In vitro evaluation of griseofulvin against clinical isolates of dermatophytes from Isfahan

In-vitro-Empfindlichkeit klinischer Dermatophyten-Isolate aus Isfahan, Iran, gegenüber Griseofulvin

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Summary
Fifty dermatophyte isolates, recently obtained from clinical materials, belonging to Trichophyton mentagrophytes, T. verrucosum, Microsporum canis and Epidermophyton floccosum were examined for their susceptibility to griseofulvin. The minimum inhibitory concentration (MIC) values were obtained using the modified microdilution method. All 100% tested isolates had MIC geometric mean at a concentration between 0.43 and 0.95 μg ml⁻¹. The MIC₉⁰ and MIC₅₀ were 8 μg ml⁻¹ and <0.25–1 μg ml⁻¹ respectively. From all isolates, 12% including three T. verrucosum, one M. canis and two T. mentagrophytes isolates had MIC values out of the standardized range, therefore, they were considered as relatively griseofulvin-resistant. At least some of the isolates tested might be difficult to eradicate in clinical terms with griseofulvin treatment in Isfahan.

Zusammenfassung

Key words: dermatophytes, griseofulvin, susceptibility.

Schlüsselwörter: Dermatophyten, Griseofulvin, Empfindlichkeit.

Introduction

Cutaneous fungal infections are most prevalent in Iran and cause a significant number of patients to be referred to skin clinics daily.¹–³ For more than 50 years griseofulvin has been in use for the treatment of dermatophytoses in humans.⁴ With an increasing variety of drugs available for the treatment of dermatophytes,⁵–⁸ griseofulvin is no longer the first-line drug of choice in Iran. Relative or absolute microbial resistance is one of the many causes of recalcitrant fungal infections in this area. Although reports on resistant strains of different dermatophytes and yeasts appeared over the years from various parts of the world,⁹–¹² there was no indication that resistant strains might play a
major role in the treatment of dermatophytes with griseofulvin. This situation changed when a report on the correlation of griseofulvin resistant dermatophytes with in vitro resistance was published by Artis et al. They showed that therapeutic failure was combined with antifungal resistance in several patients with chronic dermatophytes due to Trichophyton rubrum. Over the time other researchers also reported either resistant species of dermatophytes or the existence of strains with elevated minimum inhibitory concentrations (MICs) to griseofulvin. Various in vitro test systems have been developed in recent years to determine the antifungal activities of different drugs. The MIC obtained may give an indication of the in vivo potency of the drugs. Data obtained by various studies can differ a lot, and a generally acknowledged standardized test procedure does not exist. Most fungal infections respond to appropriately selected therapy, however, reports of therapy failures, side effects and expensive costs of some newly introduced antifungal drugs have raised the concern to evaluate the resistance pattern of griseofulvin against dermatophytes from Isfahan which was the aim of the present study. This may allow the clinician to select the adequate therapy for the treatment of dermatophytes.

Materials and methods

Organisms

A total of 50 isolates of dermatophytes isolated from clinical specimens of patients affected with various clinical types of dermatophytes were tested, including 14 Trichophyton verrucosum, 13 Epidermophyton floccosum, 12 T. mentagrophytes and 11 Microsporum canis. We paid special attention to the most prevalent species of dermatophytes from Isfahan province and strains from patients suffering from chronic dermatophytes in whom griseofulvin therapy had failed. Dermatophytes were identified to species level by conventional methods. Isolates were stored at −20 °C in potato glucose agar slants until the time of use.

In vitro susceptibility testing

The modified microdilution method was employed for antifungal susceptibility with griseofulvin. This comprises incubation of dermatophytes in Sabouraud glucose broth medium at 30 °C for 4–7 days, repeated washing and centrifugation in distilled water, homogenization in a glass homogenizer over 3 min. Then the homogenized suspension was adjusted by matching turbidity at 530 nm of a 0.5 MacFarland standard. Griseofulvin was dissolved in 70% ethanol; dilutions were prepared by addition of 50% ethanol. Microdilution plates were set up by filling the wells with 200 µl Sabouraud glucose broth, 10 µl of standardized inoculum and 10 µl of different griseofulvin dilutions resulting in final concentrations from 0.25 to 64 µg ml⁻¹. Controls were prepared by addition of 10 µl distilled water (control 1) or 10 µl 50% ethanol (control 2) instead of the griseofulvin solution. To prevent drying out the plates were sealed with a plastic foil and incubated at 37 °C for 14 days or until the control plates showed clearly visible growth. The plates were read visually for fungal growth every 2 days. The resulting MIC was determined as the lowest concentration of griseofulvin which prevented visible growth, with the control plates as reference. All tests were performed in triplicate and if the MICs were different, the range of that MIC was used. Data were analysed and showed by MIC₅₀ (the MIC at which 50% of isolates are inhibited), MIC₉₀, mode and range. Strains requiring MICs <3 µg ml⁻¹ of griseofulvin were regarded as griseofulvin-resistant.

Results

Fifty strains of dermatophytes were isolated from patients (Table 1) and their MICs determined. The MIC values of these assays are summarized as MIC₅₀, MIC₉₀, geometric mean of MIC and range values in Table 2. MICs ≥3 µg ml⁻¹ of griseofulvin were regarded as resistant. All the strains had MIC geometric mean of 0.43–0.95 µg ml⁻¹ and about 90% had 8 µg ml⁻¹ (MIC₉₀) and 50% ranged between <0.25 and 1 µg ml⁻¹ (MIC₅₀). From all isolates, six (12%) strains including three T. verrucosum, two T. mentagrophytes and one M. canis were found to be resistant to griseofulvin (MIC value of >3 µg ml⁻¹). Two strains of resistant T. verrucosum (MIC value of 64 µg ml⁻¹) were isolated from tinea capitis and tinea corporis patients who failed to respond to therapy with griseofulvin and the third resistant strain (MIC value of 8 µg ml⁻¹) was isolated from a tinea corporis patient who had no antifungal therapy. One strain of resistant M. canis (MIC value of 16 µg ml⁻¹) and T. mentagrophytes (MIC value of 8 µg ml⁻¹) were isolated from recalcitrant tinea capitis which had treatment with griseofulvin and itraconazole, the second resistant strain of T. mentagrophytes (MIC value of 4 µg ml⁻¹) was isolated from a patient without previous griseofulvin therapy. Of the 13 E. floccosum
strains isolated, resistance to griseofulvin was not detected (MIC value of <0.25–2 \( \mu \text{g ml}^{-1} \)).

**Discussion**

The increasing prevalence of cutaneous mycoses\(^1\)–\(^2\) coupled with the frequent use of antifungal drugs now available, has resulted in rising resistance of dermatophytes to griseofulvin in Iran. The growing number of patients at risk and the increasing rate of recalcitrant dermatophytoses in this area have prompted the need for appropriate laboratory testing for susceptibility of griseofulvin against prevalent isolates of dermatophytes. In our study one strain of resistant *M. canis* (MIC value of 16 \( \mu \text{g ml}^{-1} \)) and *T. mentagrophytes* (MIC value of 8 \( \mu \text{g ml}^{-1} \)) were isolated from a recalcitrant tinea capitis patient who had treatment with griseofulvin and itraconazole, the second resistant strain of *T. mentagrophytes* (MIC value of 4 \( \mu \text{g ml}^{-1} \)) was isolated from a patient without previous griseofulvin therapy. Of the 13 *E. floccosum* strains isolated, resistance to griseofulvin was not detected. Aytoun et al.\(^7\) isolated six strains of *M. canis* resistant to griseofulvin with MIC values of 8, 32 and 64 \( \mu \text{g ml}^{-1} \) and one strain of *T. rubrum* with MIC of 8 \( \mu \text{g ml}^{-1} \) by layer plate method. They believe that these strains possess, in their ability to destroy griseofulvin, a potential mechanism for attaining resistance and also other dermatophyte species may have this ability to become resistant. Jessup et al.\(^4\) determined the antifungal susceptibility of 217 isolates of dermatophytes to fluconazole, griseofulvin, itraconazole and terbinafine and found that terbinafine possessed the highest antifungal activity against all isolates. They did not report any griseofulvin-resistant isolate of dermatophytes; this is in contrast to our finding where we encountered two resistant isolates of *T. mentagrophytes* and one *M. canis*, although the susceptibility methods used were different. Artis et al.\(^3\) were able to show that patients with chronic recalcitrant dermatophytoses caused by *T. rubrum* were, indeed, infected with strains resistant to griseofulvin. Scholz and Meinhof\(^9\) evaluated the susceptibility of 270 isolates of *T. rubrum* against griseofulvin by microdilution method almost similar to ours and found seven strains as relatively resistant from six patients. Three of the patients showed no indication of therapeutic failure of griseofulvin treatment. The other three had chronic dermatophytoses and had been treated with griseofulvin previously with little or limited success. These researchers considered MIC values of \( \geq 3 \mu \text{g ml}^{-1} \) as the hallmark of resistance, the same value as our study. Simpanya\(^18\) tested the sensitivity of 13 isolates of dermatophytes from Zambia and Cameroon to griseofulvin and no resistant strain was found in these areas. As the sample size of this author was not high, it could not be concluded that griseofulvin therapy against dermatophytoses had no failure in Zambia and Cameroon. The *in vitro* antifungal activity of griseofulvin, ketoconazole and itraconazole were similar against various isolates of prevalent dermatophytes in Singapore.\(^5\) The majority of the isolates was

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<th>Table 1 Frequency of isolated species of dermatophytes from clinical types of dermatophytoses.</th>
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<td>Trichophyton mentagrophytes</td>
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<td>Microsporum canis</td>
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<th>Table 2 In vitro susceptibility of 50 isolates of dermatophytes to griseofulvin.</th>
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MICs in \( \mu \text{g ml}^{-1} \).
sensitive to these drugs, all at a concentration of \(<0.25 \text{ mg ml}^{-1}\) and few isolates of \(T. \text{rubrum}\) and \(T. \text{interdigitale}\) were resistant to these drugs. Although these researchers reported resistant strains of dermatophytes to griseofulvin in this area, yet, they suggested that griseofulvin may be given as the first-line drug for treatment of dermatophytoses in Singapore. Korting and Rosenkranz\(^{10}\) studied in vitro susceptibility of clinical isolates of dermatophytes from Munich to griseofulvin, miconazole and ketoconazole using the microdilution method. They found griseofulvin-resistant strains of \(T. \text{mentagrophytes}\) in this area – a finding similar to our study. A surprising finding in our study was the isolation of resistant strains including \(T. \text{mentagrophytes}\) from recalcitrant tinea capitis and tinea corporis patients who had failure therapy with griseofulvin and itraconazole several times. This is in agreement with other studies, which explain that strains of \(T. \text{mentagrophytes}\) are less susceptible to antifungal drugs than \(T. \text{rubrum}\).\(^{6,10}\) It would seem that acquired resistance to griseofulvin has not been reported in \(T. \text{verrucosum}\) from other parts of the world whereas in our study we encountered three resistant strains in which two strains belonged to patients who failed to respond to griseofulvin treatment. This could be explained by the fact that \(T. \text{verrucosum}\) is one of the most prevalent agents of dermatophytoses in Iran\(^{1–3}\) mostly treated with griseofulvin. The increasing MIC values may correlate with the probability of failure of therapy for dermatophytoses. In cases of griseofulvin-resistant dermatophytoses, the systemic treatment would be replaced by newer drugs such as itraconazole, fluconazole and terbinafine.\(^{5,8}\) Although resistance to terbinafine in strains of \(T. \text{rubrum}\) has also been reported,\(^{19}\) the treatment of choice is the one with the best benefit-to-risk ratio and cost ratio. Multiple mechanisms for resistance to antifungal agents are known and it is believed that long-term use of antifungal drugs with high dose leads to the selection of resistant isolates.\(^{11,12,16}\) The results of antifungal susceptibility tests, however, suffered from the fact that several different procedures of MIC evaluation were used by various researchers and each worker considers different MIC values as the hallmark of resistance.\(^{5,9,10,15}\) and that no standardized method was generally acknowledged. Therefore, it can be concluded that in future the establishment of a reference susceptibility testing method should gain more and more importance and may allow the clinician to select the appropriate therapy for the treatment of chronic or recalcitrant dermatophytoses or in failure cases of griseofulvin therapy.

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