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Bioavailability, Skin- and Nailpenetration of Topically Applied Antimycotics*

Bioverfügbarkeit, Haut- und Nagelpenetration lokal applizierter Antimykotica

G. Stüttgen und Elke Bauer

Hautklinik und Hautpoliklinik der Freien Universität Berlin im Rudolf-Virchow-Krankenhaus, Berlin
(Direktor: Prof. Dr. med. G. Stüttgen)

Key words: Penetration – Econazole – Oxiconazole – Dimethylmorpholinohydrochlorid – Horny layer – Nails – DMSO

Schlüsselwörter: Penetration – Econazol – Oxiconazol – Dimethylmorpholinohydrochlorid – Hornschicht – Nägel – DMSO

Summary: The penetration and permeation of econazol, oxiconazol and dimethylmorpholinohydrochlorid in the human skin and nails have been performed using the measurement of radio labelled substances ($C^{14} \approx 250$ µCi/ml) in vitro (penetration chamber) and in vivo.

The penetration of the antimycotics inside the horny layer of the skin and the layers of the nail (10 µg/ml) ist sufficient enough to inhibit the growth of fungi by fungistatic mechanism.

DMSO increases the penetration rate into the nail plate. Antimycotics solved in tinctures show a better penetration into the nails than the ointments which have advantages in penetrating the skin.

The small fungicid effect and low dose in the deeper nails explains the long time topical treatment necessary at least for 6 months respectively the moulting process of the nail keratin.

Zusammenfassung: Die Penetration und Permeation von Econazol, Oxiconazol und Dimethylmorpholinohydrochlorid in die menschliche Haut und Nägel wurde mit Hilfe markierter Substanzen ($C^{14} \approx 250$ µCi/ml) in vitro (Penetrationskammer) und in vivo untersucht.

Die Penetration der untersuchten antimykotischen Substanzen in die Hornschicht der Haut und in die Keratinschichten der Nägel ist ausreichend, um fungistatisch zu wirken.

DMSO erhöht die Penetration in das Nagelkeratin. Antimykotica in Tinkturen inkorporiert, zeigen eine bessere Penetration in die Nägel als inkorporiert in Salben, die Vorzüge bei der Penetration in die Haut zeigen.

Der geringe fungizide Effekt der meisten Antimykotica und die relativ geringe Dosis in den tiefen Nagelschichten erklären die notwendig lange Behandlungszeit über 6 Monate, die den Mauserungseffekt des Nagelkeratins berücksichtigt.

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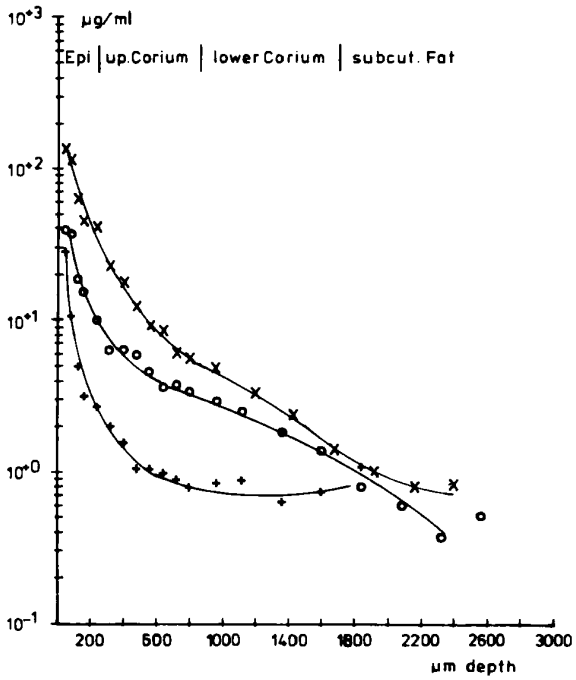

Fig. 1

Introduction

It is obvious that an antimycotic agent, before it can exert its effect, should reach the infected tissue in sufficient concentrations. The way in which topically applied antimycotics migrate into the target organ skin and, after having acted, are removed from that area is a highly complex sequence of events (4).

Liberation describes the diffusion process of a given substance out of the vehicle into which it has been incorporated. By penetration, the movement of a drug into a layer of the skin is described, while permeation means the migration through one or more layers. Resorption is the process by which the drug reaches the blood and lymph vessels, and by which it is removed from the local skin area. Absorption represent the sum of the above mentioned processes (3).

The best drug in preparation for topical application is useless, if the base in which the drug is incorporated does not allow the liberation process of the drug (Higuchi).

Higuchi described the known correlation between liberation and the concentration, diffusion coefficient, saturation solubility, and dependency of time. Under constant conditions, the release of a drug from an vehicle is proportional to the square root of time observed.

The problem of adsorption is correlated with a structural state of skin barrier namely the horny layer. The growth of fungi on the skin surface inside the horny layer induces toxic or

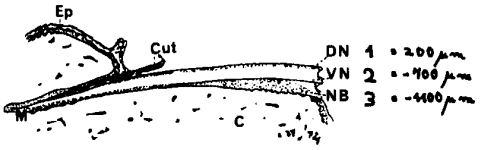

Fig. 2

Table 1
Permeation (µg/ml) of different antimycotics in the skin layer after variable exposition time

	Horny layer 20 µm		Epidermis 200 µm		Upper Corium 400 µm		Deeper Corium 800 µm		Subcutis 1200 µm	
Time	100′	300′	100′	300′	100′	300′	100′	300′	100′	1000′
Oxiconazole ointment 1%	1000	900	12	15	1	2	1	0.7	0.3	1.5
Tincture 1%	3000	2500	4	4	1	0.5	0.2	0.3	0.07	0.8
Dimethyl-morpholinoderivate ointment 1%	800	2000	10	14	2.5	6	2	5.5	0.1	1.0
Econazol-ointment 1%[1]	1200		20	5			0.2	0.5	0.1 urin = 0.61% of the applicated dose	
Isoconazole-nitrate[2] ointment 1%			10	9	8	5	0.8	0.7	1	
Clotrimazol-ointment 1%[3].....		1000		30		0.5 (-40)				

[1] Schaefer, H., Stüttgen, G., 1976 (in vivo experiments)
[2] Täuber, U., 1979
[3] Bayer-report, 1975

allergenic substances irritating the skin especially the structure of the horny layer. The more horny layer is damaged, the more the process of permeation takes place.

The quantitative measurement of absorption process of a given substance is a prerequisite for the therapeutical application later on. Such examinations have need of standarisation, because only by respecting such standard methods it is possible to compare the different pharmacokinetic of antimycotics in relation to liberation and permeation inside the horny layer and into the deeper tissue. Measurement of the behaviour of radiolabelled substances is a method of choice. Tritium or C^{14} labelled substances with a specific activity of about 500 µCi/ml were placed at our disposal (4).

Processing of the skin respectively nails

After 30, 100, 300 or 1000 min penetration time, the surplus cream on the surface was removed gently with dry cotton swabs. The specimen was then fastened on a rubber stopper and the horny layer removed by repeated stripping with adhesive tape (Tesafilm®). Each of approximately 20 strips was placed in a separate vial. A 28 mm^2 sample was punched out and cut horizontally on a freeze microtome. The 16 slices of 10 µm thickness first-sectioned represented the epidermis. The remaining part, representing the dermis, was cut into 40 µm slices.

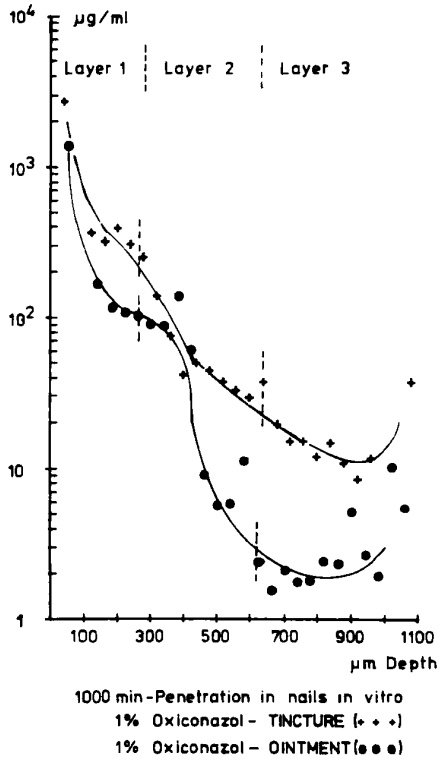

Fig. 3

The nail examination has been achieved by the same technic and same procedure putting the drug on the surface of nail area of 1 cm^2 to obtain data about the absorption.

The deep frozen nail was punched out and cut horizontally on a freeze microtom respecting the different layers of the nail (fig. 2). The samples were measured using a liquid scintillationcounter (Beckman LS 150 with an on-line teletype) whereby the maximum error was set to 10%. Subsequent calculations of decompositions per minute of percentage of the applied quantity and absolute quantities per layer were performed using a computer program.

In vivo examinations

The surplus cream was removed and the horny layer sampled as indicated above. On the exposed skin an excision of a 4 x 4 cm area was performed and a specimen of 28 mm^2 was punched out from the excised area and siliced in the same manner.

In vivo studies of human skin are only possible in a limited number, because dermatological surgery allowing pretreatment and yielding suitable skin spacements is limited.

In vitro results reflect in vivo conditions predominantly down to the basal layer of the epidermis.

Our studies were performed in vitro with a penetration chamber where the dainage or upflow of the labelled substance through the blood and lymphatic vessels of the skin is simulated by a system with steady flowing at the temperature of 32° C which corresponds with the natural conditions.

Table 2
Penetration (µg/ml) of different antimycotics in the human nail after exposition time 1000 min in vitro

Depth	dorsal nail plate (1)** - 200 µm	ventral nail plate (2)** - 700 µm	nail bed (3)** upper layers till 1100 µm	nail bed deeper layers more than 1100 µm
Oxiconazole ointment 1%	1000	15	5	7*
Tincture 1%	1500	50	80*	80*
Dimethyl-morpholinoderivate Tincture 0.5%	600	15	8	15*
Econazol ointment 1%	1000	10	10	12*
Econazollotion 1% (99% DMSO) ..	1000	50	70	80
Lotion 1% (49% DMSO)	800	70	20	10

**) see Fig. 2
*) dam effect

Our examinations were devided into two parts
1. The normal and damaged human skin in vivo and vitro including stripping of the horny layers,
2. the normal and damaged human nail in vitro including the effect of DMSO.

To have exact data about the antimycotics it is necessary to compare the micrograms found in the different layers with the antimycotic as it was done by Täuber with doing the microgram of isoconazole-nitrate found in the different layers of the skin in vitro.

Using such technic it is possible to be informed about the skin layer in which the antimycotic has his antifungal activity.

The antimycotic action of a substances is diminished by a binding capacity of proteins and membranes of the cell surface. By this facts the antimycotic action and the proved level of the antimycotic in the different skin layers needs further examinations which depends on the chemical characteristics of the free or bounded antimycotis. Skin (Tab. 1).

Econazol

After application time of 30 minutes in a depth of 200 micrometer the concentration of econazol was about 10 microgram, a concentration which is efficient to stop growth of fungi. By occlusion-dressing the level of econazol in the deeper layers of the skin is increased without over proportional value because the growth of keratophilic fungi takes place in the upper layer of the skin.

Oxiconazol 1%

Oxiconazol ointment shows in the epidermis a concentration which is higher than 10 micrograms. In the deeper corium the concentration was about 1 microgram the level in ste subcutis of 1.5 microgram after exposition time 1000 min. shows surely the dam effect of the in vitro trials because the upflow of the oxiconazol simulated by the chamber technic is not aequivalent to the natural conditions. The oxiconazol tincture shows lower concentrations in the epidermis and the corium (Fig. 1).

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