Evaluation of antifungal activity of some propiconazole nitrate gels

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ABSTRACT SUMMARY

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 concentration (MIC) of PN by broth dilution 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.

INTRODUCTION

Oromucosal, urinary-genital and dermal infections are the most frequent fungal infections in humans. 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, a new imidazole antifungal derivate, less studied in human applications versus veterinary fields (1).

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 (2). In this study we first determined the PN - MIC and in the second part of the study we evaluated the antifungal activity of 1.5 % PN formulated gels intended for topical or mucosal application.

EXPERIMENTAL METHODS

Materials

Hydroxypropyl cellulose H (HPC-H, Nisso, Japan, viscosity 1.000–4.000 mPa·s), polyethylene glycol 300 and 4.000 (PEG 300, 4000, Labo-Chemie Wien), glycerol (Sigma Aldrich, Germania), propylene glycol (Merck, München, Germany), propiconazole (CHWAY Chemicals & Pharmaceuticals LTD., China) (it was transformed into nitrate in our laboratory), triethanolamine, cetyl alcohol, lanoline, 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 gels based on HPC-H 4% (formula 1 and 2);
- two gels with modified base of polyethylene glycol (formula 3 and 4);
- two gels with hydrated alcohol cetylic base (formula 5 and 6).

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: C. albicans 124, C. albicans 124, C. albicans 88, C. krusei 106, C. krusei 5, C. kefyr 16, C. glabrata 15, C. tropicalis 155. Overnight culture of each strain, on Sabouraud agar at 30°C has been used.

MIC method: we used the broth dilution method. 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 concentration (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.
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.

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 (table 1), both C. albicans and non-
albicans strains, both susceptible or resistant strains to
other antifungal agents.

Table 1. Susceptibility to antifungal agents and MIC of
PN against tested Candida spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>F</th>
<th>E</th>
<th>K</th>
<th>M</th>
<th>MIC PN (µg/ml)</th>
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<tr>
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<td>&lt;0.125</td>
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<td>&lt;0.125</td>
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<td>++</td>
<td>++</td>
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<tr>
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<tr>
<td>C. tropicalis</td>
<td>+</td>
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<td>++</td>
<td>++</td>
<td>&lt;0.125</td>
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</table>

F=fluconazole, E=econazole, K=ketoconazole, M=miconazole
++ susceptible, + intermediate, - resistant

The susceptibility to various antifungal agents used in
therapy and PN – MIC against tested Candida spp. is
shown in table 1.

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 2). Diameters of the
inhibition areas of PN against clinical isolates and
reference strain correlated well with MIC of PN to the
same strains (tables 1 and 2).

Table 2. Diameters of inhibition areas produced by PN
gels (3.5 mg) against Candida spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gel 1</th>
<th>Gel 2</th>
<th>Gel 3</th>
<th>Gel 4</th>
<th>Gel 5</th>
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</table>

CONCLUSION

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
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statistically significant differences between the antifungal
activity of the six gel formulations.

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