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Ultra-short topical treatment of pityriasis versicolor with 2·5% bifonazole cream

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Summary

The therapeutic efficacy of a preparation containing 2·5% bifonazole was investigated by comparing three different treatment modalities—A, B, and C. Group A used bifonazole only on Day 1, Group B applied the cream on Days 1, 2 and 3, and the Group C on Days 1, 3 and 5. Of the patients in Group A 56% had a negative mycological examination at the end of the study. The results obtained in Groups B and C were not significantly different: 92% of the patients had a negative mycological examination at the end of the study. Electron microscope (EM) studies showed morphological alterations such as loss of cytoplasmic organization with shrinkage and folding of the cell membranes after 1 week of treatment only in Groups B and C. We conclude that 2·5% bifonazole is a highly effective treatment for *Pityrosporum ovale* infection when applied using a 3-day schedule.

Pityriasis versicolor (PVC) is a mild chronic infection of the skin caused by *Pityrosporum* yeasts. The infection is worldwide and it is believed that it affects up to 50% of the population in some regions of Central and South America.[1] Current theories suggest that this organism can live saprophytically in the scalp, the ear canal and elsewhere on the skin and under certain circumstances, such as immunosuppression it may become pathogenic.[2–4]

The lesions of PVC exhibit a broad range of clinical manifestations. The patients affected with PVC may present with follicular, perifollicular, macular, or papular lesions. In the typical form of PVC, most of the lesions are distributed over the sternal area or the centre of the back. PVC lesions can also occasionally involve the lower abdomen and proximal portions of the limbs. Some unusual localizations such as the face, neck, palms, inguinal folds, and genitalia have also been described.[5–7] PVC can be aggravated under various circumstances, from increased atmospheric humidity[8] to conditions such as malnutrition or systemic disease affecting the general health of the patient.[9]

We have investigated a new treatment for PVC based on a daily application of 2·5% bifonazole cream for 1 or 3 days. We followed up the treatment by examining the viability and ultrastructural changes in the organisms, isolated from the patients, as well as the clinical responses.

Methods

The efficacy of a 2·5% bifonazole cream for the treatment of PVC was tested in an open comparative study.

Patients

Patients with the clinical diagnosis of PVC were referred from the Dermatology out-patient clinic of the Italian Hospital of Buenos Aires. The selection criteria for inclusion in the study protocol were: (i) clinical diagnosis of PVC, (ii) positive microscopy in scrapings, and (iii) no systemic or topical therapy within the last month. Before entering the study, patients gave informed consent.

At the first visit, the evaluation consisted of clinical and Wood's light examinations. At the same time scrapings for KOH examination, culture and electron microscopy (EM) were obtained. Scanning and transmission EM studies were performed on only two patients from each group.

Treatment schedules

The patients included in the study were randomly allocated to one of three treatment regimens. The schedules were as follows:

Group A: one application on Day 1;
Group B: one application on Days 1, 2 and 3;
Group C: one application on Days 1, 3 and 5.

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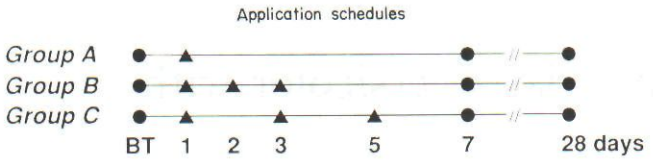

Group A: only one application on day 1 (in total 1).
Group B: one application on 3 consecutive days (in total 3).
Group C: one application every second day (in total 3).
●: *Wood's light, microscopy and clinical examination.*
▲: *application day.*

Figure 1. Treatment schedules of *P. versicolor* with 2·5% bifonazole cream: groups, clinical and mycological evaluation. BT: before therapy.

The patients were asked to apply the cream once at bedtime for the assigned time according to the scheme of treatment (Fig. 1).

Evaluation

The results were assessed according to the following criteria: (1) remission: clinical improvement and negative mycology, and (ii) no remission: no clinical or mycological changes.

Statistical analysis

The purpose of the study was to evaluate differences in remission/failure among the three treatment schedules. Results were compared with a Chi-squared test.

Culture of Pityrosporum

Cultures were performed at 28°C on a medium containing 10% yeast extract, 10% olive oil, and 2% agar, according to the method of Caprilli *et al.*[10] *Pityrosporum ovale* was identified by light microscopic morphology.

Electron microscopy

Scanning and transmission EM of the fungus in skin scrapings were performed using a modification of the method described by Dorn *et al.*[11–13]

The samples were fixed in 2·5% glutaraldehyde in 0·1 M phosphate buffer, pH 7·4, and post-fixed in 1·5% osmium tetroxide in phosphate buffer at 4°C for 2 h. A portion of the material was dehydrated with acetone and coated with gold palladium for scanning EM. The specimens were examined with a JEOL ISM 35 scanning EM. The rest of the samples were saturated overnight in a block with 2% uranyl acetate. After alcohol dehydration, the material was clarified with propylene oxide and embedded in Epon 812. The block was sectioned at 23 nm with a Porter Blum MT-2B ultra-microtome and stained with lead citrate. The specimens for transmission EM were examined with a JEOL JEM 100c. electron microscope.

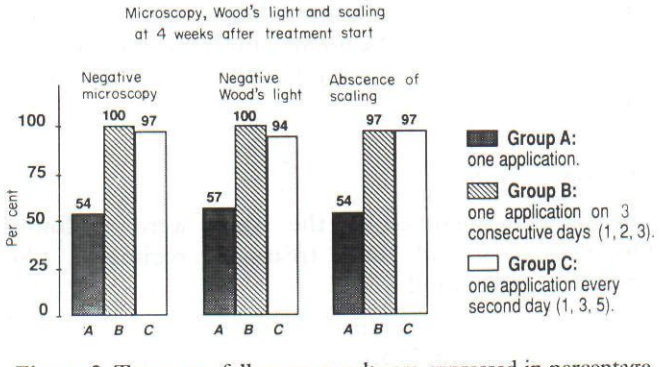

Figure 2. Treatment follow up: results are expressed in percentage negative findings for each group studied.

Post-treatment follow up

Follow up examinations were made every month for a year. Clinical and Wood's light examinations as well as direct microscopy and culture were performed at every visit (Fig. 1).

Results

Group A

Thirty-four patients completed this schedule (one single application of 2·5% bifonazole on Day 1). The mean age of this group was 30·85 ± 11·72 years; male/female ratio was 1·61. One week after treatment all patients had positive mycological examination; by the second week, 19 of 34 patients were positive (56%), and 18 of the patients (53%) were positive on the third week after treatment. One month after treatment, 13 of 28 (46%) patients were still found to have fungi by direct examination (negative = 54%) (Fig. 2).

Group B

(One application on Days 1, 2, and 3.) The mean age of this group was 31·75 ± 10·35 years, and the male/female ratio was 1·57. One week after treatment all the patients still had positive microscopy; by the second and third week, 9 of 36 (25%) patients and 3 (8%) patients, respectively, of the mycological examinations showed fungi present in the lesions. None of the patients had demonstrable fungi in the scales 1 month after treatment (Fig. 2).

Group C

(One application on Days 1, 3, and 5.) The mean age of the groups was 33·27 ± 9·15 years. The male/female ratio

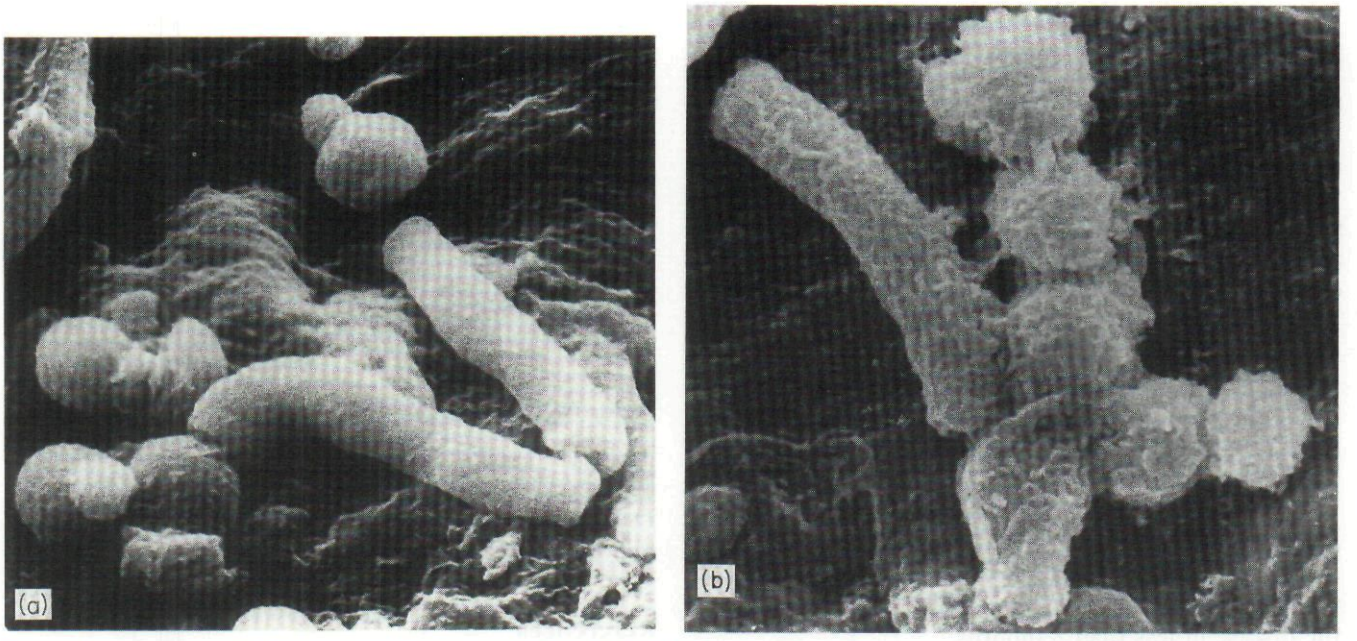

Figure 3. (a) Scanning EM of *P. ovale* pretreatment control (× 1752). (b) Scanning EM of *P. ovale*: post-treatment, Day 14 (× 2184). The fungal cell surface appears wrinkled through loss of cytoplasmic contents.

was 1·3. Fungi were present in all patients 1 week after treatment. At the second week, 10 of 35 patients (28%) had a positive direct examination. On the third and fourth weeks after treatment, only 1 of the 35 patients was still positive (negative direct mycological examination = 98%) (Fig. 2).

Statistical evaluation

There were no significant differences when Groups B and C were compared. A statistically significant difference ($P < 0{\cdot}001$) was observed when Group A was compared with Groups B and C.

Scanning electron microscopy

At the end of treatment, yeast cells appeared to be smaller and more irregular. Their surface was irregular and had a wrinkled and cerebriform appearance, probably due to the loss of intracellular contents (Fig. 3).

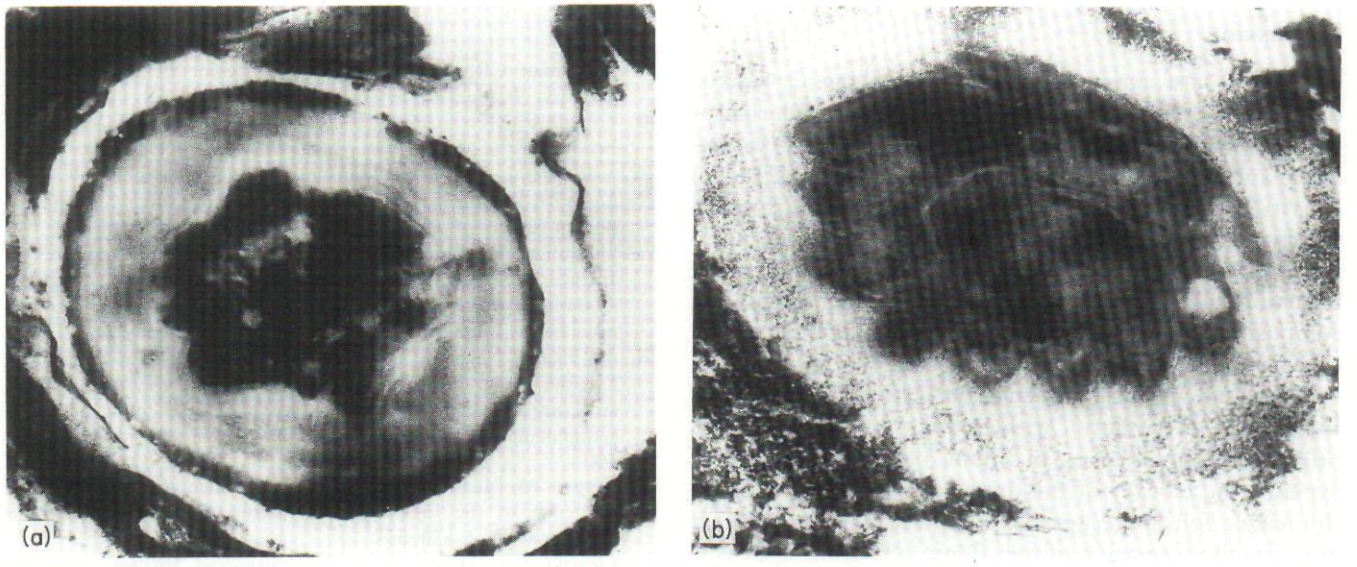

Figure 4. (a) Transmission EM of *P. ovale*: pretreatment control, uranyl acetate, lead citrate (× 27200). (b) Transmission EM of *P. ovale*: post-treatment, Day 7, uranyl acetate, lead citrate. The cytoplasm appears vacuolated with numerous osmophilic granules (× 34000).

Transmission electron microscopy

The yeast forms showed loss of definition of the cell membrane and disorganization of the cytoplasmic contents 7 days after the end of treatment. Fourteen days after therapy, the filamentous forms were vacuolated and vesicular and had thickened cell membranes. At this point some cells appeared necrotic with retracted cytoplasm and a jagged appearance which corresponded to the cerebriform features seen with scanning EM (Fig. 4).

The direct examination of samples from infected skin sites treated with KOH does not indicate the viability of the organisms, and usually the microscopic disappearance of the fungus is a late event, which is the reason why it is necessary to extend the follow-up period for at least 4 weeks after treatment.

We attempted to follow up all patients for a year to observe the recurrences. Most of the patients in Groups B and C were lost to follow up; but in Group A, all the patients reported to be in remission had a recurrence of the disease in less than 3 months.

In summary, a 2·5% bifonazole cream appears to be highly effective against *P. ovale* infection when used on a 3-day schedule. The main advantage of the local treatment is that it can be used when oral antifungals are contra-indicated.

Even though bifonazole has been used in short treatments there has been no comparison between different concentrations of the drug.[19] We have already reported a 14-day treatment study with 1% bifonazole cream.[20] Now in the light of the present observation of the viability of the fungus by EM studies, it is possible that 1% bifonazole cream may be effective over shorter periods.

Discussion

Imidazoles are active against most pathogenic yeasts, dimorphic fungi, filamentous fungi such as dermatophytes, and some protozoa such as *Leishmania, Plasmodium,* and *Trypanosoma.*[14,15] They exert their antifungal activity by inhibiting the synthesis of ergosterol through selective interaction with cytochrome P_{450}-dependent 14-α-demethylase[16] in the cell membrane, resulting in the formation of pores. This alteration in the membrane composition causes cell death due to leakage of the cytoplasmic contents.[4]

Bifonazole is an imidazole derivative produced by Bayer AG (Germany) with a high affinity for skin proteins.[17] This binding of bifonazole to the epidermis explains the efficacy of the drug when used once a day. Bifonazole inhibits ergosterol synthesis at two levels: it prevents the formation of mevalonic acid from hydroxymethyl-glutaryl CoA and transforms 2,2,4-methylene-dihydrolanosterol into deoxymethylesterol.[18]

In our series, there was an excellent and equal remission rate for PVC in Groups B and C (3 days of therapy) in whom 97% of the patients showed clinical improvement and negative mycology. The treatment schedule used in Group A (1 day of treatment) was not completely effective, as complete remission was observed in 64% of the patients who finished the study.

The therapeutic response was compared with the ultrastructural changes within the fungus. Major damage to the organisms was only observed in Groups B and C. In Group A both intact and damaged cells were seen.

Acknowledgments

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