In vitro susceptibility of Aspergillus spp. clinical isolates to albendazole

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The in vitro antifungal activity of albendazole, a benzimidazole widely used as an antihelminthic drug in humans, was investigated and assessed for its activity against Aspergillus spp. Forty-eight isolates, representing the most frequent species found in human pathology [Aspergillus fumigatus (n = 27), Aspergillus flavus (n = 10), Aspergillus terreus (n = 7), Aspergillus nidulans (n = 3) and Aspergillus niger (n = 1)], and one quality control strain (A. niger ATCC 9804 83435) were tested according to the NCCLS M38-P methodology for moulds. All the strains were susceptible to albendazole, with homogeneous MICs for each species; three strains were resistant to itraconazole.

Keywords: drug susceptibility, benzimidazole, antifungal

Introduction

Over the last few years, there has been a dramatic increase in severe infections caused by fungi in the immunocompromised patient; Aspergillus spp. are the most frequently implicated. The complications of therapy for haematological malignancies, solid organ transplantation, and long-term corticotherapy, are often associated with invasive aspergillosis (IA) as the result of Aspergillus fumigatus in most reported cases (85% to 90%), and also of Aspergillus flavus, Aspergillus terreus, Aspergillus nidulans and Aspergillus niger. In spite of recent progress, therapeutical failure, together with difficulty in establishing an early diagnosis, still leads to a high mortality rate, ranging from 30% to 90%. Current chemotherapy relies mainly on amphotericin B lipid complexes and triazole molecules (itraconazole, voriconazole). Recently, caspofungin, a promising new molecule that belongs to the new antifungal class, echinocandins, has proved clinically successful. However, important efforts are being made to discover other new antifungal products.

Benzimidazoles were first used as systemic fungicides in agriculture, then as veterinary and medical antihelminthic agents. Among them, albendazole has been used widely in the treatment of larval cestode infections, such as echinococciosis and cysticercosis. Like other members of this family, it was shown to bind to the β-tubulin subunit of microtubules, thus stopping mitosis. In addition, in the 1980s and early 1990s, A. nidulans was used as a fungal model for the study of the molecular basis of the action of benzimidazoles. It was shown that Aspergillus spp. could be susceptible to some benzimidazoles, for example benomyl and carbendazim. The necessity of developing new antifungal molecules, together with the time-to-market delay for new products, prompted us to investigate the potential of albendazole as an antifungal compound for medical use. Therefore, we tested the in vitro susceptibility of 49 Aspergillus spp. to albendazole, using the NCCLS reference method for conidium-forming filamentous fungi.

Materials and methods

Aspergillus spp. isolates

Nine clinical isolates (2515, 2519, 2372, 2941, 2521, 2522, 2672, 2516 and 2358) were supplied by the ‘Laboratoire de Parasitologie-Mycologie et Maladies Tropicales’ of the
Edouard Herriot hospital (Lyon, France). The itraconazole-resistant strains used in this study were 4421A, AF72 and AF91. The latter two have been described previously; they are deposited with the National Collection of Pathogenic Fungi (PHLS Mycology Reference Laboratory, Bristol, UK) respectively as NCPF 70099 and NCPF 7100, and were obtained from the BCCM/IHEM Culture Collection (Brussels, Belgium) as IHEM 13935 and IHEM 13936. A QC strain (quality control) was also used, referenced as A. niger ATCC 9804 83435. All the other clinical isolates were from our own collection, in the Grenoble University Hospital ‘Service de Parasitologie-Mycologie’ (France).

**Broth dilution antifungal susceptibility testing of drugs**

The amphotericin B used in this study was Fungizone (injectable solution, Bristol-Myers Squibb, France). Albendazole was provided by Smithkline Beecham (France) as assay powder (batch OM 104243–XFP040). Drug dilutions were prepared in dimethyl sulphoxide at 100× the final concentration. For a single-drug susceptibility test, plates and stock inoculum suspensions were prepared according to the NCCLS broth microdilution method M38-P, using RPMI supplemented with MOPS buffer (0.165 M, pH = 7.0 at 35°C). Results were read after a 48 h incubation at 35°C. A chequerboard broth microdilution method was adapted from Del Poeta et al. to study the amphotericin B and albendazole combination. One hundred microlitres of each inoculum in RPMI (prepared according to the NCCLS M38-P method) was incubated in 2×50 µL of a dilution of each drug (final volume = 200 µL). The end point (MIC) was read as the lowest drug concentration that could prevent any visible growth. The fractional inhibitory concentration (FIC) index was then calculated, as the sum of the FICs for each of the drugs (MIC of each drug when used in combination divided by the MIC of the drug when used alone). The interaction was defined as synergic if the FIC index was < 1.0 and antagonistic if the FIC index was > 2.0, with intermediate values indicating no interaction. Both the experiments (single-drug susceptibility test, amphotericin B and albendazole combination test) were conducted twice without demonstrating a difference.

**Sequencing of part of the A. flavus MB7191 β-tubulin gene**

PCR amplification of part of the A. flavus MB7191 β-tubulin gene was performed on genomic DNA using the oligonucleotides ACAAATATGTCCTCTCGTGCC (position 150 of the complete cds) and ggAAGTCAGAAgGACCCATC (position 786 of the complete cds) as primers. Sequencing was performed using the same primers.

**Results and discussion**

Results are summarized in Table 1. All the strains were susceptible to amphotericin B with equivalent MICs to those usually determined for susceptible *Aspergillus* spp. (range values 0.03–4 mg/L). They were also all susceptible to albendazole. The MICs of albendazole ranged from 0.03 to 1 mg/L and were identical in the two experiments. This highlights that the NCCLS method was also well adapted to testing fungal susceptibility to benzimidazoles. MICs were homogeneous for each species, with variations of two albendazole dilutions for *A. fumigatus*, *A. flavus* and *A. terreus*, and of three albendazole dilutions for *A. nidulans* and *A. niger*. For example, MICs ranged between 0.25 and 0.5 mg/L for

**Table 1. In vitro activity of amphotericin B and albendazole against various strains of *Aspergillus* spp.**

<table>
<thead>
<tr>
<th>Organisms (no. of strains)</th>
<th>Compounds</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td><strong>All (49)</strong></td>
<td>Amphotericin B</td>
<td>0.03–4</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.03–1</td>
</tr>
<tr>
<td><strong>A. fumigatus (27)</strong></td>
<td>Amphotericin B</td>
<td>0.03–1</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.5–1</td>
</tr>
<tr>
<td><strong>A. flavus (10)</strong></td>
<td>Amphotericin B</td>
<td>0.5–1</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.5–1</td>
</tr>
<tr>
<td><strong>A. terreus (7)</strong></td>
<td>Amphotericin B</td>
<td>0.5–1</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.25–0.5</td>
</tr>
<tr>
<td><strong>A. nidulans (3)</strong></td>
<td>Amphotericin B</td>
<td>0.25–4</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.03–0.125</td>
</tr>
<tr>
<td><strong>A. niger (2)</strong></td>
<td>Amphotericin B</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Albendazole</td>
<td>0.125–0.5</td>
</tr>
</tbody>
</table>
Susceptibility of *Aspergillus* spp. to albendazole

*Aspergillus terreus*, and between 0.5 and 1 mg/L for *A. flavus* and *A. fumigatus*.

Among the 27 *A. fumigatus* clinical isolates, three were resistant to itraconazole. In our experiments, no cross-resistance was detected, these three strains being susceptible to albendazole (and also to amphotericin B) with MICs, similar to those of the other *A. fumigatus* strains (0.5 or 1 mg/L).

The basis of albendazole action on the pathogenic basidiomycete *Cryptococcus neoformans* and of other benzoimidazoles antifungal compounds (benomyl, carbendazim, nocardazole and thiabendazole) on *A. nidulans* has been well documented. Some β-tubulin amino acid positions (His, Ala, Phe) have been proved to be very important. We therefore looked for these amino acids in known sequences of *Aspergillus* spp. β-tubulins. The sequences of β-tubulin are available for *A. fumigatus* (GenBank accession number AY048754, T. D. Edlind & S. K. Katiyar, 2001), for *A. nidulans* (GenBank accession numbers M17519 and M17520) and for *A. flavus* (GenBank accession number M38265). The six amino acids were present in the correct position, except for Phe in *A. flavus* β-tubulin (from the strain CRA01–2B), where it was replaced by a tyrosine (data not shown). We therefore sequenced part of the β-tubulin gene of one tested strain (*A. flavus* MB7191) and found that this sequence fits with that previously described with only one mismatch: the nucleotide in position 499 of the complete cds was a T instead of an A previously described with only one mismatch: the nucleotide in position 499 of the complete cds was a T instead of an A.

It would be of considerable interest to determine the susceptibility of *Aspergillus* spp. to these metabolites, and to determine whether such chemotherapy would be effective in invasive or disseminated aspergillosis.

Our objectives are now to determine the activity of albendazole (and of its metabolites) on other pathogenic yeasts and filamentous fungi, and then to test them in animal models.

Such studies should define whether albendazole could be an effective antifungal compound for medical use.

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This study is dedicated to the memory of Professor Michel Blot.

### References


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