug treatment were verified by an independent data review board. The members of the data review board were unaware of treatment allocation. Favourable response was defined as either complete or partial response. Stable disease, failure and unevaluable cases were grouped as unfavourable response.[4] Favourable response at the end of study drug treatment and 12 week survival were compared for subsets of patients based on prior exposure to any azole and receipt of an *Aspergillus* active azole, i.e. itraconazole or voriconazole, during the 30 day screening period prior to entering the trial.

Two-sided 95% confidence intervals of the difference in proportions are used as based on normal approximation, implying a level of significance of 0.05.

Results

Two hundred and one patients comprised the modified intent-to-treat population, defined as all randomized patients

Table 1. Baseline characteristics according to dose group and prior azole exposure

	3 mg/kg		10 mg/kg		Overall	
Baseline characteristic	no prior azole	any prior azole	no prior azole	any prior azole	no prior azole	any prior azole
N	50	57	35	59	85	116
Age (years)						
min–max	15–75	15–76	1–78	16–78	2–78	15–78
median	53	54	53	54	53	54
Female (%)	17 (34.0)	29 (50.9%)	12 (34.3%)	19 (32.2%)	29 (34.1%)	48 (41.4%)
Race						
white	42 (84.0%)	45 (78.9%)	31 (88.6%)	50 (84.7%)	73 (85.9%)	95 (81.9%)
black	0	1 (1.8%)	1 (2.9%)	0	1 (1.2%)	1 (0.9%)
Asian	0	0	0	1 (1.7%)	0	1 (0.9%)
other	0	2 (3.5%)	0	0	0	2 (1.7%)
not reported	8 (16.0%)	9 (15.8%)	3 (8.6%)	8 (13.6%)	11 (12.9%)	17 (14.7%)
Haematological malignancy	46 (92.0%)	53 (93.0%)	32 (91.4%)	55 (93.2%)	78 (91.8%)	108 (93.1%)
Leukaemia	38 (76.0%)	36 (63.2%)	27 (77.1%)	48 (81.4%)	65 (76.5%)	84 (72.4%)
Stem cell transplantation	6 (12.0%)	12 (21.1%)	6 (17.1%)	16 (27.1%)	12 (14.1%)	28 (24.1%)
Solid organ transplantation	1 (2.0%)	0	0	0	1 (1.2%)	0
HIV	0	2 (3.5%)	2 (5.7%)	0	2 (2.4%)	2 (1.7%)
Other	13 (26.0%)	19 (33.3%)	8 (22.9%)	20 (33.9%)	21 (24.7%)	39 (33.6%)
Neutropenia (days $<500/\mu L$)						
min–max	0–120	0–75	0–250	0–46	0–250	0–75
median	16	16	15	16	15	16
Neutropenic at baseline	37 (74.0%)	39 (68.4%)	25 (71.4%)	46 (78.0%)	62 (72.9%)	85 (73.3%)
Fever	21 (42.0%)	29 (50.9%)	15 (42.9%)	24 (40.7%)	36 (42.4%)	53 (45.7%)
Serum galactomannan >1.0	7 (14.0%)	8 (14.0%)	3 (8.6%)	7 (11.9%)	10 (11.8%)	15 (12.9%)

No differences are statistically significant.

receiving at least one dose of study drug for a diagnosis of proven or probable invasive mould disease verified by the data review board. One hundred and seven patients were randomized to the 3 mg/kg dose group and 94 patients were randomized to the 10 mg/kg group. Eighty-five patients did not receive any azole antifungal therapy in the 30 days prior to initiating liposomal amphotericin B study drug. Of the 116 patients who were treated with any azole prior to liposomal amphotericin B, 35 (30.2%) received itraconazole and 36 (31%) received voriconazole. The remaining 45 (38.8%) patients received fluconazole. No patients were treated with posaconazole, since the AmBiLoad study was conducted prior to its licensing. See Table 1 for baseline characteristics according to dose group and prior azole exposure.

Response and survival for haematopoietic stem cell or bone marrow transplant recipients are given in Table 2.

Favourable response and 12 week survival data for patients receiving no prior azole therapy compared with those patients receiving any prior azole, itraconazole and voriconazole are shown in Table 3. At 12 weeks only three (1.5%) patients were lost to follow-up; these were counted as non-survivors. No significant differences were seen between the liposomal amphotericin B 3 mg and 10 mg dose groups or for the combined groups. There were no significant differences in favourable response and survival associated with the sequential exposure to voriconazole and liposomal amphotericin B, or itraconazole or fluconazole followed by liposomal amphotericin B treatment.

Discussion

There has been much discussion as to the potential for azole–polyene antagonism, given their mechanisms of action directed at the production and the direct targeting of ergosterol, respectively.[12] However, the data in both the non-clinical and clinical areas fail to substantiate this theoretical interaction.[10]

We analysed treatment success and survival of 201 patients treated in the AmBiLoad study according to their prior azole exposure. Prior to randomized study treatment azoles had been prescribed for prophylaxis, empirical therapy or for treatment of the current confirmed invasive fungal disease. The overall distribution of the azoles chosen proved fluconazole to

Table 2. Favourable response and 12 week survival in recipients of haematopoietic stem cell or bone marrow transplant according to prior azole exposure

	3 mg/kg		10 mg/kg		Overall	
Baseline characteristic	no prior azole	any prior azole	no prior azole	any prior azole	no prior azole	any prior azole
N	6	12	6	16	12	28
Favourable overall response						
yes	2	7	3	7	5 (41.7%)	14 (50.0%)
no	4	4	3	8	7 (58.3%)	12 (42.9%)
not evaluable	—	1	—	1	—	2 (7.1%)
Alive at week 12						
yes	3	7	3	4	6 (50.0%)	11 (39.3%)
no	3	5	3	12	6 (50.0%)	17 (60.7%)

No differences are statistically significant.

Table 3. Favourable response and 12 week survival according to prior azole exposure

	No prior azole	Any prior azole[a]		Prior itraconazole[b]		Prior voriconazole[b]	
	n/*N* (%)	*n*/*N* (%)	difference (95% CI)	*n*/*N* (%)	difference (95% CI)	*n*/*N* (%)	difference (95% CI)
Favourable response							
3 mg	23/50 (46)	30/57 (52.6)	−6.6% (−26%; 12.3%)	10/18 (55.6)	−9.6% (−36%; 17.2%)	9/17 (52.9)	−6.9% (−34%; 20.5%)
10 mg	16/35 (45.7)	27/59 (45.8)	−0.0% (−21%; 20.8%)	9/17 (52.9)	−7.2% (−36%; 21.7%)	4/19 (21.1)	24.7% (−0.0%; 49.3%)
total	39/85 (45.9)	57/116 (49.1)	−3.3% (−17%; 10.7%)	19/35 (54.3)	−8.4% (−28%; 11.2%)	13/36 (36.1)	9.8% (−9.2%; 28.7%)
12 week survival							
3 mg	36/50 (72)	40/57 (70.2)	1.8% (−15%; 19%)	11/18 (61.1)	10.9% (−15%; 36.6%)	14/17 (82.4)	−10.4% (−32%; 11.6%)
10 mg	20/35 (57.1)	34/59 (57.6)	−0.5% (−21%; 20.2%)	9/17 (52.9)	4.2% (−25%; 33%)	12/19 (63.2)	−6.0% (−33%; 21.2%)
total	56/85 (65.9)	74/116 (63.8)	2.1% (−11%; 15.4%)	20/35 (57.1)	8.7% (−11%; 28%)	26/36 (72.2)	−6.3% (−24%; 11.4%)

[a]Includes fluconazole, itraconazole and voriconazole. Patients may have received multiple azoles prior to initiating liposomal amphotericin B.
[b]Comparisons versus the 'no prior azole' group. All comparisons were not significant.

be the most commonly used drug, a finding similar to other large randomized clinical trials.[13] In 2005 these results were confirmed upon evaluating prescription habits of haematologists.[2] In the AmBiLoad study 31% of subjects received voriconazole largely prior to the diagnosis of invasive fungal disease. Such off-label use of voriconazole has been described previously.[14] During the study period 2003–2004 posaconazole was not yet licensed.

The key finding of our analysis is that neither favourable response to study treatment nor survival rates were significantly influenced by prior azole exposure. These findings are supported by a trial on liposomal amphotericin B in empirical treatment, where success rates were also not significantly different.[13] Similarly, liposomal amphotericin B salvage therapy following failure of first-line voriconazole resulted in reasonable overall success rates.[15]

In our study, prior exposure to azoles in general, and itraconazole or voriconazole specifically, had no effect on outcomes of treatment with liposomal amphotericin B as initial treatment for invasive filamentous fungal disease. Although this patient population may be one of the largest evaluated for sequential azole–polyene exposure, the sample size is small. This is reflected by the wide confidence intervals. Despite the limitations of this *post hoc* analysis, the data do not support a clinically relevant antagonism between azoles and liposomal amphotericin B when given sequentially for invasive filamentous fungal disease.

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Transparency declarations

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