In vitro susceptibility to 9 antifungal agents of 14 strains of Zygomycetes isolated from clinical specimens

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Abstract

Fourteen clinical isolates of Zygomycetes were tested for their in vitro susceptibility to nine antifungal agents. Susceptibility assessment was performed using a microtiter broth dilution method. Synthetic broth with YNB and glucose was used for 5-fluorocytosine and BHI broth for all the other antimycotics. Amphotericin B exhibited the strongest activity against all isolates tested. MIC values of other two polyenes – nystatin and pimaricin – ranged within the susceptibility limits, with a little pronounced higher activity of pimaricin. The isolates of the genus Absidia and Syncephalastrum were well sensitive to all antimycotics with the exception of 5-fluorocytosine and naftifine. A very weak or zero growth inhibitory effect against all members of the genera Mucor and Rhizopus was found in azoles, 5-fluorocytosine and naftifine.

Key words: Antifungal agents, in vitro susceptibility, Zygomycetes

Introduction

Zygomycetes have now become increasingly important as opportunistic colonizers or invaders of open wounds, burns and other damages. Their infection capabilities have a wide range: infections vary from localized to systemic and are potentially lethal, especially in immunocompromised hosts. The difficulty of treatment of these diseases is caused, among others, by the agents of zygomycoses having a limited sensitivity to antimycotics.

According to the summarizing data concerning, e.g., itraconazole and ketoconazole, the minimal inhibitory concentration (MIC) of these antimycotics for up to 90% of isolates of these organisms reaches or exceeds the value of 16.0 mg/l [1]. Although in vitro sensitivities are known to correlate poorly with in vivo results, the laboratory monitoring of susceptibility patterns of Zygomycetes is useful and desirable. Information of complete in vitro resistance is of special validity because few data exist as yet to suggest that an agent that is ineffective in vitro is effective in clinical outcome.

The purpose of the present study is to examine in vitro activity of 9 antimycotics against a variety of clinical isolates of Zygomycetes, recently cultivated from sources of diverse origin.

Materials and methods

Organisms. Fourteen strains of Zygomycetes belonging to 6 species were isolated from clinical specimens: Absidia corymbifera 8 (4 burn, 3 lacerated wound, 1 external otitis), A. ramosa 1 (burn), Mucor circinelloides 1 (burn), M. racemosus 1 (external otitis), Rhizopus arrhizus 2 (1 brain abscess, 1 external otitis) and Syncephalastrum racemosum 1 (lacerated wound).

All strains under study were identified with conventional morphologic method [2] and maintained on Sabouraud glucose agar slants. Mixed inocula composed of both hyphae and sporangiospores were prepared from cultures grown at 27 °C for 7 days. Suspension were made by rubbing on the surface of SDA slants with a loop after the addition of sterile saline with 0.01% Tween 80, by washing and resuspending in saline. Spore and mycelial fragment counts were
made in a hemocytometer and the final suspensions were adjusted to $1 \times 10^6$ CFU/ml.

**Drugs.** Antifungal agents utilized included amphotericin B (AMB, Squibb Pharma-von Heyden GmbH, Wien), 5-fluorocytosine (5FC, Fluka AG, Buchs), nystatin (NYS, Serva GmbH, Heidelberg), pimafucin (PIM, Gist Brocades, Delft), itraconazole (ITR), ketoconazole (KTZ), miconazole (MCZ), substances kindly supplied by Janssen Pharmaceutica, Beerse, clotrimazole (CLZ, Léčiva, Praha) and naftifine (NAF, Krka, Novo Mesto). The breakpoints corresponding to individual agents were determined in systemic antymycotics according to the concentrations that might be obtained in serum by conventional therapy: AMB 1.56, 5FC 25.0, MCZ, KTZ, ITR 6.25 mg/l. The breakpoints in topical compounds were fixed at 12.5 mg/l.

**Susceptibility testing.** MICs were determined by using a broth dilution microplated system with 96 wells. Synthetic broth with Yeast Nitrogen Base Difco (6.7 g/l) and glucose (5.0 g/l) was used for 5-fluorocytosine and Brain Heart Infusion Broth Difco for all the other antymycotics. The first of the above mentioned media is recommended generally for experiments with 5-fluorocytosine [e.g. 3], the second was used successfully in the study of sensitivity of large series of filamentous fungi to other antymycotics [4].

Twofold dilutions of the antifungal agents were prepared in media to concentrations ranging from 0.045 to 100 mg/l. After inoculation, the microtiter plates were incubated at 27 °C for 48 hours. The MIC was defined as the lowest concentration preventing visible growth in the test medium.

**Results**

Table 1 compares in vitro activity of 9 antifungal agents, representing polyenic antibiotics, azole chemotherapeutics, 5-fluorocytosine and naftifine. The most active drug against all species of fungi tested was AMB, with MIC ranging from 0.09 to 0.78 mg/l. With the exception of ITR inhibiting the members of the genus *Absidia* in very low concentrations (0.045–0.19 mg/l), the activity of AMB against all isolates was higher than that of the other compounds tested.

In another two polyene antibiotics (NYS and PIM), a relatively narrow range of susceptibility was found with low MIC values, suggesting an absence of resistance. In 11 strains PIM was two to three more active than NYS.

MICs of azole compounds were distinguished primarily by reaching the values of complete resistance (25.0–100 mg/l) in genera *Mucor* and *Rhizopus*. In genera *Absidia* and *Syncephalastrum*, CLZ was the second best, after the already mentioned ITR. MIC values of this imidazole were in some strains up to fourfold lower in comparison with MCZ and KTZ.

All isolates of Zygomycetes tested were fully resistant to 5FC and NAF. In these two compounds MIC value did not fall below 50 mg/l in any case.

**Discussion**

Despite the introduction over the past years of numerous agents with potent antifungal activity, little success has as yet been seen in the treatment of some mycoses. Beside the causes relating to the host (uncontrolled underlying disease, immunosuppression) and the properties of the antymycotic (drawbacks of pharmacokinetic parameters), the failures may be due to the lack of sensitivity of these fungal organisms to antymycotics. Laboratory monitoring of the susceptibility of Zygomycetes shows that it is in this group where the resistance or poor sensitivity of some of their representatives is permanently occurring phenomenon.

Although the number of strains that could be studied in our experiments was small, we are able to demonstrate that AMB is still the agent with very high activity, coming into play in the whole range of species spectrum of these fungi. AMB continually represents the drug of choice in the treatment of zygomycoses and, according to some authors [5–7] it is the only reliable drug to control these infections. The uniqueness of AMB application particularly in the treatment of mycoses caused by fungi of genera *Mucor* and *Rhizopus* is documented not only by a number of human case reports, but also by the results of experimental infections of animals. While imidazoles and triazoles are ineffective in these cases, AMB prolonged survival of infected animals and was life-saving [8].

Our finding that there exists a conspicuous difference between the MIC for the group covering the genera *Absidia* and *Syncephalastrum* and the group covering the other genera has enriched the hitherto information on the resistance of Zygomycetes to azoles. The members of the genera *Absidia* and *Syncephalastrum* were well sensitive toazole antifungals in our exper-