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COMMUNICATION

The Effect of Keratolytic Agents on the Permeability of Three Imidazole Antimycotic Drugs Through the Human Nail

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ABSTRACT

The permeability of three imidazole antimycotics (miconazole nitrate, ketoconazole, and itraconazole) through the free edge of healthy human nail was evaluated in vitro using side-by-side diffusion cells. The influence of keratolytic substances (papain, urea, and salicylic acid) on the permeability of the antimycotics was also studied. The results suggested that the nail constituted an impermeable barrier for these antimycotics; it could be considered that the nail behaved as a hydrophilic gel membrane, through which drugs of low solubility could not permeate. The use of ethanol did not promote the passage of any of the antimycotic drugs. Although scanning electron microscopy indicated that the keratolytic substances had a significant effect on the nail surface (papain > salicylic acid > urea), the passage of the three antimycotics was not improved by pretreatment with salicylic acid alone (20% for 10 days), or by the application of the drug in a 40% urea solution. It was found that only the combined effects of papain (15% for 1 day) and salicylic acid (20% for 10 days) were capable of enhancing the permeability of the antimycotic

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therapeutics. It is conceivable that the enhancement effect can be attributed to the formation of pores which create transport channels, through which the antimycotics are able to permeate.

INTRODUCTION

The nail[1] is the hard durable epidermal appendage that protects the end of the digit. It is composed of multiple layers of horny cells joined in a tightly cemented continuous sheet. The nails cells are dead corneocytes without nuclei or organelles and filled with α-keratin, which constitutes almost the entire dry weight of the nail (the water content of the nail varies between 7 and 16%).

The α-keratin structure is underpinned by covalent crosslinkages (mainly disulfide bonds) between polypeptide chains within the multihelical "ropes" and between adjacent partners (1–3). It is this biochemical construction which defines the physical properties of the nail, e.g., the strength and its behavior as a permeability barrier for many substances including beneficial therapeutic agents.

This impermeability of the nail constitutes a formidable obstacle for the treatment, principally, of fungal infections (onychomycoses) which are the most frequent cause of nail diseases and represent 30% of all mycotic infections of the skin (4,5). In terms of therapeutic practice, it is generally accepted that substances applied locally have little if any impact on arresting the spread of the infection. The pathogen will, as a rule, penetrate into the nail to such a depth within the keratin that no conventional drug will be able to achieve a corresponding in-depth fungicidal action capable of destroying the pathogens (6).

A number of treatments such as a solution of miconazole (2% in alcohol) (6), tioconazole (28% with undecylenic acid), amonolfine (5% nail lacquer) (7), and "chemical removal" of the infected area with 40% urea paste (8) were reported to be effective in some cases. The efficiency of these therapeutic interventions depends on the degree of fungal localization, that is, the number of keratin layers that shelter the fungi from the antimycotic drug.

Moreover, frequently the nail area lying under the dorsal groove of the nail bed is also infected, and it is in these cases, in particular, that treatment based exclusively on topical application of the therapeutic remains unsatisfactory (6). On the other hand, oral antifungicidal therapy should be avoided because of the possibility of recurrence and risk of provoking adverse side effects. Surgical avulsion of the nail is not curative and has resulted in disability, recurrence, or loss of the nail (9). It is therefore clear that the development of an effective topical treatment is needed. However, to achieve this, the keratin barrier has to be breached so that antifungal agents are able to reach the pathogens.

In this work we have studied the in vitro permeability of the nail to three imidazole-based antimycotic drugs (miconazole nitrate, ketoconazole, and itraconazole) and the effect of keratolytic agents (papain, urea, and salicylic acid) as permeability promoters, as well as the concomitant effects on nail structure.

[1]In this paper the term nail refers to the nail plate.

MATERIALS AND METHODS

Materials

Miconazole nitrate, ketoconazole, and itraconazole were provided by Janssen Farmacéutica (Puebla, Mexico). Salicylic acid, urea, papain, and bromocresol green were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were reagent grade and were obtained from J. Baker S.A. de C.V. (D.F., Mexico). Sections of healthy human fingernails (the free edge), with an average thickness of 0.027 cm ± 10%, as measured using a gauge scale (Metromex, Mexico), were washed with distilled water and used immediately after being obtained from the donor. The volunteers were told to avoid contact with solvents or detergent solutions and to refrain from using nail varnish or varnish remover for a period of 1 week before the study.

Analysis of Antimycotic Concentration

An assay using bromocresol green was used to quantify the concentration of the antimycotic drugs. Repeated extraction (n = 3) with chloroform at pH 3.0 (using a citrate-HCl buffer system) resulted in a reaction between the imidazole moiety and bromocresol green causing a yellow complex to be formed. The concentration of the complex product was followed by spectrophotometric analyses of the chloroformic extracts, at λ = 420 nm for

miconazole nitrate and ketoconazole and at $\lambda = 422$ nm for itraconazole. This method has been validated for the three antimycotic drugs used in this study (10).

Experimental Protocol

Effect of Keratolytic Agents on Permeation

These studies were carried out using a modified version of the horizontal cell design (11). Circular sections of the nail plate were filed slightly on their external surface (until the shiny appearance had been removed) before being securely attached to the cell by two silicon O-rings. The hermeticity of the system was continuously monitored. The donor and receptor solutions (30 ml ethanol:water, 60:40) were continuously stirred at 50 rpm using a glass paddle propeller and maintained at 37 ± 0.5°C by using a sand bath. The experiments were conducted with attention being paid at all times to the solubility and stability of the antimycotic agents in the presence of the keratolytic substances. In some cases, the nail was pretreated with 3 ml of papain (15% w/v) in phosphate buffer (pH 6.7) for 1 day or as the first step in a pretreatment procedure where the second phase consisted of treatment with salicylic acid (20% w/v) for 10 days. These experiments were conducted in order to verify previously obtained results (12). Samples (1 ml) of the solution were periodically withdrawn from the receptor compartment (care was taken to restore the volume to 30 ml) and analyzed to detect the presence of any antimycotic using the assay described above. All experiments were carried out at least in duplicate.

Effect of Keratolytic Agents on Nail Plate Surface Morphology

Scanning electron microscopy was used to examine the changes in nail plate ultrastructure following exposure to the keratolyte. The external surfaces of nail plate sections (filed and unfiled) were subjected to keratolysis by urea (40%) and salicylic acid (20%) in ethanol:water (60:40), and by papain (15%) in phosphate buffer at pH 6.2 for 4 days at 37°C. Following this treatment period, the nails were rinsed with distilled water, dried under vacuum at ambient temperature, and subsequently analyzed with a scanning electron microscope (JSM 2S SII, Jeol Instruments, Japan).

RESULTS AND DISCUSSION

The results showed that no permeation through the nail was detected for the three antimycotics, over a period of 60 days, in the experiments performed in the absence of a keratolytic agent. Walters et al. (13) and recently Mertin (14) suggested that the human nail behaves like a hydrogel of high ionic strength, the permeability of which depends on the solubility of the drug in water or in the swollen keratin matrix. Considering that the imidazole derivatives used in this study are only slightly or practically insoluble in water, it is easy to understand their low capacity to pass through a barrier with essentially hydrophilic characteristics. These results agree with the clinical ineffectiveness reported for topical formulations containing imidazole derivatives (9). It should also be noted that the presence of ethanol (used as cosolvent) did not promote the passage of the antimycotics. Although ethanol has been reported as a skin permeation enhancer (15), it does not appear to have the same effect on the nail. Walters et al. (16) observed that solvents which tend to promote the diffusion through the stratum corneum have little promise as accelerants of the nail permeability. Most of these enhancers, such as ethanol, have an effect on the intercellular stratum corneum lipids; however, the lipid content of the nail is only 0.15–0.76% of the total weight of the nail (17). Furthermore, the corneocytes are joined in a tightly cemented continuous sheet, with overlap of their borders (1), which constitutes a barrier that is insensitive to the effect of ethanol.

The addition of urea (40%) to the donor solution and the pretreatment with salicylic acid (20%) for 10 days, did not induce the transport of any of the antimycotic drugs. Thus, although both substances have been reported as good keratolytic agents for the detachment of scales, softening of cornified material (18), and chemi-

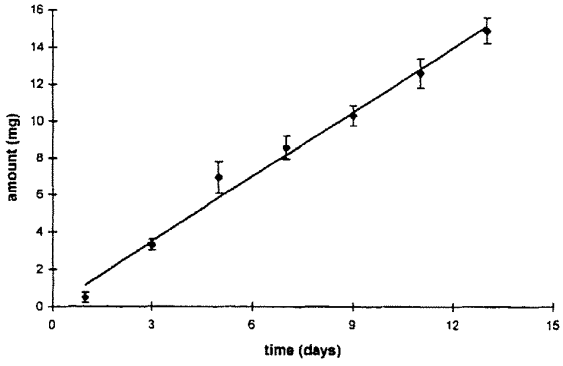

Figure 1. Permeation profile for miconazole nitrate through nail pretreated with papain 15%/1 day and salicylic acid 20%/10 days ($n = 3$).

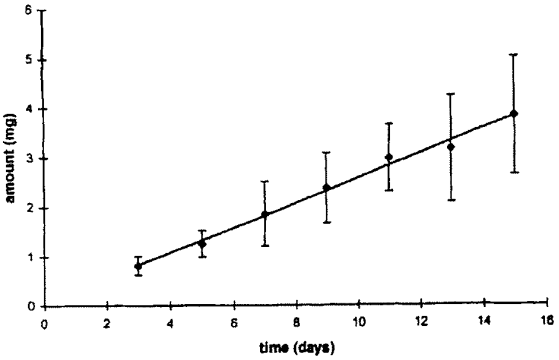

Figure 2. Permeation profile for ketoconazole nitrate through nail pretreated with papain 15%/1 day and salicylic acid 20%/10 days (n = 3).

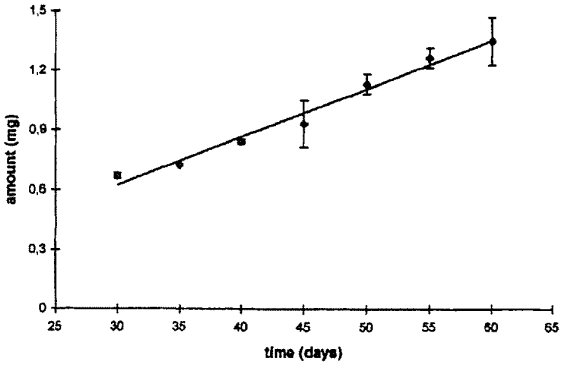

Figure 3. Permeation profile for itraconazole nitrate through nail pretreated with papain 15%/1 day and salicylic acid 20%/10 days (n = 2).

cal dissolution of the diseased nail [e.g., 40% urea paste (8)], they are not capable of promoting the passage of the antimycotics through the nail structure.

Figures 1–3 and Tables 1 and 2 give the permeation profiles and the permeability data for the three antimycotics when the nail was pretreated with papain (15%) for 1 day, followed by salicylic acid (20%) for 10 days. As is shown by the data, only this two-step pretreatment allowed the permeation of the antimycotics. After short lag times, between 0.53 and 1.12 hr, zero-order kinetics were observed for the three antimycotics. This behavior is explained by the formation of pores in the nail matrix, as a consequence of the keratolytic action. It is possible that the antimycotic molecules can pass through these pores than via interconnected channels permeate into the nail. This hypothesis is supported by the SEM images shown in Fig. 4. The normal scaly surface of the nail was modified by the keratolytic action of the permeation enhancers, as evidenced by the observation of a more fractured scaly surface. This effect was

Table 1

Experimental Conditions Employed for the Nail Permeation Studies

Donor (mg/ml)	Pretreatment	Keratolytic in the Donor (mg/ml)	Sampling (Days)
Miconazole nitrate (60)	None	None	60
	None	Urea (40)	60
	None	Salicylic acid (20)	60
	Type I[a]	Urea (40)	60
	Type II[b]	None	30
Ketoconazole (37.5)	None	None	60
	None	Urea (40)	60
	None	Salicylic acid (20)	60
	Type I	Urea (40)	60
	Type II	None	30
Itraconazole (0.8)	None	None	60
	None	None	60
	None	Urea (40)	60
	Type I	Urea (40)	60
	Type II	None	30

[a]Pretreatment type I with papain (15% w/v) for 1 day.
[b]Pretreatment type II with papain (15% w/v) for 1 day and salicylic acid (20% w/v) for 10 days.

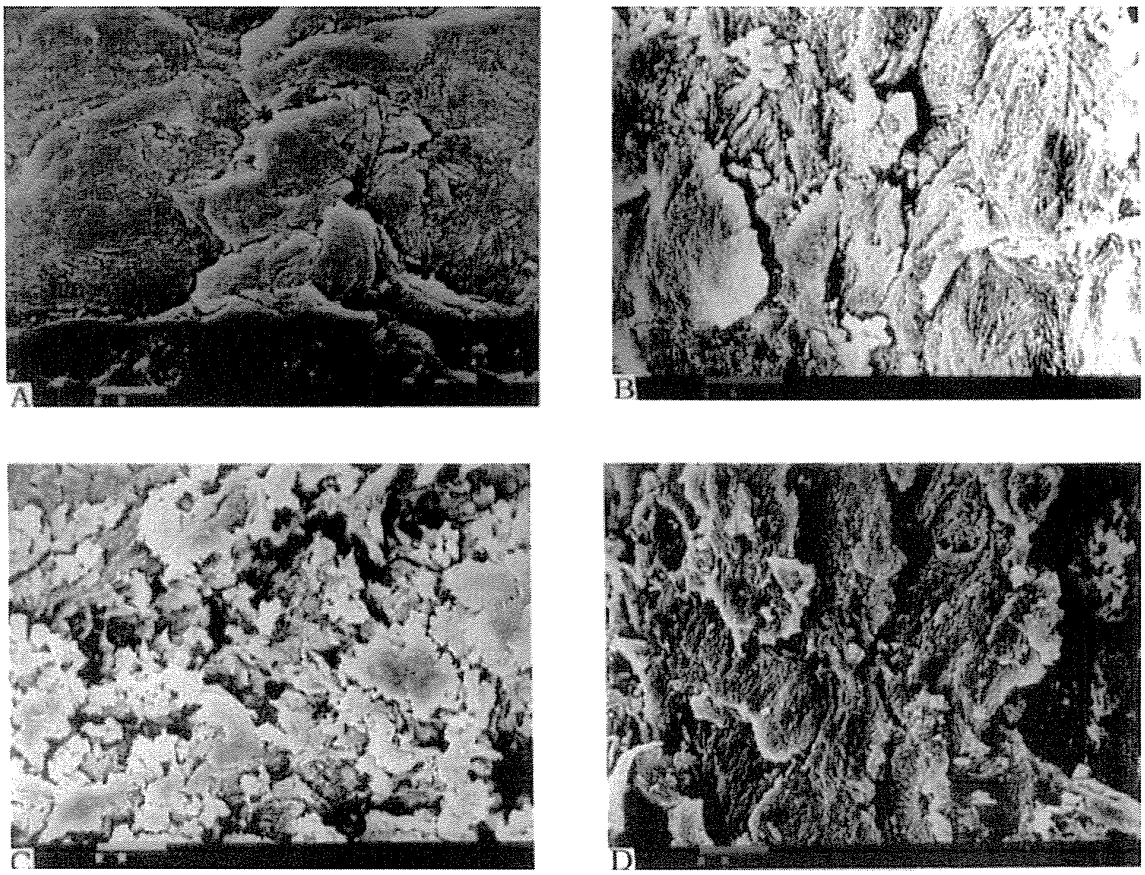

Figure 4. Scanning electron microphotographs showing the nail surface after exposure to keratolytic substances for 4 days at 37°C. (a) Control; (b) urea; (c) salicylic acid, and (d) papain (×1500).

most pronounced in the case of papain, followed by salicylic acid, and was less significant with urea. Note that these observations only showed the effect of the keratolytic substances on the nail surface and not in the deeper regions. Thus, we can only say that the treatment with papain (15%) for 1 day and salicylic acid (20%) for 10 days allowed the formation of pathways into the nail. This long and aggressive pretreatment was chosen, after

Table 2

Nail Permeability Data for the Antimycotics Assayed After Nail Pretreatment with (15% w/v)/1 Day and Salicylic Acid (20%)/10 Days

Antimycotic	Flux[a] (mg $cm^{-2}sec^{-1}$) × 10^5	Lag Time (T_L) (sec)	Effective Diffusion Constant (D_{eff})[b] (cm^2 sec^{-1}) × 10^8
Miconazole nitrate	6.66 ± 0.42	1929 ± 208	6.29
Ketoconazole	1.15 ± 0.21	3373 ± 425	3.60
Itraconazole	0.13 ± 0.08	4050 ± 632	3.00

n = 3 (miconazole nitrate and ketoconazole); n = 2 (itraconazole).
[a]From the steady-state values.
[b]From $D_{eff} = h^2/6T_L$ (mean value for h = 0.027 cm).

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