Letter to the Editor

Aspergillus nidulans is frequently resistant to amphotericin B

Aspergillus nidulans ist häufig resistent gegenüber Amphotericin B

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Key words. Aspergillus nidulans, aspergillosis, amphotericin B, resistance.


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 \textit{A. nidulans} to polyenes may contribute to the poor response in patients.


Introduction

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

Materials and methods

We tested the susceptibility of seven different clinical isolates of \textit{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) \cite{2} and the E test method. All experiments were performed in triplicate. The reference strains \textit{Candida glabrata} ATCC 582, \textit{Candida parapsilosis} ATCC 22019 were used as QC strains. Strains of AmB-resistant \textit{A. terreus}, ITR-resistant \textit{A. fumigatus} were also used as controls.

For the microdilution assay, we followed the procedure described by NCCLS \cite{2}. Briefly, logarithmic phase cultures were prepared by subculturing the \textit{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 × 10⁶–5 × 10⁶ 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-morpholino-
propanesulfonic acid (MOPS, Sigma Chemical Co.) growth medium to achieve an inoculum of 1.0 × 10⁵–2.5 × 10⁴ 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 (Pharm- 
Tek, Inc., Huntington, NY, USA) was prepared by reconstituting the deoxycholate salt preparation in sterile water, then diluting the solution in RPMI-1640 (with pH 7.0 with 0.165 mol 
N-glutamine; without bicarbonate) buffered to 37°C. Minimum inhibitory concentrations (MICs) 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-morpholinepropanesulfonic 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.

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<th>Isolate</th>
<th>NCCLS Microdilution method</th>
<th>E test</th>
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<td>AmB</td>
<td>ITR</td>
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<tr>
<td>6</td>
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<td>7</td>
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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