Treatment failure in invasive aspergillosis: susceptibility of deep tissue isolates following treatment with amphotericin B

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Objectives: To determine whether treatment failure in invasive aspergillosis (IA) is the result of resistance of Aspergillus spp. isolates to amphotericin B.

Methods: Six Aspergillus fumigatus and six Aspergillus flavus isolates cultured from deep tissue biopsies in 11 patients with haematological malignancies during 1991–1998 were tested. A method based on the NCCLS M38-A broth microdilution method, with colorimetric determination of MICs, was used to determine the MICs of amphotericin B and itraconazole.

Results: All A. fumigatus isolates were susceptible to amphotericin B (MIC 0.25–0.5 mg/L), as were three A. flavus isolates (MIC 1 mg/L), but three were less susceptible (MIC 2 mg/L). All isolates were susceptible to itraconazole (MIC 0.125–0.25 mg/L). All patients had been treated with amphotericin B, having received a median of 12 days of treatment when the tissue was obtained.

Conclusion: The difficulty in treating IA may not be because of the susceptibility of the isolates, but because of poor penetration of antifungal agents into infected tissue. Aspergillus spp. invade blood vessels causing thrombosis and tissue infarction, and therefore it may be difficult for antifungal drugs to exceed MICs in infected tissues. This highlights the need for different treatment strategies, such as surgery and the administration of cytokines.

Keywords: NCCLS, antifungals, MICs, Aspergillus

Introduction

We have found that, despite patients having received antifungal therapy with amphotericin B, sometimes for prolonged periods, it is possible to culture Aspergillus spp. from their tissue biopsies.1 The reasons for this microbiological failure are unclear and need to be investigated.

Although there has been progress in antifungal susceptibility testing over the past decade, only a few studies have looked at the correlation between susceptibility of isolates to antifungal agents and clinical outcome. One study has demonstrated a positive correlation between an amphotericin B MIC < 2 mg/L and survival in neutropenic patients.2 An MIC ≥ 2 mg/L was highly associated with a fatal outcome, as was infection with Aspergillus terreus.3 Johnson et al.3 also found a correlation between high MIC values and poor clinical outcome with A. terreus using a neutropenic mouse model. However, they failed to demonstrate any correlation for Aspergillus fumigatus isolates.

The aim of this study was to perform antifungal susceptibility testing on clinical Aspergillus spp. isolates cultured from tissue biopsies, to determine whether or not resistance to amphotericin B plays a role in treatment failure in invasive aspergillosis (IA).

Materials and methods

Clinical specimens and patient details

Twelve clinical isolates (six A. fumigatus and six Aspergillus flavus), cultured from biopsies in 11 patients with haematological malignancy during 1991–1998, were identified and tested (Table 1). These included available organisms from our previous study.1 Each patient had histologically confirmed IA. Case notes of the 11 patients were obtained and analysed.

Controls

Isolates from the PHLS Mycology Reference Laboratory, Bristol, for which the amphotericin B MIC had been determined, were used as controls: A. fumigatus NCPF 7097 MIC 0.25 mg/L, A. fumigatus NCPF 7100 MIC 0.5 mg/L.

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Table 1. MICs of Aspergillus isolates from tissue

<table>
<thead>
<tr>
<th>Patient</th>
<th>Site of biopsy</th>
<th>Species</th>
<th>Amphotericin B MIC (mg/L)</th>
<th>Itraconazole MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>lung</td>
<td>A. flavus</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>2A</td>
<td>lung</td>
<td>A. fumigatus</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>2B</td>
<td>lung</td>
<td>A. fumigatus</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>3</td>
<td>skin</td>
<td>A. flavus</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>cerebral</td>
<td>A. fumigatus</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>lung</td>
<td>A. fumigatus</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
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<td>A. fumigatus</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>vitreous fluid</td>
<td>A. fumigatus</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>lung</td>
<td>A. flavus</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>lung</td>
<td>A. flavus</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>10</td>
<td>sinus</td>
<td>A. flavus</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>11</td>
<td>lung</td>
<td>A. flavus</td>
<td>1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

MIC 1 mg/L, A. terreus NCPF 6026 MIC 4 mg/L and Scedosporium prolificans NCPF 7618 MIC 16 mg/L.

Susceptibility testing

We used a method based on the NCCLS M38-A broth microdilution method, with colorimetric determination of MICs. Briefly, a stock suspension of 0.4–5 \times 10^5 cfu/mL was prepared. One hundred microlitres of this was added to 4.9 mL RPMI 1640 medium with 2% glucose, to prepare the working suspension. This resulted in a suspension of twice the final inoculum concentration of 0.4–5 \times 10^4 cfu/mL. Microdilution plates containing different concentrations of amphotericin B and itraconazole were used (Sensititre, Accumed International Ltd, Trek Diagnostics Systems Ltd, Imberhorne Lane, East Grinstead, West Sussex, UK). One hundred microlitres of RPMI 1640 medium was added to each of the wells containing an antifungal concentration, followed by 100 \mu L of the working suspension. The plates were then incubated at 35°C for 48 h. The MICs were determined visually using a colorimetric (Alamar Blue) evaluation of growth inhibition.

Interpretative breakpoints for filamentous fungi have yet to be established, but we adopted the following provisional breakpoints used at the HPA Mycology Reference Laboratory, Bristol, UK (E. M. Johnson, personal communication): MIC \leq 1 mg/L susceptible, MIC 2 mg/L less susceptible and MIC > 2 mg/L resistant.

Results

All isolates of A. fumigatus were susceptible to amphotericin B (MIC 0.25–0.5 mg/L), as were three isolates of A. flavus (MIC 1 mg/L), but three were less susceptible (MIC 2 mg/L). All isolates of Aspergillus spp. were susceptible to itraconazole (MIC 0.125–0.25 mg/L) (Table 1). Results of control isolates were consistent with reference laboratory results.

Patient 2 had persistent radiological evidence of IA following a lobectomy (2A, Table 1) and subsequently had a pneumonectomy (2B, Table 1). A. fumigatus was cultured from both specimens with no change in the MIC. The skin biopsy in patient 3 was taken from the external auditory canal. This patient had localized IA involving the right ear and parotid gland. Patient 7 had IA involving the left eye, and histologically proven pulmonary IA.

The majority of patients were at high risk of IA, with four having a bone marrow transplant and six having relapsed leukaemia (Table 2). As a result of a previous episode of IA, patient 3 was given secondary prophylaxis with intravenous amphotericin B 1 mg/kg on alternate days.

All patients were treated with conventional (dose 0.5–1 mg/kg) or liposomal (dose 3 mg/kg) amphotericin B, and had received a median of 12 days treatment (mean 16 days, range 4–39 days) at the time of biopsy (Table 2).

At the time of biopsy, eight patients were neutropenic (<500 cells/mm^3), three profoundly (≤100 cells/mm^3) (Table 2). A. fumigatus was cultured from consecutive samples from patient 2, despite an increase in the neutrophil count from 400–22 600 cells/mm^3. Only one patient remained profoundly neutropenic at the time of death, while four others had a count of 500 cells/mm^3.

Three patients received granulocyte–macrophage colony-stimulating factor (GM-CSF) (Table 2). Of these, patient 10 made a full recovery and went on to have an allogeneic bone marrow transplant with no recurrence of IA; patient 11 improved initially, but subsequently suffered a fatal pulmonary haemorrhage; patient 9 died from disseminated aspergillosis. The median number of days of amphotericin B treatment at the time of biopsy for these patients was 7 (range 4–7 days).

Of the patients who died, IA was at least a contributory cause of death in all cases, although two had evidence of super-added infection.

Discussion

Mortality rates from IA remain high^5 and the reasons for the failure of treatment in these patients is unclear. We have previously isolated Aspergillus spp. from 54% of lung tissue specimens resected from patients with histologically proven IA, despite the patients having received a median of 13.7 days treatment with amphotericin B. However, even in the culture-negative cases, the lack of growth of the organism may not necessarily equate with efficacy of the antifungal agent. In a previous study, in which the majority of patients had not received antifungal therapy, only 70% of tissue specimens with histologically proven IA were culture positive.

Furthermore, a good clinical response to therapy in a patient with IA does not necessarily equate with eradication of the organism. A study in patients with acute myeloid leukaemia found that those who had suffered a previous fungal pneumonia had a 52% reactivation
rate during subsequent neutropenic episodes. In another study, patients with a previous fungal pneumonia were given secondary prophylaxis with amphotericin B and flucytosine, but even then radiological evidence of reactivation was detected in three out of 14 neutropenic episodes. Patient 3 in our current study suffered a recurrence of IA and died despite receiving secondary prophylaxis with intravenous amphotericin B and having an amphotericin B-susceptible isolate of A. flavus (MIC 0.2 mg/L). The reasons why organisms are capable of surviving despite antifungal therapy, and in some cases neutrophil recovery, need to be explored. One possible explanation is resistance of the organisms to the antifungal agents used. However, we have demonstrated in this study that this is not the case. The majority (nine out of 12) of isolates were fully susceptible to amphotericin B and isolates.

One study has looked at the concentration of amphotericin B in human tissues using high pressure liquid chromatography (HPLC). Only one patient in that study had proven IA. A. fumigatus (MIC 0.2 mg/L) had been cultured from sputum and bronchial washings, 3 months pre-mortem. Post-mortem histology of pulmonary cavitating lesions revealed hyphae in keeping with Aspergillus spp., and amphotericin B levels of 18.8 µg/g were found in lung tissue. However, the paper does not state whether the portion of tissue tested contained invasive infection or was normal background lung.

We have also used HPLC in an attempt to measure amphotericin B levels in infected lung from a patient treated for 28 days and who died from IA. Fresh post-mortem samples revealed amphotericin B levels of 113.7 µg/g in liver, 0.67 µg/g in background lung tissue and 0.1 µg/g in lung tissue histologically proven to be infected with Aspergillus spp. Hence the levels in the infarcted lung would appear inadequate, even though the concentration in the non-infected lung would have been sufficient to inhibit the growth of half our isolates.

If tissue penetration of antifungal agents is poor then alternative treatment strategies, such as cytokines and surgical resection of lesions, need to be considered. The number of patients who received GM-CSF in this study is too small for any conclusions to be drawn.

Further work obviously needs to be done, but we conclude that the difficulty in treating IA is not due to resistance of the isolates, and speculate that it may be because of poor tissue penetration of antifungal agents into infected tissue.

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Trust statement

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References


