Microbiology

Chemotherapy

Chemotherapy 1998;44:31–35

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Antimicrobial Susceptibility Testing of Dermatophytes – Comparison of the Agar Macrodilution and Broth Microdilution Tests

Abstract

Fifty dermatophyte strains, recently obtained from clinical material, belonging to 4 different species were examined for their susceptibility to 5 systemic or topical antimycotic agents using both an agar macrodilution and a broth microdilution test. Antimycotics compared were griseofulvin, itraconazole, sertaconazole, terbinafine and ciclopiroxolamine. A comparison of the minimum inhibitory concentrations (MIC) clearly showed differences between the two test methods applied. For all 5 antimycotics, MIC data were three- to seventyfold lower in the microdilution test system. These differences, depending on the test method, have to be taken into account when comparing MIC data in the literature or when relating the in vitro data to the tissue concentrations determined in vivo.

Key Words

Dermatophytes
Agar macrodilution test
Broth microdilution test
Griseofulvin
Terbinafine
Ciclopiroxolamine
Itraconazole
Sertaconazole

Introduction

Dermatophyte infections are common; depending on the localization, they may cause therapeutic problems, e.g. in tinea capitis or tinea unguium [1]. While the number of available antimycotics has increased considerably over the last years, more and more reports have been published stating recalcitrance to therapy or even resistance of a dermatophyte against the antimicrobial agents used [2–4]. In order to determine the capability of antimycotics to eradicate dermatophytes, the implementation of in vitro susceptibility testing may prove helpful, as is now established with Candida species. With griseofulvin, a failure of therapy corresponding to in vitro resistance has been shown [5]. Measurement of antimicrobial concentrations in tissue, e.g. in nail plates or in the stratum corneum, and a com-

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0009–3157/98/0441–0031$15.00/0

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parison with the minimum inhibitory concentration (MIC) determined in vitro might provide further insight [6].

The agar macrodilution test has been the prevailing test system until the broth microdilution test was introduced recently. These two methods differ in various aspects, including medium and time required. In order to allow a comparison of the results of both methods, susceptibility testing using the agar macrodilution assay and the broth microdilution assay were performed with 5 antimycotics in 50 dermatophyte strains belonging to 4 different species. Moreover, it was intended to get insight into ease of handling and costs.

Materials and Methods

Isolation of Dermatophytes

Fifty dermatophyte strains were isolated consecutively from skin samples, nail and hair material at the Department of Dermatology of the University Hospital at Eppendorf, Hamburg, Germany. All isolates were identified according to the regulations of the 'Deutsche Gesellschaft für Hygiene und Mikrobiologie' [7]. The dermatophytes were isolated on Kimmig agar and determined as *Trichophyton rubrum* (n = 38), *Trichophyton mentagrophytes* sensu stricto (n = 10), *Trichophyton mentagrophytes* var. *quinckeanum* (n = 1) and *Microsporum canis* (n = 1).

Antimycotics

To determine the MIC all isolates were exposed to the 5 antimycotics griseofulvin, itraconazole, sertaconazole, terbinafine and ciclopiroxolamine. Griseofulvin was dissolved in 70 vol% ethanol (Merck, Darmstadt, Germany) and further diluted in 50 vol% ethanol (Merck). Itraconazole and sertaconazole were both dissolved in dimethylsulfoxide; further dilutions were made with distilled water. Terbinafine was dissolved and diluted in distilled water. Ciclopiroxolamine was dissolved in 40 vol% ethanol (Merck) and diluted with distilled water.

Agar Macrodilution Test

The agar macrodilution test was carried out in Petri dishes. Kimmig agar (Merck) served as nutrient medium. Serial dilutions of the given antimycotic were mixed with the warm liquid agar to obtain the final concentrations. After cooling down, the solid agar-antimycotic mixture was inoculated with the dermatophyte using a sterile swab. After an incubation period of 4 weeks at ambient temperature, the results were read. The MIC was considered as the lowest concentration that totally inhibited visible growth [8].

Broth Microdilution Test

For the broth microdilution test, four mycelium pieces of about 0.5 × 0.5 cm of each isolate, subcultured on Kimmig agar, were cut out and transferred into 100-ml Erlenmeyer bottles. Twenty microliters of nutrient broth (NB; Difco, Detroit, Mich., USA) were added. The cultures were incubated for 5–7 days at ambient temperature and were shaken daily in order to keep the cultures submerged and prevent growth of air mycelium. After the incubation period, the cultures were centrifuged at 3,023 *g* for 15 min. The supernatant was discarded, and the pellet was resuspended in 0.9% sodium chloride solution. This washing procedure was repeated twice. The final pellet was resuspended in 4 ml nutrient broth and homogenized in a tissue grinder (Type Tenbroek, 7 ml; Kontes Glass, Vineland, N.J., USA). The mycelium suspensions were photometrically adjusted (Photometer 1101 M; Eppendorf, Hamburg, Germany) to an extinction of 0.6 at 436 nm. Aliquots of 10 µl were transferred to the wells of microtiter plates. Two hundred microliters nutrient broth and 10 µl antimycotic dilution were added. In addition, a positive and a negative control were run. The plates were sealed with self-adhesive sterile plastic foil, and after incubation of 1 week at ambient temperature, the results could be read with the naked eye. The MIC is defined as the lowest concentration at which no visible growth can be detected [9, 10].

Results

The results of both test procedures are shown in figure 1. The columns show the MIC data of the 5 antimycotics tested.

In table 1, the MIC_{50}, the MIC_{90} and the MIC range of each species are given. The MIC_{50} and MIC_{90} are the MIC that inhibit growth in 50 and 90% of the given isolates, respectively.

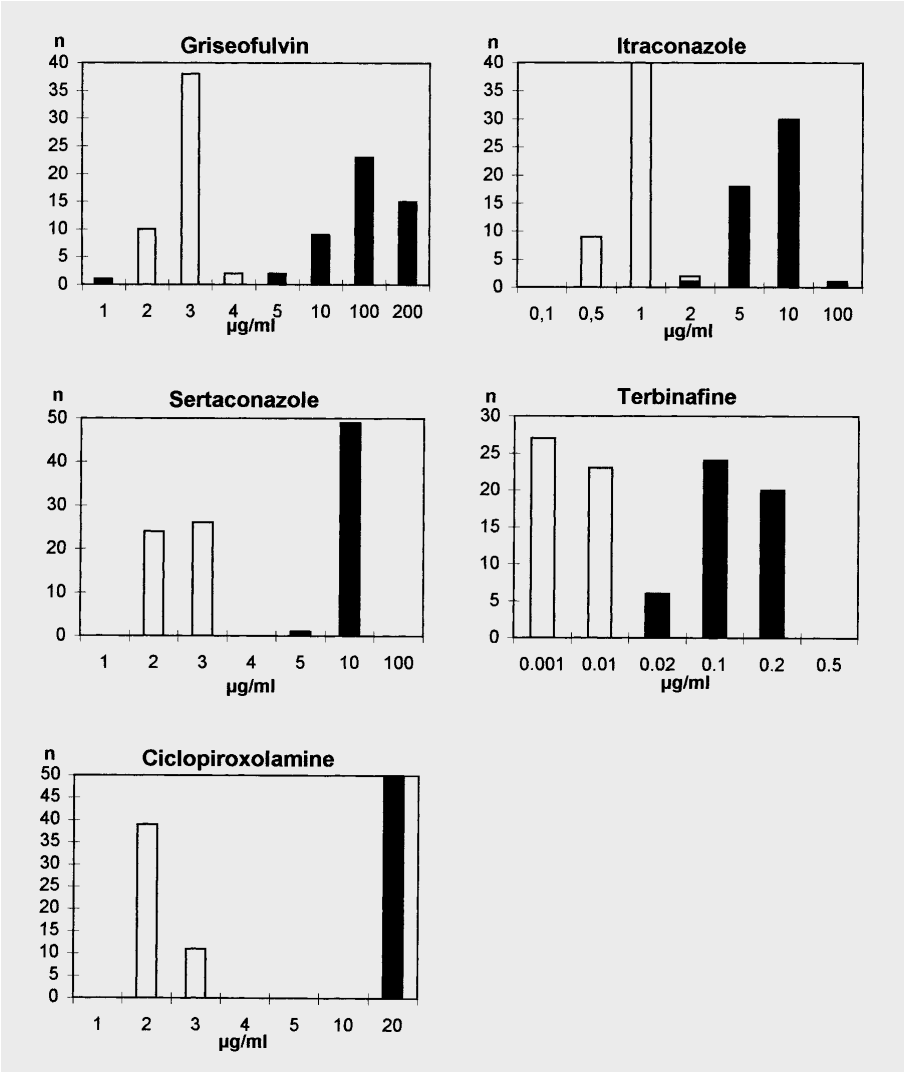

Fig. 1. Distribution of the MIC of five antimycotics obtained using the agar macrodilution test and the broth microdilution test. Closed bars = Agar macrodilution test; open bars = broth microdilution test; n = number of isolates.

The comparison of the results shows clear differences between the MIC distribution found with the two methods. With all 5 antimycotics, the MIC of the broth microdilution test were lower than the ones of the agar macrodilution test. In the case of the azoles, the MIC_{90} with *T. rubrum* lay at 10 µg/ml with the agar macrodilution test, in contrast to a MIC_{90} of 1 µg/ml for itraconazole with the broth microdilution method. Sertaconazole had a MIC_{90} of 3 µg/ml with *T. rubrum*. With ciclopiroxolamine, all isolates showed a

Table 1. In vitro activity of griseofulvin, itraconazole, sertaconazole, terbinafine and ciclopiroxolamine against 50 dermatophytes using the agar macrodilution test and the broth microdilution test

Antifungal agent	Organism	Isolates	Agar macrodilution test, μg/ml			Broth microdilution test, μg/ml		
			MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}	range
Griseofulvin	*T. rubrum*	38	100	200	1–200	3	3	2–4
	T. mentagrophytes	10	100	200	10–200	3	3	2–3
	T. quinckeanum[1]	1	200	200	200	3	3	3
	M. canis	1	200	200	200	2	2	2
Itraconazole	*T. rubrum*	38	10	10	2–10	1	1	0.5–2
	T. mentagrophytes	10	10	10	5–10	1	1	0.5–1
	*T. quinckeanum**	1	100	100	100	1	1	1
	M. canis	1	10	10	10	1	1	1
Sertaconazole	*T. rubrum*	38	10	10	5–10	3	3	2–3
	T. mentagrophytes	10	10	10	10	2	2	2
	T. quinckeanum[1]	1	10	10	10	3	3	3
	M. canis	1	10	10	10	3	3	3
Terbinafine	*T. rubrum*	38	0.1	0.2	0.02–0.2	0.01	0.01	0.001–0.01
	T. mentagrophytes	10	0.1	0.2	0.1–0.2	0.001	0.01	0.001–0.01
	T. quinckeanum[1]	1	0.2	0.2	0.2	0.001	0.001	0.001
	M. canis	1	0.2	0.2	0.2	0.001	0.001	0.001
Ciclopiroxolamine	*T. rubrum*	38	20	20	20	2	3	2–3
	T. mentagrophytes	10	20	20	20	2	2	2
	T. quinckeanum[1]	1	20	20	20	3	3	3
	M. canis	1	20	20	20	3	3	3

[1] *T. mentagrophytes* var. *quinckeanum*.

MIC of 20 μg/ml in the agar macrodilution test. This is in clear contrast to a MIC of 3 and 2 μg/ml *(T. mentagrophytes)*, respectively, in the broth microdilution test. Terbinafine showed the expected low MIC values. They ranged from 0.02 to 0.2 μg/ml and from 0.001 to 0.01 μg/ml, respectively. Thus, the MIC_{90} obtained by the broth microdilution test is 20 times lower than the MIC_{90} obtained by the agar macrodilution test. In comparison, griseofulvin appeared particularly remarkable. Not only were the ranges widest, but also the MIC_{90} differed considerably: 3 μg/ml with the broth microdilution assay stood in contrast to 200 μg/ml with the agar macrodilution assay both with *T. rubrum* and with *T. mentagrophytes*. In this case, the results even differ seventyfold. A higher susceptibility of *T. rubrum* than of *T. mentagrophytes* against griseofulvin, as described earlier [11], could not be substantiated in this study. No species-specific differences could be established with any other antimycotic, either. Among the 50 consecutively isolated dermatophytes, there was only 1 isolate of *T. mentagrophytes* var. *quinckeanum* and *T. canis*. The test results for these 2 fungi are in keeping with the other results at large.

Discussion

A comparison of the practicability of both test methods showed advantages of the broth microdilution test. It required less expenditure of labour, space and material, and the results were obtained after a much shorter time. The present study substantiates that the development of newer susceptibility tests for dermatophytes has caused a shift of inhibition values towards lower concentrations. The reason is far from being obvious at present. Generally, it becomes clear that susceptibility tests for dermatophytes in clinical practise should be standardized to make a direct comparison between results from various laboratories possible. Facing the skin tissue levels obtained in man with usual regimens and ensuing cure rates, the MIC values found with the broth microdilution assay look more plausible [12].

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