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IN VITRO COMPARATIVE STUDIES OF THE ANTIFUNGAL ACTIVITY OF TRICHOMYCIN, PIMARICIN, AND GRISEOFULVIN

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INTRODUCTION

Trichomycin is an antibiotic obtained from cultures of Streptomyces hachijoensis. It was isolated for the first time in 1952 by Hosoya et al. [1] who discovered the microorganism in a soil sample from a Japanese island. Its chemical structure is not yet completely known but according to Hattori et al. [2] the molecule is a conjugated heptaene. As a powder it is stable but in solution it progressively loses activity. In vitro and in vivo experiments demonstrated that trichomycin is highly effective against Candida albicans [3, 4] and other yeasts and against Trichomonas vaginalis [5]. It is also active against some pathogenic fungi [7, 8] and several protozoan parasites, especially flagellata and amoeba [6].

Pimaricin, another new antibiotic, was isolated in 1955 at Delft (Netherlands) by Strucky and Waisvisz [9] from a culture of Streptomyces natalensis obtained from a soil sample of South Africa. Chemically, pimaricin is a crystalline, colorless substance, its complex molecule ($C_{34}H_{49}NO_{14}$) containing a system of four conjugated double bonds (tetraene). Biological investigation shows that pimaricin exhibits a broad-spectrum of antifungal action [9]. It is effective in inhibiting the growth of many yeasts and saprophytic as well as pathogenic fungi which produce plant diseases and skin diseases in man and animals. It is also active against T. vaginalis and other flagellata [10].

Griseofulvin is the well-known antibiotic obtained from cultures of Penicillium griseofulvum, which was discovered by Oxford et al. [11] in 1939 and whose antifungal properties were studied in vitro by Brian et al. [12] in 1946. However, it became an important therapeutic agent only after the investigations carried out by Gentes [13] in 1959. Griseofulvin is effective against dermatophytes, mainly Trichophyton and Microsporum [14].

The present study was undertaken to compare the in vitro activity of these three compounds and their relative antifungal selectivity.

MATERIALS AND METHODS

Microorganisms. C. albicans Yu-1200, which is the standard used to determine the activity of trichomycin, was obtained from Fujisawa Pharmaceutical Co., Osaka, Japan. The other organisms were obtained from L.I.F.E.'s stock culture collection.

Agents. Solutions of the antibiotics studied were prepared by adding trichomycin to a diluent consisting of a 1 : 1 mixture of acetone and water with 8 mg% of NaOH. A concentration limit of 2.7 mg/ml (when 1 μg = 3.7 U) was obtained from which the necessary dilutions were made. Pimaricin was dissolved in pure glycerol to achieve a concentration of 10 mg/ml. Griseofulvin was dissolved in pure methyl alcohol to a concentration of 2 mg/ml. Fresh solutions were used.

Technique. After preliminary assays the following standard technique was adopted. Cultures were made in mycophyl broth (Baltimore Biological Laboratory). Forty-nine milliliters of broth were distributed into glass flasks, each containing 1 ml of the antibiotic solution. For Candida strains 1 ml of a 1:1000 dilution of a 24-hr tryptose-phosphate broth culture was used as the inoculum. For the other fungi the inoculum was a standard 3-mm plug from a 25- to 30-day-old culture of the organism on Sabouraud's maltose agar (Difco). Cultures were incubated at 28 C and the growth was recorded at 48-hr intervals. However, only the results recorded on the sixth day were used for statistical analysis. Five duplicates for each strain and each antibiotic concentration were used. For each series of concentrations there was a control culture which received the respective diluent but not the antibiotics. At least four antibiotic concentrations were tried.

The 100% minimal inhibitory concentration (MC_{100}) was found for each organism as the lowest concentration of the antibiotic at which there was no visible growth of fungi or turbidity in the case of Candida. A 10% minimal inhibitory concentration (MC_{10}) was also found as the lowest concentration which allowed approximately 90% growth, measured according to the diameter of the colony in the case of fungi and turbidimetrically in the case of Candida. Relative potencies as well as the ratio MC_{100}/MC_{10} were calculated.

RESULTS

Figure 1 and Table 1 present the concentrations required to obtain the desired two levels of growth inhibition, namely, 10 and 100% inhibition. Within the limits of the concentrations studied all organisms but one were completely inhibited in their growth. Candida was only slightly inhibited by griseofulvin despite the high concentrations used.

Figures corresponding to the ratio MC_{100}/MC_{10} showed that the more selective a compound was in inhibiting the growth of an organism, the lower was its numerical value. For instance, trichomycin presented a ratio of 3 to 5 when used upon Candida and reached a value of 36 when used upon Trichophyton violaceum. In other words, when an antibiotic is not very selective, it is capable of producing a slight inhibition of growth at a relatively low concentration but 100% inhibition is obtained only at a very high concentration.

There is no agreement as to classification of organisms according to their susceptibility to the three antibiotics studied. Arbitrarily we have considered as

Table 1. Antifungal Activity of Trichomycin, Pimaricin, and Griseofulvin

Organism	Strain	100% Minimal inhibitory concentrations (MC_{100}), μg/ml and ratio MC_{100}/MC_{10}					
		Trichomycin		Pimaricin		Griseofulvin	
		MC_{100}	Ratio	MC_{100}	Ratio	MC_{100}	Ratio
C. albicans	Yu-1200	0.35	3.5	2.0	4.0	>1000	–
C. albicans	A-101	2.1	5.1	3.0	3.0	>1000	–
M. canis	B-1	150.0	30.0	25.0	6.3	10	10
M. canis	G-2	200.0	28.0	50.0	10.0	25	10
T. violaceum	Z-17	90.0	36.0	10.0	10.0	50	12
T. schoenleini	A-1	160.0	26.0	50.0	10.0	25	10
T. mentagrophytes	AHC-1	250.0	25.0	50.0	14.0	25	10
A. fumigatus	9194	250.0	10.0	25.0	10.0	150	30

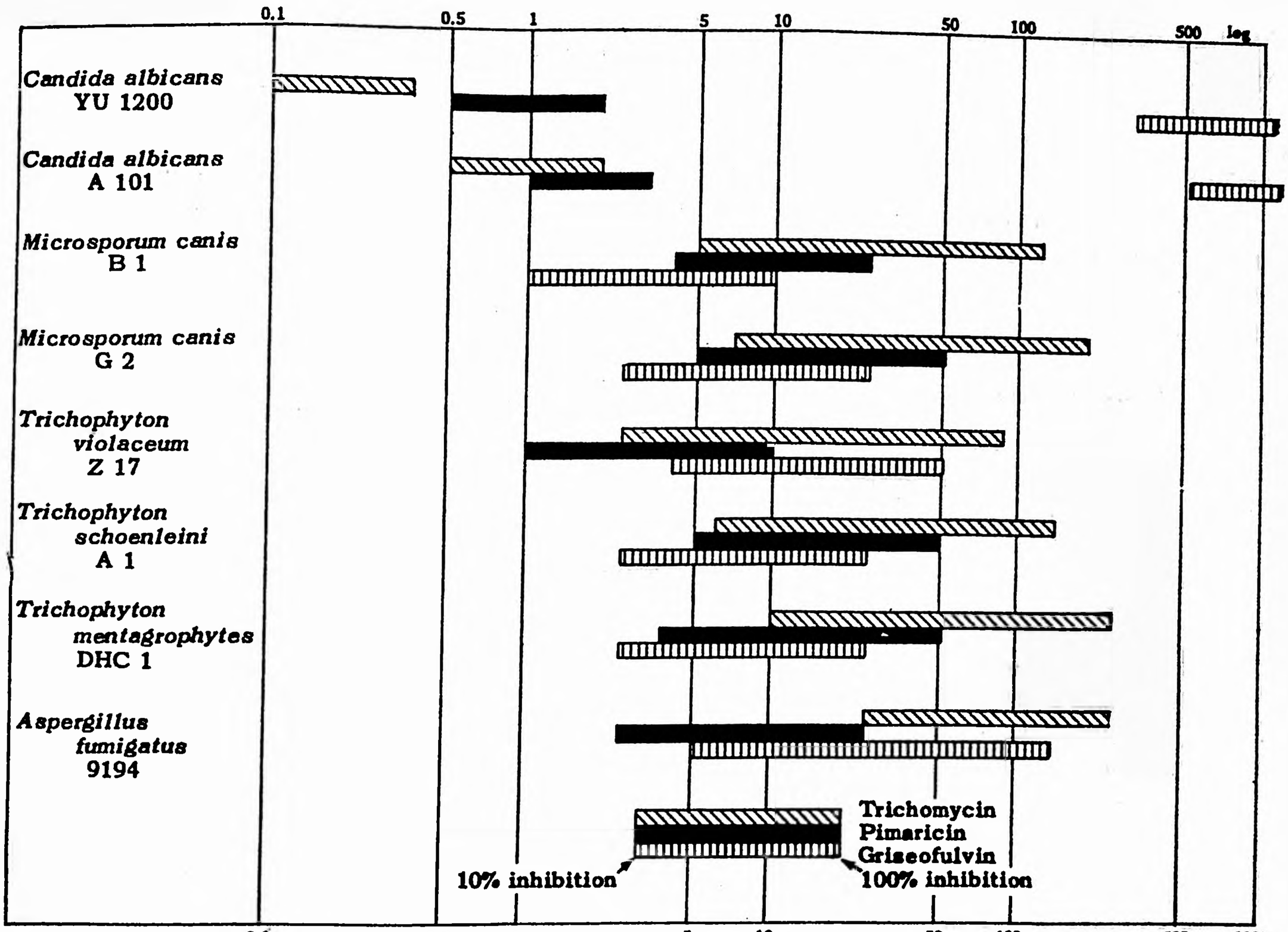

Fig. 1. Minimal concentration required to produce 10% (at left side) and 100% (at right side) inhibition of growth.

Table 2. Degree of Susceptibility of the Studied Microorganisms to Trichomycin, Pimaricin, and Griseofulvin

Drug	Organisms			
	Highly susceptible, <1 to 9.9 μg/ml	Very susceptible, 10 to 99 μg/ml	Moderately susceptible, 100 to 499 μg/ml	Resistant, >500 μg/ml
Trichomycin	C. albicans	T. violaceum	M. canis T. schoenleini T. mentagrophytes A. fumigatus	
Pimaricin	C. albicans	T. violaceum M. canis A. fumigatus T. schoenleini T. mentagrophytes		
Griseofulvin		M. canis T. schoenleini T. mentagrophytes T. violaceum	A. fumigatus	C. albicans

"highly" susceptible those organisms for which the MC_{100} is less than 10 μg/ml; "very" and "moderately" susceptible and "resistant" those for which the MC_{100} was between 10 and 99 μg/ml, 100 to 499 μg/ml, and more than 500 μg/ml, respectively (Table 2).

Table 3 shows the relative activity of the three compounds on each organism. Trichomycin was the most active agent in inhibiting the growth of C. albicans. There were quantitative differences according to the strain of Candida but the potency of trichomycin was 2 to 5 times higher than that of pimaricin. Griseofulvin was almost inactive against this microorganism.

Table 3. Relative Antifungal Activity of Trichomycin, Pimaricin, and Griseofulvin

Organism	Antibiotics* (in sequence of their relative activity)	Relative activity
C. albicans Yu-1200	T > P > G	28.5 : 5.0 : 0.01
C. albicans A-101	T > P > G	4.7 : 3.3 : 0.01
M. canis B-1	G > P > T	15.0 : 2.5 : 1
M. canis G-2	G > P > T	8.0 : 2.0 : 1
T. violaceum Z-1	P > G > T	9.0 : 5.0 : 1
T. schoenleini A-1	G > P > T	6.4 : 2.0 : 1
T. mentagrophytes AHC-1	G > P > T	10.0 : 2.0 : 1
A. fumigatus 9194	P > G > T	10.0 : 6.0 : 1

*T, trichomycin; P, pimaricin; G, griseofulvin.

Griseofulvin was the most active agent against Microsporum canis, its potency being 4 to 6 times higher than that of pimaricin and 8 to 15 times higher than that of trichomycin.

Pimaricin was the most active against T. violaceum, being twice as potent as griseofulvin and 9 times more potent than trichomycin.

Griseofulvin was the most active agent and trichomycin the least against Trichophyton schoenleini and Trichophyton mentagrophytes.

On Aspergillus fumigatus, pimaricin was the most active agent, griseofulvin was second in activity, and trichomycin last, being only one tenth as potent as pimaricin.

DISCUSSION

Among the yeasts and fungi tested only C. albicans was "highly" susceptible to both trichomycin and pimaricin. Lutz and Witz [15] reported that trichomycin was some 30 to 70 times more active against C. albicans than its congener, nystatin, which was considered, until the discovery of trichomycin, the most selective antibiotic for inhibiting such an organism. At the present time trichomycin is the most potent agent against Candida, a fact that has been confirmed consistently in the therapeutic field [4, 5, 15].

None of the other pathogenic fungi appeared as "highly" susceptible to any of the three antibiotics described. If, for example, the minimal concentration of penicillin required to kill susceptible bacteria, is compared with the concentration of griseofulvin or pimaricin required to completely inhibit the growth of Trichophyton or Microsporum, it is easy to see that these latter antibiotics are not extremely potent agents.

Certainly the introduction in therapy of these new antibiotics represents important progress in the treatment of cutaneous fungus diseases. However, we have still to search for more potent and more selective agents.

SUMMARY

Antifungal activity of trichomycin, pimaricin, and griseofulvin was studied by using the in vitro method of serial dilutions. The 10 and 100% minimal inhibitory concentrations were found for each antibiotic and each organism tested.

Trichomycin was the most potent agent against Candida albicans. Pimaricin, according to the strain of Candida, was 2 to 5 times less active than trichomycin and griseofulvin was almost inactive.

Griseofulvin was the most active agent against Microsporum canis. Its potency was 4 to 6 times higher than that of pimaricin and 8 to 15 times higher than that of trichomycin.

For Trichophyton violaceum, pimaricin was twice as potent as griseofulvin and trichomycin was the least active agent. On Trichophyton schoenleini and Trichophyton mentagrophytes, griseofulvin was the most active agent and trichomycin the least.

Pimaricin was the most active agent and trichomycin the least against Aspergillus fumigatus.

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DISCUSSION

DR. KATZ: Dr. Muggia, can you tell us whether these drugs have had clinical trials, particularly trichomycin, and either clinical trial or in vivo use in experimental infections in mice?

DR. MUGGIA: I was not there myself at the time of the studies and I am not aware that they have tried it clinically.

DR. EMMONS: We are indebted to Dr. Muggia for presenting this paper and he has an adequate explanation for his inability to answer all questions but if there are any questions or any comments from anyone

in the audience we would be glad to hear them. The role of griseofulvin in the treatment of superficial fungus infections is certainly well established. We tried griseofulvin against the systemic mycoses as soon as it became available to us and found, as others have, that it was not effective in treatment of systemic mycoses. Trichomycin is available for experimental use now but we do not have conclusive results to report at this time.

DR. LYNCH: (Kansas City) We have had some experience testing this drug both in vivo and in vitro and at the present time I thought it might be interesting to point out some of our in vitro results. With our standard organism, Candida, that we use to compare all drugs, we found that a level of about 0.25 μg gives the same effect that would be given, let's say, by a weaker concentration of amphotericin B, so that in our hands it is not necessarily the most potent drug against that organism. There is an organism which is supplied by the company for testing this drug. It is a Japanese isolate of Candida and I'm not sure quite where they got it from but it is exceedingly sensitive to this drug, except that we have had trouble propagating the organism and comparing it to others. I was just wondering whether you might know whether that was the particular isolate that was used in these studies.

DR. MUGGIA: I'm sorry. Once again, I can't answer that question.