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LETTER TO THE EDITOR

Aspergillus nidulans is frequently resistant to amphotericin B

Aspergillus nidulans ist häufig resistent gegenüber Amphotericin B

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Schlüsselwörter. *Aspergillus nidulans*, Aspergillose, Amphotericin B, Resistenz.

Summary. The high failure rate of amphotericin B-based therapy in patients with *Aspergillus nidulans* infections may not be entirely a result of host factors as suggested previously. Innate resistance of *A. nidulans* to polyenes may contribute to the poor response in patients.

Zusammenfassung. Die hohe Versagerquote der Amphotericin B-Therapie bei Patienten mit *Aspergillus nidulans*-Infektionen kann nicht nur auf Wirtsfaktoren zurückgeführt werden, wie früher angenommen. Angeborene Resistenz von *A. nidulans* gegenüber Polyenen dürfte ebenfalls für das schlechte Ansprechen der Patienten verantwortlich sein.

Introduction

Aspergillus nidulans, a genetically amenable model fungus closely related to other pathogenic species of the *Aspergillus* genus, is a rare human pathogen with a unique predilection for patients having chronic granulomatous disease [1]. This mould is frequently refractory to amphotericin B therapy, with a failure rate of 50% (vs. only 15% for *Aspergillus fumigatus*) reported in this patient population [1]. The reasons for this poor response are unclear.

Materials and methods

We tested the susceptibility of seven different clinical isolates of *A. nidulans* (obtained from the Fungus Reference Laboratory at The University of Texas Health Science Center at San Antonio) to amphotericin B (AmB) and itraconazole (ITR). Specifically, we used both the National Committee for Clinical Laboratory Standards (NCCLS) microdilution (document M38-P) [2] and the E test method. All experiments were performed in triplicate. The reference strains *Candida glabrata* ATCC 582, *Candida parapsilosis* ATCC 22019 were used as QC strains. Strains of AmB-resistant *A. terreus*, ITR-resistant *A. fumigatus* were also used as controls.

For the microdilution assay, we followed the procedure described by NCCLS [2]. Briefly, logarithmic phase cultures were prepared by subculturing the *A. nidulans* isolates on yeast extract agar (YAG) medium (0.5%, yeast extract, 1% glucose, 1.5% agar; Sigma Chemical Co., St Louis, MO, USA) and incubating at 37 °C for 5–7 days. Conidia were collected with a sterile swab and suspended in sterile saline containing 0.05% Tween-20. After heavy particles were allowed to settle for 15 min, the turbidity of the supernatants was measured by spectrophotometer (Spectronic 20, Bausch & Lomb, Overland Park, KS, USA) at 530 nm and transmission was adjusted to 80–82% corresponding with an inoculum of

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1×10^6–5×10^6 conidia ml^{-1} [3]. The inoculum was then diluted 1 : 50 in RPMI-1640 (with L-glutamine; without bicarbonate) buffered to pH 7.0 with 0.165 mol/l^{-1} 3-*N*-morpholinepropanesulphonic acid (MOPS, Sigma Chemical Co.) growth medium to achieve an inoculum of 1.0×10^4–5×10^4 conidia ml^{-1}. ITR solution was prepared at 100× the final test concentrations in dimethylsulphoxide using powder from the manufacturer (Janssen Pharmaceutical, Titusville, NJ, USA). This solution was then diluted 1 : 50 in RPMI-1640 growth medium. AmB solution (Pharma-Tek, Inc., Huntington, NY, USA) was prepared by reconstituting the deoxycholate salt preparation in sterile water, then diluting the solution in RPMI-1640 medium [2]. Stock solutions of AmB, and ITR were then prepared at twice the final test concentration (0.03–16 μg ml^{-1}) in RPMI-1640 medium. Wells of a 96-well, flat-bottom microtitre plate were filled with 100 μl of each drug concentration. A well containing RPMI-1640 medium served as growth control. Each well was then inoculated with 100 μl of a 1 : 50 dilution of the conidia suspension, to obtain a final test inoculum of 1×10^3–5×10^3 conidia ml^{-1}. Plates were then incubated for 48 h at 37 °C. Minimum inhibitory concentrations (MICs) were read at 24 and 48 h visually with the aid of a reading mirror. The MIC was defined as the lowest concentration of antifungal that resulted in absence of fungal growth compared to control.

In addition, the E test MICs were determined using ITR (range, 0.002–32.000 μg ml^{-1}) and AmB (range, 0.002–32.000 μg ml^{-1}) strips provided by the manufacturer (AB Biodisk, Solna, Sweden). Solidified RPMI-1640-morpholinepropanesulphonic acid–2% glucose–1.5% Bacto agar plates served as the test medium. A standardized cell suspension (80% transmittance at 530 nm) was prepared by harvesting conidia from mature cultures on potato glucose agar slants and suspending them in 0.85% sterile saline prior to each experiment. All MICs were recorded 24 and 48 h after the application of the E test strip.

Results

Using the NCCLS method, four of the seven isolates exhibited a high AmB MIC (> 2), while the remaining three isolates had intermediate sensitivity to the drug (MIC = 1). With the E test method all but isolate 7 had MIC ≥ 1 for AmB (Table 1). All of the isolates were susceptible to ITR on both tests.

Table 1. Susceptibility of *Aspergillus nidulans* to amphotericin B (AmB) and itraconazole (ITR) as determined using the NCCLS microdilution and E test methods; values are median MIC (μg ml^{-1})

Isolate	NCCLS			
	Microdilution method		*E*-test	
	AmB	ITR	AmB	ITR
1	2	0.5	1.000	0.380
2	2	0.5	32.000	0.064
3	2	0.5	24.000	0.380
4	1	0.5	1.000	0.032
5	1	0.5	1.000	0.190
6	1	1.0	1.000	0.750
7	2	0.5	0.047	0.500

Discussion

These data suggest that the high failure rate of AmB-based therapy in patients with *A. nidulans* infections may not be entirely due to host factors as suggested previously [1]. Innate resistance of this *Aspergillus* species to polyenes, which is analogous to the resistance observed in *Aspergillus terreus* [3], may contribute to the poor response seen in patients with chronic granulomatous disease. This resistance to polyenes has rarely been reported and only in laboratory strains of *A. nidulans* [4]. Therefore, *A. nidulans* may be a promising model fungus for dissecting the molecular genetics of AmB resistance in non-*fumigatus Aspergillus* species. Finally, AmB may not be a suitable drug for the treatment of uncommon infections caused by *A. nidulans*; consideration should therefore be given to treatment using triazoles.

References

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