Sensitivity of strains of *Candida albicans* to jaritin, haloprogin, clotrimazole and miconazole

L. LANGSADL
M.D.

Z. JEDLICKOVA
M.D.

Research Institute for Pharmacy and Biochemistry, Postgraduate Medical and
Pharmaceutical Institute, Prague, Czechoslovakia

Summary

The antifungal activity of jaritin and haloprogin against *Candida albicans* is similar. *In vitro* they had less activity than clotrimazole and miconazole, but the activity was independent of inoculum size.

Introduction

Several highly effective antimycotics, such as miconazole, clotrimazole and haloprogin, are used in several countries for the control of mycotic infections. The Research Institute for Pharmacy and Biochemistry in Prague has developed a haloprogin analogue, jaritin. It is a 2-methylthio-5-(3-iodopropargyloxy) pyrimidine. In this paper the antifungal activities of the new preparation jaritin are compared with those of haloprogin, clotrimazole and miconazole.

Materials and methods

Pure substances of these antimycotics were used for testing. The tests were performed with strains of *Candida albicans* isolated from patients with vaginal candidiasis and with laboratory strains of other *Candida* spp. The minimal inhibition concentration (MIC) was determined in both liquid and solid agar media by the dilution method. Strains previously cultivated on Sabouraud's glucose agar medium were washed off with saline and diluted to optical density (OD) 0.05, measured at 520 nm in a spectrophotometer (SPECOL Zeiss). The diluted cultures were used as inocula. When testing the effect of the inoculum size for antifungal activities of the antimycotics, cultures were diluted to OD 0.01 and 0.05 equalling 1.6×10^4 CFU/ml and 2×10^8 CFU/ml respectively. A standardized inoculum of 0.05 ml was then added to each test tube. Agar plates were inoculated with a Steer's replicator. The test tubes and plates were incubated at 28°C for 48 hr. The MIC was defined as the lowest concentration of an antimycotic inhibiting the growth after incubation at 28°C for 48 hr. The effect of proteins upon antifungal activities was determined in the same media

supplemented by 10% of inactivated normal bovine serum (BIOVETA Ivanovice, Czechoslovakia). The composition of the liquid medium was Neopeptone (Difco) 1 g + glucose 2 g/1000 ml of distilled water. The solid medium was prepared in the same way as liquid medium, but with the addition of 15 g of Oxoid agar no. 3. The data were statistically tested by nonparametric Wilcoxon matched pairs signed-rank test at the significance level 0.05.

TABLE 1. Antifungal activities of jaritin and haloprogin in liquid medium. Inoculum size 2×10^8 CFU/ml

<i>Candida</i> spp.	MIC (μg/ml)			
	Jaritin		Haloprogin	
	1	2	1	2
<i>C. albicans</i>	0.7	1.5	0.7	12.5
<i>C. tropicalis</i>	0.3	0.7	0.15	1.5
<i>C. krusei</i>	0.7	3.1	6.2	25.0
<i>C. pseudotropicalis</i>	6.2	12.5	25.0	25.0
<i>C. lipolytica</i>	0.15	0.7	0.03	12.5
<i>C. utilis</i>	1.5	6.2	25.0	25.0
<i>C. humicola</i>	6.2	12.5	25.0	25.0
<i>C. parapsilosis</i>	12.5	12.5	12.5	25.0
<i>C. pelliculosa</i>	0.3	0.7	0.03	6.2
<i>C. catenulata</i>	1.5	3.1	0.3	25.0
<i>C. zeylanoides</i>	1.5	3.1	1.5	25.0
<i>C. reukauffi</i>	1.5	3.1	0.7	25.0

1. Medium without bovine serum.
2. Medium with 10% of inactivated bovine serum.

Results

The antifungal effect of jaritin and haloprogin in liquid medium against 12 laboratory strains of *Candida* spp. is shown in Table 1. *C. pelliculosa*

TABLE 2. The effect of inoculum size on antifungal activity against *Candida albicans* in liquid medium. Results - MIC (mg/l)

	Inoculum size (CFU/ml)	
	2×10^8	1.6×10^4
Miconazole	0.39-12.5	0.025-1.56
Clotrimazole	0.39-12.5	0.025-0.10
Jaritin	0.7-3.1	0.39-0.7
Haloprogin	0.3-6.2	0.39-1.5

*CFU, colony-forming units

was most sensitive to haloprogin, and *C. pseudotropicalis*, *C. utilis* and *C. humicola* the least sensitive. The MICs of the strains regarded as resistant to haloprogin were sensitive to jaritin. The addition of 10% bovine serum produced significant differences in the sensitivities to both jaritin and haloprogin. The activity of miconazole and clotrimazole against *C. albicans* was shown to be dependent on inoculum size (Table 2).

Antifungal activities of jaritin, haloprogin, clotrimazole and miconazole against freshly isolated strains from patients with vaginal candidiasis were also tested on solid agar medium. The inoculum size was 1.6×10^4 CFU/ml. The range of MICs (mg/l) for jaritin was 0.1–1.5; for haloprogin, 0.2–6.2; for clotrimazole, 0.01–0.7; for miconazole, 0.1–3.1. There were no statistically significant differences between MIC values of jaritin and haloprogin, but there were differences between MIC values of jaritin, clotrimazole and miconazole.

Jaritin in the concentration 0.78 mg/l inhibited 48.9% of the strains. As many as 96.9% of the strains were inhibited by 1.5 mg/l of haloprogin. The

concentration of 0.78 mg/l of miconazole inhibited 93% of strains (Fig. 1).

Conclusions

The antifungal activities of jaritin and haloprogin against collection strains of *Candida* are approximately equal. When testing freshly isolated strains of *C. albicans*, the same activity was found. Antifungal activities of jaritin and haloprogin do not depend on the inoculum size. The antifungal activities of clotrimazole and miconazole depend on the size of inoculum. With high inoculum size the activity of jaritin is higher than the activity of miconazole. With low inoculum size, antifungal activities of clotrimazole and miconazole are higher than those of jaritin.

The activity of all antimycotics tested was inhibited by the presence of serum proteins in the medium. Using freshly isolated strains of *C. albicans* in the lower concentration of inoculum (1.6×10^4 CFU/ml), clotrimazole was the most effective agent; miconazole came next; jaritin and haloprogin followed. No statistical differences between jaritin and haloprogin in antifungal activity were found.

FIG. 1. Antifungal activity of antimycotics against fresh isolated strains of *Candida albicans*. Jaritin —; haloprogin ---; miconazole -.-.; clotrimazole

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