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Ciclopirox Nail Lacquer 8%: In vivo Penetration into and through Nails and in vitro Effect on Pig Skin

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Abstract. This report presents original methods to assess the bioavailability of an antifungal drug from a varnish preparation in finger nails. For the studies with human volunteers a ciclopirox 8% nail lacquer was used to determine its efficacy in the treatment of onychomycoses. In vivo studies were performed on the fingernails of healthy volunteers by determining the total amount of ciclopirox penetrated per milligram of nail and the partition of the drug in the plate of the nails (technically divided into four layers). Ciclopirox concentrations were evaluated by measuring the inhibition of *Candida pseudotropicalis* growth in vitro. The ciclopirox concentration after 30 days treatment was determined as 3.35 ± 0.82 µg/mg nail material. This is a sufficient amount to kill the fungal pathogens. In addition, in vitro penetration experiments were carried out with excised pig skin. Lacquer formulations from 0.5 to 8% were used to inhibit the growth of *Trichophyton mentagrophytes*. Formulations from 2 to 8% led to a strong to total inhibition of the dermatophyte after 30 min treatment time.

Introduction

Fungi involved in onychomycosis can grow within different nail locations in the nail plate and/or the nail bed [1]. This phenomenon raises the interest of determining the penetration of antifungal drugs into and through the nail. On the other hand, the ultrastructure of the nail can limit this parameter [2]. The keratinocytes within the nail plate are strongly linked by numerous desmosomes [3] and surrounded by phospholipid layers [3]. The nailplate is built up by three different layers which could have different penetration characteristics, but this subject is discussed controversally in the literature. The penetration characteristics in diseased nails are poorly investigated but it is rather likely that the more dehydrated diseased nail has a reduced penetration for hydrophilic compounds. This report presents methods to test the nail penetration of an

antifungal drug by measuring its biological activity instead of using radiolabeled molecules [4]. Additionally, the evaluation of large numbers of lacquer formulations causes problems due to the limited availability of healthy human nails. Therefore we applied an additional method using excised pig skin [5], which is a well-established method for the evaluation of penetration. Hänel and Ritter [5] showed that even lacquer formulations can be evaluated on the pig skin by applying the samples and subsequent strip of the stratum corneum before inoculation with dermatophytes.

Materials and Methods

Chemicals

6-Cyclohexyl-1-hydroxy-4-methyl-2-(1H)-pyridone (ciclopirox free acid) and ciclopirox varnish 8% (Centre de Recherche Pierre-Fabre Médicament/Hoechst AG, FRG; patent pending).

In vitro Pig Skin Experiments

For the investigation the back skin of pigs was used as described in detail elsewhere [5]. The skin was shaved and kept on water agar. Lacquer formulations (5–10 mg) containing 0.5, 1, 2, 3, 4, and 8% ciclopirox were applied with a nail varnish brush as was the placebo lacquer. Treatment time was 30 min throughout the different concentrations. In previous experiments (unpublished data) it had been found that treatment time longer than 1 h leads to a complete saturation of the skin pieces with ciclopirox and therefore complete inhibition of fungal growth irrespective of the concentrations used. After the treatment the lacquer was removed with a pair of forceps. Subsequent adhesive tape strips exposed different depth of the stratum corneum of the pig skin.

Inoculation was carried out as described [5] with microconidia of *Trichophyton mentagrophytes*. During 7 days of observation the fungal growth was recorded daily by measuring the size of the mycelium in comparison to the placebo-treated group [for more details see ref. 5].

In vivo Experiments

The study was performed on the fingernails of 9 healthy volunteers. The amount of varnish (8% ciclopirox and placebo) applied daily was about 5–15 mg/nail. Before each application and before sampling, the residual film of lacquer was gently removed with methanol-wetted cotton wool.

Sampling. Four free distal parts of nails were cut after 7, 14, 30 (9 subjects) and 45 (4 subjects) days of application, and also 7 and 14 days after the end of the treatment (4 subjects). The samples of each subject were used for two different experiments. (1) For the determination of the total amount of ciclopirox per milligram of nail (two samples) direct extraction of ciclopirox and microbiological determination were performed. (2) In order to evaluate the distribution of ciclopirox in the nail (two samples) the distal parts of the nails were pressed flat. Then the flattened samples were cut by means of a freezing microtome to obtain four layers in depth of equal thickness. Extraction was carried out with each layer separately. Repartition of ciclopirox in the four layers was determined by the microbiological method described.

Extraction. Extraction was performed in a 10% polyethylene glycol 4,000 (PEG) aqueous solution with a contact time of 48 h. For the determination of the total amount of ciclopirox per milligram of nail, the samples were weighed and extraction was carried out with 50 µl PEG solution per milligram of nail. To assess the distribution of ciclopirox in the nail depth, each layer was extracted with 100 µl PEG solution. Extraction yield was determined under ex vivo experimental conditions. A quantity Q of ciclopirox 8% nail lacquer was applied, equivalent to a total amount of $Qc = 0.08 \times Q$ of ciclopirox. Then the pieces of nail were put into a humid chamber during 7 days to obtain a passive diffusion of the drug. After elimination of the residual film of lacquer, ciclopirox was extracted with 50 µl PEG solution per milligram of nail. The extract for the microbiological determination was defined as Qe (extractible amount of ciclopirox). Qe was identical irrespective of the elimination of the residual film, involving a total passive penetration of ciclopirox in the nail. In all the cases, extraction yield (Qe/Qc) was estimated at 0.95.

Microbiological Determination. Ciclopirox concentrations in the nail material were evaluated after filtration of extracts at room temperature (0.2-µm pore size glass fiber) by measuring the kinetics of inhibition of *Candida pseudotropicalis* growth. Determi-

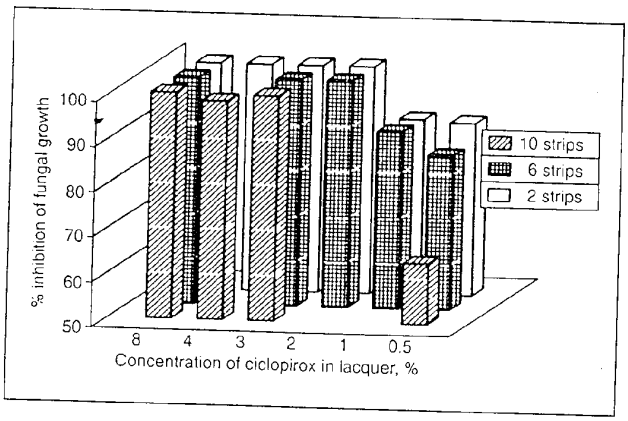

Fig. 1. Inhibition of fungal growth of *T. mentagrophytes* on excised pig skin after 30-min treatment with lacquer formulations containing different amounts of ciclopirox.

nations were performed on microtiter plates in comparison with a ciclopirox solution to evaluate the determination. Dilutions of samples and ciclopirox solution were carried out with a PEG solution (100 µl). After addition of the same volume of Neopeptone glucose broth (DIFCO, Mich.) to each well, microtiter plates were inoculated with suspensions of *C. pseudotropicalis* (10^4 yeasts/ml final concentration). Incubation was carried out at 28.5 °C and optical density (650 nm) was read at 18 h. At concentrations close to the MIC the tests were run for 24 or 48 h to lower the detection limit. The limit of ciclopirox detection had been estimated to be 0.04 µg/mg of nail (unpublished data) due to the sensitivity of *C. pseudotropicalis* and the extraction yield. To assess the distribution of ciclopirox in the nail matrix, the relative percentage in the four layers was determined by the same microbiological method. The limit of solubility of ciclopirox in the lacquer is around 12%.

Results

In vitro Experiments

In the test using pig skin six concentrations of ciclopirox in the lacquer formulation were compared (fig. 1): 0.5% led to a low inhibition of fungal growth close to the stratum lucidum (10 strips) : 63.4%, whereas in the upper layers 84.1% (6 strips) and 88.11% (2 strips) inhibition of fungal growth was recorded. 1% lacquer led to comparative results. The 2% formulation was only tested down to the 6-strip layer and showed 100% inhibition at this depth. 3% lacquer was also tested at the stratum lucidum (10 strips) and led to 99.72% inhibition just like the 4% lacquer. The 8% lacquer however resulted in total inhibition (100%) in all layers investigated.

In vivo Experiments

Kinetics of Ciclopirox Penetration. After correction by the extraction yield, individual ciclopirox concentrations and means and standard errors were calculated and are given in micrograms per milligram of nail in table 1. Figure 2 shows the increase of the ciclopirox concentration in the nail during the first 30 days and the persistence of a steady state between the 30th and the 45th day of the daily application. The repartition of ciclopirox in the nail plate is indicated in table 2 by relative percentage values observed in the four layers. After 7 days of daily application, the greater amount of ci-

Table 1. Concentrations of ciclopirox in nails (μg/mg nail)

Subject	Duration of treatment			
	7 days	14 days	30 days	45 days
1	0.1	0.3	0.95	ND
2	1.1	1.5	1.7	1.55
3	2.6	3.3	3.2	3.3
4	1.3	3.8	6	ND
5	0.18	0.5	1.3	ND
6	0.6	1.6	2.3	1.94
7	0.45	0.95	1.2	1.26
8	0.7	1.3	6.5	ND
9	1	2.8	7	ND
Mean	0.892	1.783	3.35	2.01
SE	0.252	0.413	0.822	0.45

ND = Not determined; SE = standard error.

clopirox was obtained in the first layer (51.5%) and a progressive decrease occurred in the deeper layers. Ciclopirox levels were not detectable in layer 3 for 2 subjects and in layer 4 for 3 subjects. In all cases, penetration through the nail down to the fourth layer was relatively low. A more homogeneous partition of ciclopirox appeared after 14 days of application, always with a higher level in the first layer (32%). At this time, penetration occurred down to the fourth layer (23%). The same phenomenon could be observed after 30 days of application.

Ciclopirox Concentration after Treatment. These studies were performed on 4 healthy volunteers, 7 and 14 days after the end of the treatment. Results are presented in table 3 and indicate a progressive decrease of the active drug concentrations, which fell below the limit of detection 14 days after the end of ciclopirox treatment.

Table 2. Relative percentages of ciclopirox in the four nail layers (1–4)

Subject	7-day sample				14-day sample				30-day sample			
	1	2	3	4	1	2	3	4	1	2	3	4
1	32	28	29	11	34	25	21	20	33	45	12	10
2	52	41	7	–	30	22	22	26	40	21	19	20
3	60	26	9	5	30	20	26	24	38	20	22	20
4	45	34	17	4	32	28	22	18	41	26	19	14
5	56	44	–	–	36	29	18	17	36	24	16	24
6	41	33	18	8	34	28	25	13	38	20	21	21
7	48	43	9	–	33	15	15	37	28	25	23	24
8	47	36	11	6	23	28	22	27	34	25	20	21
9	55	21	16	8	40	22	18	20	28	26	26	20
10	60	40	–	–	28	24	23	25	31	32	19	18
Mean	49.6	34.6	11.6	4.2	32	24.1	21.2	22.7	34.7	26.4	19.7	19.2
SE	2.8	2.4	2.8	1.3	1.5	1.4	1.1	2.1	1.5	2.4	1.2	1.4

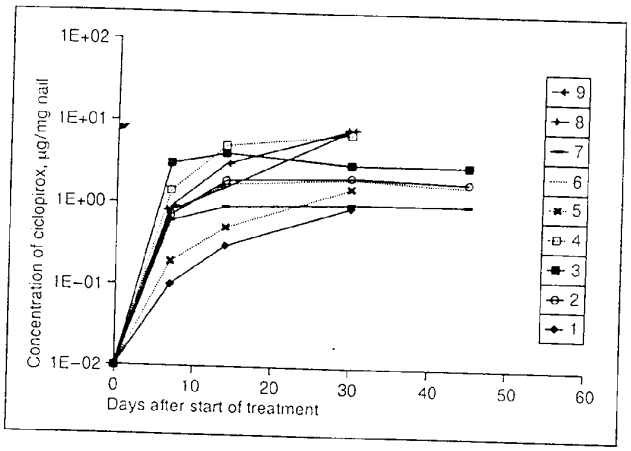

Fig. 2. Ungual penetration of ciclopirox from an 8% lacquer formulation in the fingernails of 9 subjects. Treatment from day 0 to day 30 or to day 45.

Discussion

Numerous attempts have been made to treat onychomycoses by topical antifungals. Among these were clotrimazole [6], tolnaftate [7], miconazole [8], and recently bifonazole [9]. Ciclopirox has proved to be effective since 1979 [10]; also cow horn penetration experiments with ciclopiroxolamine cream were performed. The systemic therapy of onychomycoses with azole-compounds like ketoconazole has to be considered carefully prior to treatment due to the possibility of serious side effects [11]. From its composition the nail plate is more like hair than like stratum corneum [12]; therefore the penetration characteristics are remarkably different from the skin. From the in vivo results (fig. 3) it is obvious that under treatment a steady state is reached which enables the inhibition and killing of fungi like *Trichophyton* spp. Also the MICs for fungi like *Hendersonula* spp. and other causative agents of onychomycoses are more or less within the range of concentrations calculated.

Table 3. Residual amounts of ciclopirox (µg/mg nail) at the end of treatment (D45) and 7 and 14 days later

Subject	D45	7 days	14 days
2	1.55	0.05	0.04
3	3.3	0.32	0.04
6	1.94	0.13	0.04
7	1.26	0.04	0.04

Among the group of nails investigated there were some (e.g. subjects 1, 5 and 7) which did not accumulate high concentrations of the compound. The variation of the nail structure in the individual onychomycosis patient is also of clinical importance. In all clinical trials a considerable part of 'non-responders' cannot be explained by a lack of compliance since it is often due to hydration problems and other morphological differences in the nails.

Nevertheless, ciclopiroxolamine proved to be effective even as a 1% cream-solution

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