IN VITRO SYNERGISM BETWEEN MICONAZOLE AND
GRISEOFULVIN AGAINST CANDIDA SPECIES

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ABSTRACT

Objective: To find the synergistic effect between miconazole and griseofulvin on common human Candida.

Methods: A serial dilution of miconazole and griseofulvin in Yeast Nitrogen Base (YNB) was prepared in test tubes. 50l of standardised suspension of yeasts was inoculated into each test tube and incubated at 30°C for 24-48 hours. The antifungal activity of miconazole alone or with griseofulvin was measured in vitro against 300 isolates of the Candida.

Results: Miconazole and griseofulvin in combination showed a Minimum Inhibitory Concentration (MIC) of 6.7mg/l miconazole (M) and 20mg/l griseofulvin (G) for C. albicans, 13mg/l M and 20mg/l G for C. tropicalis, 4.9mg/l M and 20mg/l G for C. pseudotropicalis and 14.2mg/l M and 20mg/l G for C. parapsilosis. The combination of miconazole with griseofulvin resulted in synergistic activity (Fractional Inhibitory Concentration, FIC <0.82) against C. albicans in a microdilution checkerboard assay. FICs for other species were as follow: FIC <0.75 for C. tropicalis, FIC <0.86 for C. pseudotropicalis and FIC <0.79 for C. parapsilosis.

Conclusion: These results suggest that combination of griseofulvin and miconazole regimens may reduce usual dosage of miconazole and it is helpful in the control of infections caused by Candida species.

KEY WORDS: Candida, Miconazole, Griseofulvin, Synergism.

INTRODUCTION

Candida species are among the most ubiquitous of the normal flora saprophytic yeasts and cause candidiasis. Over the past two decades, the incidence of candidiasis has risen in inexorably. Candidiasis occurs particularly in immunocompromised patients, such as cancer and transplant recipient’s patients.¹ One of the most important topical preparations for candidiasis is miconazole whereas amphotericin B usually applies for systemic infections.

Previous studies show that the anti-fungal activity of miconazole is due to an interaction of the drug with sterol in cell membrane. Therefore, the specific target of miconazole is sterol, especially, ergosterol.² Griseofulvin is an enzymatic inducer of coumarin-like drugs and estrogens.³ Griseofulvin interacts with microtubules during mitosis process. This drug is used for control of dermatophytosis.

Combination therapy could be of benefit for the treatment of candidiasis. Antifungal agents given in combination may improve efficacy due to synergism. In addition side effects of drugs could be reduced, when small dose of drugs is used in combination.¹ The reduction in the development of resistance is another advantage in combination therapy. Many studies on the in vitro antifungal susceptibilities of clinical yeasts have been performed with Candida species.⁴⁵ In this work the synergism effect between miconazole and griseofulvin was studied for the first time on Candida species.
MATERIALS AND METHODS

Organisms: All species were isolated from different clinical types of candidiasis. Three hundred clinical Candida isolates were studied including 190 C. albicans, 74 C. tropicalis, 21 C. parapsilosis and 15 C. pseudotropicalis. The samples were maintained on Sabouraud dextrose agar (SDA) at 4°C. Synchronous cultures were prepared according to the Johnson’s method. For preparing fresh synchronous cultures, the organisms were sub-cultured on SDA and incubated at 37°C for 24 hour. Ten parts of plate were chosen and yeast cells were collected in 2 ml of sterile PBS to prepare a suspension. The suspension was adjusted to 70% T by a spectrophotometer at 530 nm. This resulted in a suspension containing about 1×10^6 cfu/ml.

Preparation of drug solution: Sixty-four mg of the antifungals were dissolved in 50 ml of dimethyl sulphoxide (DMSO) at 1280 mg/l. The solutions were kept at room temperature for 30 min for self-sterilisation. The stock solutions were then stored at -70°C for up to 1m and diluted with Yeast Nitrogen Base (YNB) to reach a final concentration of 160mg/l and 128mg/l for griseofulvin and miconazole respectively. The stock solutions of griseofulvin and miconazole were diluted to 160mg/l and 128mg/l with H_2O respectively.

Test method: Antifungal interactions were assessed using a chequerboard broth dilution-based method. 2ml of sterile YNB was added to each test tube (A1-F10). Then 0.5ml of serial dilution of both antifungals was added to test tubes A1-E9. This process gave a set of doubling drug dilutions from 64mg/l to 0.25mg/l for miconazole and 80mg/l to 5mg/l for griseofulvin. Test tubes F1-F9 and A10-E10 only contained dilution of miconazole and griseofulvin respectively. Test tube F10 was as control without antifungals. Finally the 50 ml standardised suspension was inoculated in to each tube. The tubes were incubated at 30°C for 24-48 hour. The efficacy of miconazole in combination with griseofulvin was measured after 24-48 hour. The lowest drug concentration was seen in MIC compared to control (F10). MICs were also determined for each drug after combination and FICs were calculated by the FIC index. The FIC index was defined as follows:

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\frac{MIC \text{ of drug A, in combination}}{MIC \text{ of drug A, tested alone}} + \frac{MIC \text{ of drug B, in combination}}{MIC \text{ of drug B, tested alone}}
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RESULTS

In this work miconazole was known as synergic in interaction with griseofulvin against 300 Candida isolates. Remarkable synergy was documented for C. albicans with reduction of MIC 2.7 fold for miconazole. The MICs of miconazole and griseofulvin for 190 isolates of C. albicans were respectively 8-32mg/l (mean 18.2mg/l) and 20-80mg/l (mean 44.9mg/l). When C. albicans grew on miconazole+griseofulvin the MICs for isolates of C. albicans were 20mg/l and 6.7mg/l for griseofulvin and miconazole respectively (Figure-1). For miconazole-griseofulvin combination synergism was observed for 190 C. albicans isolates with FIC index 0.82.

The synergism was observed for 74 C. tropicalis isolates with FIC index 0.75. The MICs of miconazole and griseofulvin for isolates of C. tropicalis were 16-64mg/l and 20-80mg/l respectively. The mean of MICs was 42.4mg/l for miconazole and 45.1mg/l for griseofulvin. The MICs of combination between miconazole and griseofulvin were 20 and 13.1mg/l. MICs of miconazole fell from 32-64mg/l (mean 47.2mg/l) to 8-32mg/l (mean 14.2mg/l) in 21 isolates of C. parapsilosis in combination with griseofulvin. 74 isolates of C. parapsilosis showed a synergy with FIC index 0.79. FIC index all isolates of C. pseudotropicalis was 0.86. The mean of MICs was 22.9mg/l and 31.3 for

Fig-1: The synergism effect of miconazole in combination with griseofulvin for Candida isolates.
miconazole and griseofulvin respectively. When *C. pseudotropicalis* grew on miconazole + griseofulvin the MICs were 20mg/l and 4.9mg/l for griseofulvin and miconazole respectively (Figure-1).

**DISCUSSION**

Current therapeutic agents for example azoles, amphotericin B are only partially effective for the treatment of candidiasis, therefore, more effective drugs and drug combination are needed. More recently it has been confirmed that this approach could add some microbiological benefits to the treatment of candidemia in nonneutropenic patients. Combinations of antifungal drugs, in comparison with single drugs, may confer the benefit of increasing efficiency, sparing toxicity, or both.

In this study, we analysed in vitro interaction between the miconazole and griseofulvin, and the drugs were tested against the clinical isolates of *Candida* species. The result demonstrated an increase in fungistatic activity of miconazole against Candida by addition of griseofulvin. Therefore, the results of the microtiter checkerboard assay combinations of the antifungal agents used in this study indicated that their activity was synergistic. The combination of miconazole with griseofulvin was always more effective in 300 species of Candida than the monotherapy, thus a clear synergism between griseofulvin and miconazole was seen. The checkerboard method that we have employed is useful for an initial analysis of drug-drug interactions. Time-kill studies would provide additional data on the nature of these interactions and would be useful for further analysis of the interaction between miconazole and griseofulvin.

Interesting by the MIC griseofulvin (20mg/l) was same for all species after combination with miconazole. In contrast, the MIC for species was significantly different. The lowest MIC was only 4.9mg/l for *C. pseudotropicalis* and the highest was 14.2mg/l for *C. parapsilosis*. This suggests that candidal infections may be treated based on species. To our knowledge, this is the first study to demonstrate that the combination of miconazole and griseofulvin has a significant impact on survival, quantitative culture results. In conclusion, our results indicate that a combination of miconazole and griseofulvin might be effective in infection due to *Candida* species. Animal models are required to validate the in vivo significance of these in vitro data presented for the miconazole-griseofulvin combination.

**REFERENCES**