IN VITRO DRUG SENSITIVITY OF TRICHOPHYTON RUBRUM AGAINST GRISEOFULVIN, KETOCONAZOLE AND FLUCONAZOLE

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The invitro activity of griseofulvin, ketoconazole and fluconazole was investigated against 50 isolates of Trichophyton rubrum. Ketoconazole was more active, inhibiting all the 50 isolates at a concentration of 5 μgm/ml (MIC range 0.5-5 μgm/ml). Griseofulvin (MIC range 2.5-20 μgm/ml) required 20 μgm/ml of the drug for inhibition of all the isolates. Fluconazole was least active as it inhibited only 2 isolates at a concentration of 20 μgm/ml, which was the upper limit of the test system.

Key Words : Trichophyton rubrum, Griseofulvin, Ketoconazole, Fluconazole

Introduction

Rising incidence of recurrence of dermatophytic infections and its tremendous morbidity lead us to compare the incidence of Trichophyton rubrum (commonest dermatophyte) and its sensitivity pattern in-vitro against griseofulvin, ketoconazole and fluconazole.

Materials and Methods

Skin scrapings/hair specimens/nail clippings were taken from various clinical types of tinea patients and were subjected to direct microscopic examination in 10% KOH solution. KOH positive specimens were cultured on Sabouraud's dextrose agar media and incubated at 28°C upto 3 weeks. Culture growths of Trichophyton rubrum were identified on the basis of gross and microscopic appearance. Fifty pure isolates of T rubrum were subcultured into nutrient broth at 28°C for 4 days.[1] For preparing the fungal inoculum, nutrient broth grown mycelia were fragmented with the help of a cyclo-mixer to achieve a uniform suspension. This suspension was standardised spectrophotometrically to an absorbance of 0.500 to 0.600 at a wavelength of 450 nm.

Griseofulvin, ketoconazole and fluconazole in pure powder form were obtained from Glaxo India Limited, Micro Labs Limited and FDC Limited, respectively. Solutions of all the three antifungal drugs were prepared by dissolving 5 mg of each drug in 5 ml of dimethyl formamide (DMF). Further dilutions of the drugs in different concentrations (0.1, 0.5, 1, 2.5, 5, 10 and 20 μgm/ml) were prepared in nutrient broth. For testing the antifungal sensitivity, tube dilution method was followed.[2]

The drug sensitivity test was carried out in a set of 23 test tubes. Each test tube contained 2 ml of nutrient broth with different concentrations of griseofulvin (in test tubes numbered 1-7), ketoconazole (in test tubes numbered 8-14) and fluconazole (in test tubes numbered 15-21). Test tube numbered 22 containing 2 ml of nutrient broth and DMF, and test tube numbered 23 containing 2 ml of nutrient broth only, served as controls. Each test tube was inoculated with 0.1 ml of inoculum and incubated at 28°C. The lowest concentration of the drug which permitted no macroscopically visible growth after 6 days was taken as the minimum inhibitory concentration (MIC).

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Results

Table I. Minimum inhibitory concentrations (MICs) of griseofulvin, ketoconazole and fluconazole against 50 isolates of Trichophyton rubrum

MICs μgm/ml	0.1	0.5	1.0	2.5	5	10	20
				Griseofulvin			
No. of cases	-	-	-	1	12	31	6
				Ketoconazole			
No. of cases	-	2	16	26	6	-	-
				Fluconazole			
No. of cases	-	-	-	-	-	-	2

All the 50 isolates of T rubrum were sensitive to griseofulvin and ketoconazole. However, only 2 isolates were sensitive to fluconazole at MIC of 20 μgm/ml, which was the upper limit of the test system. The MICs of griseofulvin ranged between 2.5-20 μgm/ml with a mean MIC of 9.85 μgm/ml, while MICs of ketoconazole ranged between 0.5-5 μgm/ml with a mean MIC of 2.24 μgm/ml. The MIC range and the mean MIC for fluconazole could not be ascertained.

Discussion

Our results showed that out of these 3 drugs, ketoconazole is the most effective, fluconazole is the least effective and griseofulvin is the intermediate effective drug in vitro. Our findings are comparable with those of other workers.[3,4] Grant and Clissold (1990)[3] reported the MIC of ketoconazole and fluconazole against T rubrum species as 0.39-3.1 μgm/ml and 12.5-100 gm/ml, respectively. The medium used in above study was tissue culture agar. Korting et al (1994)[4] in their study on 32 isolates of T rubrum and 16 isolates of T mentagrophytes in Kimmig's agar, from patients of tinea unguium, found the MIC range for griseofulvin, ketoconazole and fluconazole against T rubrum species to be 0.5-3.0 μgm/ml, 0.5-2.0 μgm/ml and 64-1024 μgm/ml, respectively. The method used by them was broth dilution test in micro-titer plates. The investigators of this study commented that the higher MICs for fluconazole determined in their study might be due to well known technical problems regarding this compound such as interactions with particular media or dissolution problems at higher concentrations.

We find that fluconazole is a poorly effective drug in vitro. On the contrary, as per existing literature,[5,6] it has been termed as a wonderful drug in vivo because of weekly dosage, which improves patient compliance and reduces the cost of therapy. The clinical efficacy of fluconazole is because of its unique physicochemical and pharmacokinetic properties like high solubility in water, low protein binding (11%), high levels of free drug in the blood, even distribution of the drug throughout the body, very less metabolism of the drug into inactive metabolites (10%) and long half-life (30 hours).[7]

Since the results of in vitro antifungal drug sensitivity testing have poor interlaboratory reproducibility and precision, it is suggested that further more detailed studies should be carried out for standardising the antifungal sensitivity tests and to establish the correlation between in vitro and in vivo efficacy of fluconazole.

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