Formulation influence on antifungal activity of propiconazole nitrate gels

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ABSTRACT

In this paper are shown the results of the antifungal activity evaluation of a new imidazole derivate – propiconazole nitrate (PN), both monosubstance and formulated as gels suspension type, using various ointment bases. The objectives of the study consist in: 1. the formulation and preparation of six gel formulations with PN 1.5%; 2. the determination of the minimum inhibitory concetration (MIC) of PN by broth dillution method; 3. the evaluation of the antifungal activity of PN formulated gels. The p values have been computed in order to find out if there are any statistically significant differences between the results of the tests ($p < 0.05$). PN shows good antifungal activity against clinical isolates of Candida spp., including some resistant strains to other antifungal agents. The values of MIC are in range of 0.125 – 32 µg/ml. The diameters of the inhibition areas of propiconazole nitrate gels have values between 20 – 34 mm, depending on the Candida strain. All tested PN gels have similar activity against Candida spp. There were no statistically significant differences between the antifungal activity of the six gel formulations.

Key words: propiconazole nitrate, gels, antifungal activity

Introduction

Oromucosal, urinary-genital tract and dermal infections are the most frequent fungal infections in humans. Their etiology has changed lately. More than 10 years ago they were mostly produced by *Candida albicans*, but nowadays more and more *Candida* non-*albicans* species are involved. Generally, for treating the infected area with an antifungal agent, the local skin or mucosa treatment is preferred, since it assures a high drug substance concentration onto and inside the epidermis.

The aim of this study consists in the evaluation of the antifungal activity of PN (fig. 1.), a new imidazole antifungal derivate, less studied in human applications versus veterinary fields (1, 2).

There were prepared six variants of type suspension ointments with 1.5 % PN. The researches regarding the physicochemical characterization of these formulation gels have been presented in some previous papers (3, 4).

In this study we first determined the PN - MIC and in the second part of the study we evaluated the

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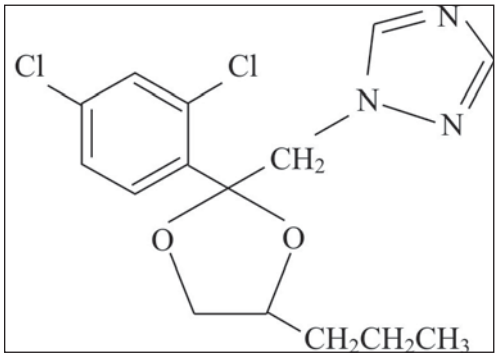

Figure 1. *Propiconazole nitrate*

1–[[2–(2,4–diclorfenil)–4–propil–1,3–dioxolan–2–il]–metil]–14–1,2,4–triazol

antifungal activity of 1.5 % PN formulated gels intended for topical or mucosal application.

Experimental methods

Materials

Hydroxypropyl cellulose H (HPC-H, Nisso, Japan, high dynamic viscosity 1.000–4.000 mPa·s), *polyethylenglycol* 300 and 4.000 (PEG 300, 4000, Labo-Chemie Wien, Fischamed, Osterreich), *glycerol* (Sigma Aldrich, Germania), *propylene glycol* (Merck, Schuchard, München, Germany), *propiconazole* (CHWAY Chemicals & Pharmaceuticals LTD., China) (it was transformed into nitrate in our laboratory), *triethanolamine* (Alpa Aesar GmbH&CoKG, Germania), *cetylic alcohol* (Sigma Aldrich, Germania), *lanoline* (Sigma Aldrich, Germania), *vaseline* (Sigma Aldrich, Germania). All used chemicals used respect the quality degree required by 10th Romanian Pharmacopoeia.

Methods

Formulas preparation

There were prepared six types of propylene glycol 1.5% gel, using three ointment bases in two variants: the first one including 10% glycerol as wetting and permeation enhancer agent and the second one including propylene glycol 10%, instead of glycerol:

- two types of gels based on HPC-H 4 % (formula 1 and 2);
- two types of gels with modified base of **polyethylene glycol** (formula 3 and 4);
- two types of gels with hydrated alcohol cetylic base (formula 5 and 6) (Table 1);

The formulas 1 and 2 were prepared using 4 grams HPC – H, wetted with 5 grams of glycerol (formula 1) and 5 grams of propylene glycol (formula 2); after mixing it we added, drop by drop, gently stirring, distilled water up to 90 grams. After 24 hours in the prepared gel, the mixture of 1.5 grams propiconazole nitrate dispersed in 5 grams of wetting agent and 1 gram of triethanolamine was included, stirring continuously in order to homogenize.

The formulas 3 and 4 were prepared with cetylic alcohol, PEG 4000 and PEG 300 which were fluidized on water bath. In these fluidized compositions were added 5 grams of wetting agent and 70°C heated distilled water. Finally, the propiconazole nitrate was dispersed in the ointment base as previously. The formulas 5 and 6 were prepared also on water bath, fluidizing cetylic alcohol, lanoline and vaseline. The resulted mixture was hydrated and homogenized with heated distilled water containing triethanolamine. Propiconazole nitrate was dispersed as we specified above. The gels were packed in brown bottles and stored in a cool place.

- **Detection of propiconazole nitrate (MIC).**

Tested strains: we have tested *Candida albicans* ATCC 10231 and 8 yeast strains isolated from human infections of genital or urinary tract (tabel 2). The susceptibility to antifungals of the tested

Table 1. *Gel formulations with propiconazole nitrate 1.5 %*

Substances	Formula (g)					
	1	2	3	4	5	6
Propiconazole nitrate	1.5	1.5	1.5	1.5	1.5	1.5
HPC-H (Nisso)	4	4	–	–	–	–
Glycerol	10	–	10	–	10	–
Propylene glycol	–	10	–	10	–	10
PEG 300	–	–	23.5	23.5	–	–
PEG 4000	–	–	40	40	–	–
Cetylic alcohol	–	–	5	5	2.5	2.5
Lanoline	–	–	–	–	6	6
Vaseline	–	–	–	–	50	50
Triethanolamine	1	1	1	1	1	1
Distilled water	..... to 100					

strains is shown in tabel 2. Overnight culture of each strain, on Sabouraud agar at 30°C has been used.

Tabel 2. *Tested yeast strains and clinical specimen of origin*

Strain	Origin
C. albicans 124	urine
C. albicans 14	vaginal exsudate
C. albicans 88	vaginal exsudate
C. krusei 106	vaginal exsudate
C. krusei 5	urine
C. kefyr 16	vaginal exsudate
C. glabrata 15	vaginal exsudate
C. tropicalis 155	urine

MIC method: we used the broth dillution method (5). We have prepared the inoculum for each tested strain, a suspension in sterile saline solution with turbidity equivalent to 0.5 McFarland, photometrically adjusted with a densitometer (*Densimat*, *bioMérieux*).

We prepared serial double dilutions of propiconazole nitrate (PN) in Sabouraud broth (64µg/ml to 0.125µg/ml) using the stock ethanol solution (1mg/ml), then we placed 50 µl inoculum over 1 ml of each PN concentration. All tubes were incubated for 24 hours at 30°C. Minimum inhibitory concetration (MIC) has been defined as the smallest PN concentration that inhibits any fungal growth. In order to minimize the testing errors, every test has been performed 3 times.

The quality control: 1) Tubes containing only 1 ml Sabouraud broth plus 50 µl inoculum have been used as fungal growth control. 2) In paralel, for each yeast, we have performed the same test using serial double dilutions of ethanol without PN in Sabouraud broth (64µl/ml to 0,125µl/ml).

• **Testing the antifungal activity of gels containing PN 1,5%**

We used Sabouraud agar plates uniformly inoculated with the 0.5 McFarland suspension of every tested yeast (tabel 2). On the surface of each inoculated plate we placed the rings of 1 µl disposable inoculating loops, charged at limit with the tested gel (excess removed by sliding the loop against the wall of the jar) and aseptically cut with the scissors. Every ring retained 3.5 mg gel, corresponding to 0.0525 µg PN. In order to minimize the testing errors, every test has been performed 3 times. After the 24 hours incubation at 30°C we have measured and compared the diameters of the inhibition areas (6).

• **Statistics**

The p values have been computed in order to find out if there were any statistically significant differences between the results of the tests ($p < 0.05$).

Results and discussion

• **Detection of propiconazole nitrate MIC**

All tested strains have shown good growth in Sabouraud broth without PN. For all tested strains, ethanol MIC was >64µl/ml, showing absence of antifungal activity of the diluent at tested concentrations. There were no statistically significant differences between the 3 tests on the each strain. We have noticed good antifungal activity of PN on the reference strain and on vaginal or urinary clinical isolates (tabel 3), both *C. albicans* and non-*albicans* strains, both susceptible or resistant strains to other antifungal agents. The susceptibility to various antifungal agents used in therapy and PN – MIC against tested Candida spp. is shown in table 3.

• **Testing of antifungal activity of gels containing PN 1.5%.**

There were no statistically significant differences between the 3 tests with PN gels on the each strain. There were no statistically significant differences between the antifungal activity of different gels, showing similar diffusion in Sabouraud agar of PN from all tested pharmaceutical formulas (table 4). Diameters of the inhibition areas of PN against clinical isolates and reference strain correlated well with MIC of PN to the same strains (tables 3 and 4).

Tabel 3. *Susceptibility to antifungal agents and MIC of PN against tested Candida spp.*

Strain	Fluconazole	Econazole	Ketoconazole	Miconazole	MIC PN (µg/ml)
C. albicans ATCC 10231	susceptible	susceptible	susceptible	susceptible	<0.125
C. albicans 124	susceptible	susceptible	susceptible	susceptible	<0.125
C. albicans 14	susceptible	susceptible	susceptible	susceptible	0.25
C. albicans 88	resistent	susceptible	susceptible	susceptible	16
C. krusei 106	resistent	susceptible	susceptible	susceptible	1
C. krusei 5	resistent	susceptible	susceptible	susceptible	16
C. kefyr 16	susceptible	susceptible	susceptible	susceptible	32
C. glabrata 15	susceptible	resistent	resistent	resistent	32
C. tropicalis 155	intermediate	susceptible	susceptible	susceptible	<0.125

Table 4. *Diameters of inhibition areas produced by PN gels (3.5 mg) against Candida spp.*

Strain	Diameters of inhibition areas (mm)					
	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5	Gel 6
C. albicans ATCC 10231	31	31	32	32	33	33
C. albicans 124	34	33	31	32	33	33
C. albicans 14	30	29	30	31	31	30
C. albicans 88	24	24	23	25	25	24
C. krusei 106	27	26	26	27	28	27
C. krusei 5	21	22	21	22	23	23
C. kefyr 16	20	20	20	21	21	20
C. glabrata 15	21	20	21	21	21	21
C. tropicalis 155	32	32	31	32	33	32

There is under study the evaluation of „in vitro“ disponibility performed by dissolution test of PN formulated gels in order to make the correlation disponibility-antifungal activity of these pharmaceutical preparation. The results of this study will be the aim of another paper.

Conclusions

There were formulated six PN 1.5% gel suspension types. There was evaluated the antifungal activity of PN, both monosubstance and gel formulations.

PN shows good antifungal activity against clinical isolates of *Candida* spp., including strains resistant to other antifungal agents. All tested PN gels have similar activity against *Candida* spp. The values of MIC are in range of 0.125–32 μg/ml. Diameters of the inhibition areas of propiconazole nitrate gels have values between 20–34 mm depending on the *Candida* strain. All tested PN gels have similar activity against *Candida* spp. There were no statistically significant differences between the antifungal activity of the six gel formulations.

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