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Laboratory evaluation for determining posaconazole susceptibility of fungi isolated in denture stomatitis

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Abstract

Candida-associated denture stomatitis constitutes a very important health problem because fungal infections occur in patients who use prosthetic restorations. Increasing resistance of fungi as well as toxicity of antifungals and high costs of treatment have an influence on many difficulties in denture stomatitis treatment. The study was to estimate in vitro susceptibility to the selected antifungal – posaconazole of yeast-like fungal strains, obtained from direct oral mucosal swabs taken from patients using prosthetic restorations. Mycological tests were performed using the method of direct swab taken from oral mucosa. In the next part of the research, cultured strains were identified. Evaluation of susceptibility of all isolates to posaconazole was estimated using E-test. On the basis of laboratory mycological tests performed, the growth of 99 species of yeast-like fungi was diagnosed at 84.95% among 93 patients, from whom oral mucosa swab samples were collected. Posaconazole presented a high activity against the tested fungal strains.

Key words: *denture stomatitis, fungal infection, antifungal therapy.*

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Introduction

In the past couple of decades, an increasing prevalence of fungal infections in humans, especially systemic opportunistic ones, characterised by a severe clinical course, recurring tendency and complications, has been observed. Surface mycosis as well as subcutaneous and systemic ones develop mainly in patients with considerable immunodeficiencies in neoplasms, leukaemia, AIDS, and also in metabolic diseases, such as diabetes or endocrinopathies [1].

The problem of an increase in the number, intensity and recurrent character of fungal infections is also related to prosthetic restorations use in patients, especially when coupled with a decreased immunity or with systemic diseases [2-5].

Fungal infections in denture wearers are caused by biocenotic balance perturbations under a denture base. Its acrylic surface is irregular, absorbent, and prone to plaque build-up, which together with fungal cells might penetrate all the pits and form biofilm [6]. An irregular acrylic surface and increased adhesion and adsorption properties of the denture plaque when water is being absorbed by acryl, make it difficult to properly clean dentures, which increases the risk of oral mucosal infection. Other factors, such as saliva which makes cleaning the mouth more difficult, humidity, higher temperature under the denture base, lingering food debris, and decreased mucosal oxygenation also promote bacterial and fungal growth and multiplication. Furthermore, bad hygiene habits and around 24 hours' denture wearing promote Candida-associated denture stomatitis [7]. Among yeast-like fungi, *Candida albicans*, *C. tropicalis*, and *C. glabrata* are its most common cause, and more rarely *C. parapsilosis*, *C. krusei*, and *C. guilliermondii* [8, 9]. However, it should be stressed that the development of fungal infection mostly depends on the host immune system, strain virulence, and host point of entrance. People

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mostly prone to developing Candida-associated denture stomatitis are those with decreased saliva secretion, leukoplakia, oral tumours, epithelial dysplasia of oral mucosa, nutritional deficiencies, and also those having undergone radiotherapy to the head and neck region.

Denture stomatitis treatment, depending on its severity and the prevalence of concurrent diseases includes prosthetic, surgical, pharmacological and prosthetic combined with pharmacological elements. Polyene (nystatin, natamycin), azole (clotrimazole, miconazole, ketoconazole, fluconazole) and pyrimidine derivatives (5-fluorocytosine) are the most frequently used antimycotics. They are applied topically in the form of solutions, tablets, ointments, creams and powders [7]. Topical denture stomatitis treatment might prove ineffective in patients with decreased immunity, after chemo- and radiotherapy, and also after immunosuppressive or long-term antibiotic therapy. In those groups of patients and in chronic and recurrent infections, systemic treatments with imidazole derivatives: fluconazole, ketoconazole, and itraconazole, polyenes – amphotericin B; or new generation antimycotics: voriconazole, posaconazole, and caspofungin, are recommended [10]. However, those patients face repeating many times therapies with the most common antifungals, often without determining strain sensitivity, which results in developing resistance to those drugs.

Since a large number of treatments failed to hinder the progression of Candida-associated denture stomatitis and also its recurrence, especially in patients with systemic diseases and decreased functions of the immune system, it is crucial to search for new effective antifungals.

The study was to estimate *in vitro* susceptibility to the selected antifungal – posaconazole of yeast-like fungal strains, obtained from direct oral mucosal swabs taken from patients using prosthetic restorations.

Material and methods

The research protocol was performed in 93 patients of the Department of Prosthetic Dentistry of the Warsaw Medical University, aged 21-86 years (mean age of 63.3 years) including 62 women and 31 men. Clinical symptoms of denture stomatitis were diagnosed. This group included people using fixed and removable prosthetic restorations and undergoing prosthetic rehabilitation with intraosseous implants.

Material for mycological laboratory tests was collected by swabbing from the surface of oral mucosa in the contact area with intramucosal surfaces of the prosthetic restorations, and resulted in 99 yeast-like fungal strains obtained from cultures. Identification was performed using reference mycological procedures according to Clinical and Laboratory Standards (CLSI). Organisms were grown at 30°C on Sabouraud dextrose agar with chloramphenicol and gentamicin (bioMeriéux). *Candida* species were identified by chromogenic medium CHROMagar *Candida* (Becton Dickinson) and biochemical assimilation test API ID 32 C (bioMeriéux).

The strain sensitivity to posaconazole was estimated using E-tests, recommended to measure antifungal efficiency. It is a quantitative diffusion method making it possible to determine the exact minimal inhibitory concentration (MIC) inhibiting the growth of various fungal strains.

Solid medium RPMI 1640 plus MOPS with an addition of 2% of glucose and 1.5% of Bacto agar (Biomed, Warsaw) was prepared according to the manufacturer's guidelines. E-test stripes for posaconazole were placed on the plates with study and model (*C. albicans ATCC 14053*, *C. tropicalis ATCC 13803* and *C. glabrata ATCC 90030*) strains suspension (0.5 McFarland). All plates were incubated in 37°C up to 24-48 hours, but in the case of *C. glabrata* and *C. tropicalis* the incubation was made up to 48 hours.

Then, fungal growth inhibition zones and MIC, MIC 50, MIC 90 after 24 h were observed; for *C. glabrata* and *C. tropicalis*, the observation was made only after 48 h. All the described procedures were performed according to the manufacturer's guidelines, and the results interpreted according to CLSI standards.

Furthermore, for posaconazole, a MIC ≤ 1 mg/l indicated a *sensitive* strain, according to CLSI standards and professional recommendations [11].

Results

Ninety-nine yeast-like fungi, mainly *C. albicans*, *C. tropicalis*, and *C. glabrata*: 57.58%, 18.18% and 14.14%, respectively (Fig. 1), were grown out from direct oral mucosal swabs taken from 93 patients in 84.95% of the cases.

Posaconazole, one of new triazole drugs, presented a high activity against the tested fungal strains. *Candida tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. sake*,

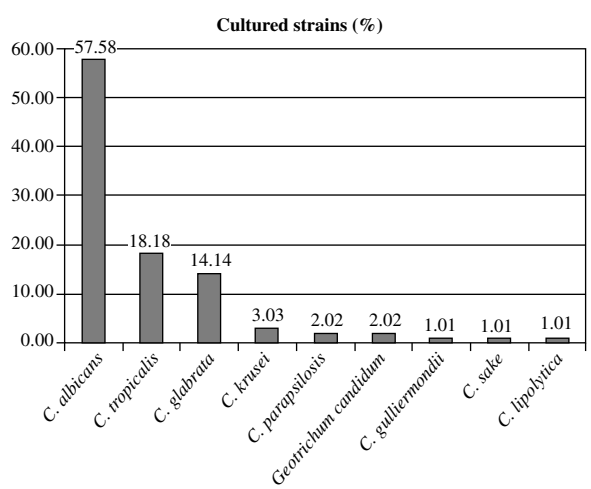

Fig. 1. Cultured and identified 99 strains of yeast-like fungi (%)

Table 1. Minimal inhibitory concentration (MIC range), MIC_{50}, MIC_{90} for posaconazole

Yeast-like fungi	Number of strains	Minimal Inhibitory Concentration (MIC) [mg/l]		
		MIC_{50}	MIC_{90}	MIC_{range}
C. albicans	57	0.032	0.5	0.004-32
C. tropicalis	18	0.064	0.25	0.016-0.5
C. glabrata	14	1	32	0.064-32
C. krusei	3	0.016	1	0.016-1
C. parapsilosis	2	0.032	0.5	0.032-0.5
Geotrichum candidum	2	0.5	32	0.5-32
C. gulliermondii	1	0.125	0.125	–
C. sake	1	0.064	0.064	–
C. lipolytica	1	1	1	–

C. lipolytica, and *C. krusei* presented 100% sensitivity. In 18 *C. tropicalis* isolates, MICs were low and were 0.016-0.5 mg/l (MIC_{50} was 0.064 mg/l; MIC_{90} – 0.25 mg/l). *Candida parapsilosis* (MIC range 0.032-0.5 mg/l), *C. guilliermondii* (MIC 0.125 mg/l) and *C. sake* (MIC 0.064 mg/l) also presented similarly low MICs. Despite *C. krusei* primary resistance to azoles, posaconazole was active towards all of the three tested strains, and two of them presented breakpoint sensitivity (MIC 1 mg/l) to that antimycotic. Analysing sensitivity of 57 *C. albicans* strains to posaconazole, the growth of 29 isolates was inhibited by very low MICs of 0.004-0.032 mg/l, the growth of 23 strains was inhibited by 0.064-0.5 mg/l, and the 5 remaining isolates were posaconazole resistant (MIC ≥ 32). To sum up, posaconazole was highly effective against *C. albicans* and amounted to 91.22% (MIC_{50} 0.032 mg/l, MIC_{90} 0.5 mg/l).

Among the analysed species, *C. glabrata* (MIC_{50} 1 mg/l, MIC_{90} 32 mg/l, MIC range 0.064-32 mg/l) presented the lowest sensitivity to posaconazole. 78.57% of isolates were sensitive to that antimycotic, and in the remaining 21.43% of the strains, posaconazole was not effective (MIC ≥ 32 mg/l) (Table 1).

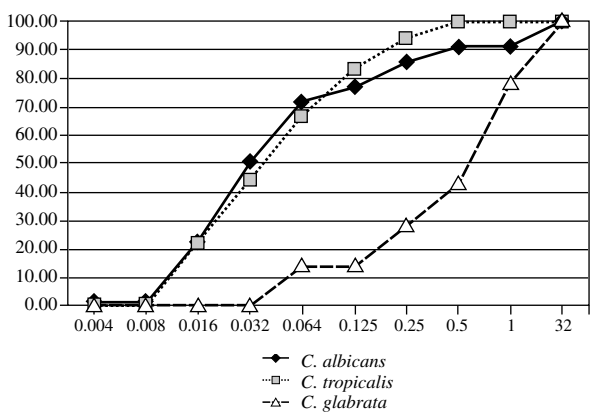

Fig. 2. Comparison of cumulative number of (%) *C. albicans*, *C. tropicalis* and *C. glabrata* strains inhibited at various posaconazole concentrations

Table 2 presents MICs for all 99 analysed strains and for model strains, and Fig. 2 compares cumulative numbers of *C. albicans*, *C. glabrata* and *C. tropicalis* inhibited at various posaconazole concentrations.

Discussion

As it is emphasised not only in various publications but also by physicians dealing with those problems on a daily basis, denture stomatitis and the associated fungal infections are becoming more and more difficult to be successfully treated and prevented from recurring. Despite a broad portfolio of available antifungal drugs, there seem to be more and more yeast-like fungal strains, including *C. albicans*, resistant to different drugs or even groups of antimycotics [12].

The recurrent character of fungal infections in patients wearing prosthetic restorations and under immunosuppressive treatments, prolonged chemo- or radiotherapy makes it difficult for physicians to choose an effective antifungal treatment. In those patients, infections are most often caused by more than one fungal strain and also by *Candida non-albicans*, which makes it harder to choose an appropriate antifungal treatment [13]. At the same time, the associated general and topical immune disorders, considering the inefficiency of topical pharmacotherapy, have to be treated with general pharmacotherapy, including imidazole derivatives. Posaconazole is one of the newest triazoles. The drug is active against yeasts and moulds, not excluding strains genetically resistant to fluconazole. Posaconazole activity against numerous strains is a dozen times higher than that of fluconazole [14, 15].

Table 2. Minimal inhibitory concentration (MIC): the growth of studied and model strains for posaconazole

Number of strains		Minimal Inhibitory Concentration (MIC) [mg/l]									
		0.004	**0.008**	**0.016**	**0.032**	**0.064**	**0.125**	**0.25**	**0.5**	**1**	**32**
C. albicans	57	1	–	12	16	12	3	5	3	–	5
		1.75%	–	21.05%	28.07%	21.05%	5.26%	8.77%	5.26%	–	8.77%
C. tropicalis	18	–	–	4	4	4	3	2	2	5	3
		–	–	22.22%	22.22%	22.22%	16.67%	11.11%	5.56%	–	–
C. glabrata	14	–	–	–	–	2	–	2	2	5	3
		–	–	–	–	14.29%	–	14.29%	14.20%	35.71%	21.43%
C. krusei	3	–	–	1	–	–	–	–	–	2	–
		–	–	33.33%	–	–	–	–	–	66.67%	–
C. parapsilosis	2	–	–	–	1	–	–	–	1	–	–
		–	–	–	50.00%	–	–	–	50.00%	–	–
Geotrichum candidum	2	–	–	–	–	–	–	–	1	–	1
		–	–	–	–	–	–	–	50.00%	–	50.00%
C. gulliermondii	1	–	–	–	–	–	1	–	–	–	–
		–	–	–	–	–	100.00%	–	–	–	–
C. sake	1	–	–	–	–	1	–	–	–	–	–
		–	–	–	–	100.00%	–	–	–	–	–
C. lipolytica	1	–	–	–	–	–	–	–	–	1	–
		–	–	–	–	–	–	–	–	100.00%	–
C. albicans ATCC 14053	1	–	–	–	–	1	–	–	–	–	–
			–	–	–	100.00%	–	–	–	–	–
C. glabrata ATCC 90030	1	–	–	–	–	–	–	–	–	–	1
		–	–	–	–	–	–	–	–	–	100.00%
C. tropicalis ATCC 13803	1	–	–	–	1	–	–	–	–	–	–
		–	–	–	100.00%	–	–	–	–	–	–

In the present study, posaconazole presented a high activity against the tested yeast-like fungal strains, isolated from oral mucosa in patients wearing prosthetic restorations. *Candida tropicalis*, *C. parapsilosis*, *C. guilliermondii, C. sake*, *C. lipolytica*, and *C. krusei* presented 100% sensitivity. Posaconazole activity against *C. albicans* was high and amounted to 91.22%; *C. glabrata* presented the lowest sensitivity of 78.57%.

Pfaller *et al*. encountered a similar sensitivity to posaconazole in 4,169 *Candida strains* [16]. Almost 100% of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. kefyr* strains were sensitive to that drug, but it was less effective against *C. glabrata* (< 80%).

Sabatelli *et al.* [17] studied a couple of thousands *Candida* strains and observed an efficacy of 97% and 93% for posaconazole and fluconazole, respectively. They also stressed that fluconazole-resistant strains were sensitive to posaconazole. Similarly, Spreghini *et al.* [18] found out, while analysing posaconazole efficacy against *C. glabrata*, that it was also active against fluconazole-resistant strains.

The presented results suggest that posaconazole could be used in treating denture stomatitis and the associated fungal infections, especially in patients with decreased immunity, systemic diseases and predisposition to recurrence. However, considering posaconazole's broad spectrum of activity, it should be used in treatments of persistent and recurrent oral mucosa fungal infections.

In conclusion, rational treatment of the oral mucosa fungal infection in patients using prosthetic restorations, should be based on therapy with effective antifungals specified on the basis of antimicogram. In our study, posaconazole showed high activity in relation to yeast-like fungi. It is

worth considering using it in Candida-associated denture stomatitis, especially in chronic and recurrent cases of fungal infections.

The authors declare no conflict of interest.

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