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INFLUENCE OF CLOTRIMAZOLE PHARMACEUTICS ON ANTIFUNGAL ACTIVITY

Olivia Dorneanu[1], Iuliana Popovici[2], Lucian Boiculese[3], Irina Popovici[1], Daniela Bosnea[1]

1-Department of Microbiology, 2-Department of Pharmaceutical Technology, 3-Department of Medical Informatics and Biostatistics

University of Medicine and Pharmacy "Gr. T. Popa" Iaşi , Romania

Abstract. Superficial *Candida* infections are very common throughout the world. Clotrimazole may be administered topically in the treatment of superficial candidiasis. We have tested the anti-fungal activity of eight 1% clotrimazole pharmaceutics (solutions, suspensions, ointments), against 3 *Candida* strains (*C. albicans*, *C. tropicalis*, *Candida* spp.), using the diffusion method of susceptibility testing to anti-microbial drugs. In order to compare the anti-fungal activity of the 3 groups of pharmaceutics (solutions/ suspensions/ ointments) the ANOVA statistical test was performed. The activity of clotrimazole significantly decreased from solutions to suspensions and ointments. There were no significant differences in anti-*Candida* activity among the same type of pharmaceutical form; excepting the activity of ointments against *C. albicans*. Anti-fungal activity of clotrimazole is better expressed in solutions, comparing with suspensions and ointments.
Key words: Clotrimazole, Candida, Pharmaceutics

Rezumat. Infecţiile superficiale determinate de *Candida* sunt frecvente în toată lumea. Clotrimazolul poate fi administrat local în tratamentul candidozei superficiale. Am testat activitatea antifungică a opt preparate farmaceutice conţinând clotrimazol 1% (soluţii, suspensii, unguente) faţă de trei tulpini de *Candida* (*C. albicans, C. tropicalis, Candida* spp.) folosind metoda difuzimetrică de testare a sensibilităţii faţă de substanţe antimicrobiene. Pentru a compara activitatea antifungică a celor trei grupe de preparate farmaceutice (soluţii/ suspensii/ unguente) a fost aplicat testul statistic ANOVA. Activitatea clotrimazolului a scăzut semnificativ de la soluţii la suspensii şi, în continuare, la unguente. Nu au existat diferenţe statistic semnificative între produsele farmaceutice de acelaşi tip, în activitatea anti-*Candida*, cu excepţia activităţii unguentelor împotriva *C. albicans*. Activitatea antifungică a clotrimazolului este mai bine exprimată în soluţii comparativ cu suspensii şi unguente.
Cuvinte cheie: Clotrimazol, Candida, Preparate farmaceutice

INTRODUCTION

Superficial *Candida* infections may involve the skin, nails and mucous membranes of the mouth and vagina. *Candida albicans* accounts for 80-90% of cases, but other species, notably *C. tropicalis*, *C. lusitaniae*, *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* may occur (1). *C. albicans* is an ubiquitous, usually saprophytic, yeast that can become pathogenic if a favorable environment or the host's weakened defenses allow the organisms to proliferate.

Specifically, intertriginous and mucocutaneous areas where heat and maceration provide a fertile environment are the most susceptible sites. Systemic antibacterial, corticosteroid, and immunosuppressive therapy; pregnancy, obesity, diabetes mellitus and other endocrinopathies; debilitating diseases; blood dyscrasias; and immunologic defects increase susceptibility to candidiasis (2).

Clotrimazole (1-o-chloro-α,α-diphenylbenzyl) imidazole is a synthetic imidazole, having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast-like fungi. The mechanism may involve an action on the fungal cell membrane whereby the uptake of essential nutrients is inhibited. It may be administered topically in the treatment of superficial candidiasis.

The Romanian drug industry produces only two pharmaceutical forms containing 1% clotrimazole: a solution in solvent mixture, alcohol-polyetylene-glycol (III), and two ointments L/H emulsion type (VII, VIII).

Previous research targeted diversification of topical clotrimazole drug products. Studies have been performed on formulation, physico-chemical characterization and *in vitro* drug release from: buccal-adhesive gels with carbopol (3, 5), oro-pharyngeal tablets (4), dermal suspensions and emulsions (6).

We aimed to determine the anti-*Candida* activity of different pharmaceutical formula containing 1% clotrimazole (solutions, suspensions, ointments), using the diffusion method of antimicrobial drug susceptibility testing.

MATERIALS AND METHODS

Strains. We have tested 3 *Candida* strains, isolated from clinical specimens (table 1). Identification of the strains was based on: microscopy, growth characters, germinative tubes production; submerge growth in corn agar, assimilation of carbohydrates (5).

Table 1. Candida species and micro-organisms used for testing

MICRO-ORGANISM	DISEASE	CLINICAL SPECIMEN
Candida albicans	Oral candidiasis	Tongue scraping
Candida tropicalis	Bacteremia	Blood
Candida spp.	Tongue hairy leucoplakia	Tongue scraping

Susceptibility testing. A standardized inocullum was prepared from each *Candida* strain (turbidity 0.5 on McFarland scale). The susceptibility testing was performed on Sabouraud agar. Thoroughly mixed pharmaceutics (50µl) were disposed in different sterile metal cylinders, placed on the surface of the medium, at distances of 3 cm. The plates were incubated overnight at 37°C, then the diameters of the inhibition zones were measured.

In order to minimize the errors, each test has been performed 10 times.

*Pharmaceutics.*The tested pharmaceutical formulas, all of them containing 1% clotrimazole, are listed in table 2.

Statistics. The mean value of the measured diameters and their standard deviations (STD) have been determined and compared. In order to compare the anti-fungal activity of the 3 groups of pharmaceutics (solutions/ suspensions/ ointments) the ANOVA statistical test was performed. The p values have been computed for finding out if the differences between the compared diameters are statistically significant ($p < 0.05$). For validation of the results, the Bartlett (chi square) test for inequality of population variances has been used. If population variances differed, Mann-Whitney/ Wilcoxon two-sample test has been used. Values were significantly different if p value was less than 0.05.

Table 2. 1% clotrimazole formulas for topical application

INGREDIENTS (G)	SOLUTIONS			SUSPENSIONS			OINTMENTS	
	I	II	III*	IV	V	VI	VII**	VIII***
Clotrimazole	1	1	1	1	1	1	1	1
Alcohol	99		44.5	10	10			
Propylene glycol		99						
Polyethylene glycol			44.5					
Tween 80				1.5		1.5		
2% methyl cellulose solution					20	20		
Distilled water with parabens				87.5	69	77.5		
Excipients							99	99

* Industrial product, S.C. Biofarm S.A., Bucuresti;
** Industrial product, Antibiotice S.A., Iasi;
*** Industrial product, Mark International, Iasi.

RESULTS

We have studied the influence of pharmaceutical form on clotrimazole activity against 3 clinical *Candida* isolates.

Although the only standardized method for the susceptibility testing of antifungals is the broth dilution method (7), we have used a method derived from the agar diffusion method of susceptibility testing to antimicrobial drugs, in order to test the ability of clotrimazole to diffuse from different pharmaceutical forms.

There were no statistically significant differences between the weights of 50 µl of the 3 tested solutions, neither between the weights of 50 µl of the tested suspensions, nor of the ointments.

The mean values of the 10 testings on each of the 3 selected strains, accompanied by the standard deviations (STD) are presented in table 3.

When compared solutions (I, II, III), using the same statistical procedure, p value was smaller than 0.05, meaning there is no statistically significant difference among solutions. The same results were obtained for suspensions (IV, V, VI). The only difference in antifungal diffusion and activity of clotrimazole was found between ointments VII and VIII, against *C. albicans* strain.

Comparison of the mean values of the diameter of the inhibition zones yielded p values < 0.05, attesting for statistically significant differences for all 3 tested *Candida* strains (table 4). The only exception was found when compared suspensions and ointments activity against *C. tropicalis*, suggesting the same anti-fungal activity of both pharmaceutical forms.

Table 3. Results (mm) of the susceptibility testing of *Candida* species, using different pharmaceutics

		I	II	III	IV	V	VI	VII	VIII
C. albicans	Mean	28.53	30.38	28.3	21.30	18.38	20.46	14.38	19.71
	STD	4.27	2.98	2.05	4.42	3.99	3.84	4.73	6.87
C. tropicalis	Mean	22	23.69	23.0	13.30	10.00	12.69	10.76	11
	STD	2.16	2.56	1.68	1.88	1.15	2.01	1.48	1.41
Candida spp.	Mean	23	24.27	22.9	15.36	11.09	14.72	10.27	11.85
	STD	2.09	1.61	1.76	1.74	1.51	1.34	0.90	2.47

Table 4. The p values in statistical comparison of solutions (sol.), suspensions (susp.) and ointments (oint.)

	SOL. *vs* SUSP.	SUSP. *vs* OINT.	SOL. *vs* OINT.
C. albicans	0.0001	0.0061	0.0001
C. tropicalis	0.0001	0.0919	0.0001
Candida spp.	0.0001	0.0001	0.0001

DISCUSSION

Clotrimazole is used for the treatment of infections caused by various species of pathogenic dermatophytes and yeasts. Actually, clotrimazole is marketed in a variety of preparations: vaginal suppositories and cream, topical lotion and cream (dermal formulation), topical solution and lozenges, for oropharyngeal candidiasis. The ability of formulations to reach subcutaneous tissues is poor, so clotrimazole is not indicated in the treatment of subcutaneous mycosis. In Europe, the drug is also used systemically, but it has considerable

CNS toxicity. There is a little systemic absorption following topical application (6).

Figure 1 illustrates the graphical representation of the i*n vitro* activity, expressed as mean values of the inhibition zones of the 3 types of pharmaceutics tested in this work.

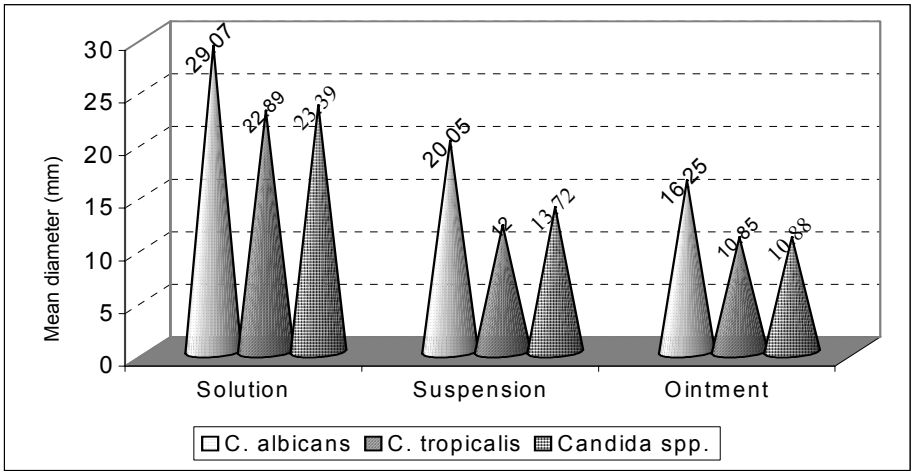

Fig. 1 Comparison of clotrimazole activity in solutions, suspensions, ointments

The explanation for the found differences between solutions, suspensions and ointments may be their different viscosity and the bigger particle diameters in suspensions, resulting in different diffusion times. The earliest and best anti-fungal activity is achieved when were used solutions while ointments have the advantage of longer contact times with the affected area.

Still, even if viscosity of solutions is slightly different (different viscosity of the vehicle), there are no differences in the clotrimazole activity among solutions. It is amazing the difference that appears between ointments VII and VIII in their activity against *C. albicans*.

CONCLUSIONS

1. Anti-fungal activity of clotrimazole is better expressed in solutions, comparing with suspensions and ointments.
2. Excepting the ointments effect on *Candida albicans*, no significant differences appeared in anti-*Candida* activity within specimens of tested solutions and suspensions.

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