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Kinetics of amorolfine in human nails

Die Amorolfin-Kinetik in Nägeln des Menschen

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Summary. Amorolfine penetrates rapidly into the nail after topical application. The kinetics of penetration follow an exponential law as expected, and the level of amorolfine measured in the nail (at least in the upper levels) already exceeds the MIC of most fungi causing onychomycosis after only 24 h of contact. After topical application amorolfine is detectable in the nail earlier and in higher concentrations than terbinafine and itraconazole after oral application.

Zusammenfassung. Amorolfin dringt nach topischer Anwendung schnell in den Nagel ein. Die Penetrationskinetik folgt erwartungsgemäß einer Exponentialfunktion, und der Amorolfin-Spiegel im Nagel (zumindest in seinen oberen Anteilen) übersteigt bereits nach 24 h Kontaktzeit die MHK der meisten Onychomykose-Erreger. Nach topischer Anwendung wird Amorolfin früher und in höheren Konzentrationen als Terbinafin und Itraconazol nach oraler Anwendung im Nagel wiedergefunden.

Introduction

Amorolfine is a new topical antifungal agent of the morpholine class which is very effective in the treatment of onychomycosis. A nail lacquer has been especially developed for this indication. Two galenic forms exist, one containing methylene chloride, one ethanol.

Two questions have arisen in connection with this therapy: firstly, whether the penetration of amorolfine into the nail is dependent on the solvent and secondly, whether sufficient amounts of amorolfine penetrate into the nail to inhibit fungal growth.

This document reports on the kinetics of amorolfine penetration into the nail after a 24 h contact with both the methylene chloride- and ethanol-containing lacquers.

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Materials and methods

Nails of human origin with varying degrees of damage were made available to us. A zone of 1 cm^2 was marked on the surface of these nails and the whole nail was treated with amorolfine lacquer containing either methylene chloride or ethanol. The nail was laid on a moistened piece of gauze in a Petri dish which was then closed and incubated for 24 h at 20 °C. After incubation, the unabsorbed material on the nail was removed with cotton wool soaked in alcohol, and the marked zone of the nail was cut out and sectioned into horizontal slices using a microtome. Layer 1 is the uppermost, and 'remainder' denotes the lowermost portion which could not be further sliced. The number of slices varied according to the thickness and condition of the nail. The nail samples were then weighed and treated with KOH (0.5 ml 20% wt/vol at 37 °C overnight), during which time the sliced nail completely dissolved. The KOH solution was then acidified with hydrochloric acid and the amount of amorolfine present was measured microbiologically using an agar dilution technique.

The microbiological measurement of amorolfine proceeded as follows: 24 h cultures of *Candida albicans* H_{29} grown on Sabouraud glucose agar were suspended in saline and adjusted to a density

of 7×10^6 yeast/ml. One millilitre of this suspension was mixed with 200 ml of casitone agar, cooled to 50 °C and poured into Nunc bioassay plates measuring 235 × 235 mm. The agar gel layer on the plates was about 3.5 mm thick. On each plate, 25 holes 5 mm in diameter were cut out and filled to the brim with standard solution or the solubilized nail samples. As standard solution, amorolfine was diluted in acidified water, since earlier experiments showed that amorolfine diffusion in agar is best measured in slightly acidified water.

An amorolfine standard curve with 50, 25, 12.5, 5.6, 2.5 and 1.25 μg ml^{-1} was determined. The zone of inhibition was read after an overnight incubation at 37 °C.

The inhibition zones measured were highly reproducible between experiments over an extended period. In each test one nail was treated with the lacquer containing methylene chloride, and another was treated with lacquer containing ethanol. In the tables these pairs are indicated with the same letter.

Results and discussion

The amorolfine levels in 10 human nails after an exposure of 24 h are given in Table 1. Five nails were treated with methylene chloride lacquer, 5 nails with ethanol lacquer, one of each in five separate experiments. It can be seen that in all cases microbiological activity was measurable right through to the lower part of the nail, even if in some cases at a rather low level, thus demonstrating that amorolfine penetrates through the nail within 24 h. This rapid penetration is similar to that observed in an earlier study using radiolabelled amorolfine dissolved in methylene chloride [1], where amorolfine was already measurable in the cotton wool underneath the nails after 6 h of contact. The data obtained do not suggest that the solvent used in the lacquer plays any role in penetration.

As expected, the kinetics of penetration typically follow an exponential law which shows the distribution of amorolfine for the pair of nails denoted A. The concentration of amorolfine in the upper layer is about 100 times higher than in the lowest, and again no significant difference exists between the two formulations.

Both the degree and kinetics of penetration are influenced by the condition of the nail. It seems that soft and 'floury' nails do not retain amorolfine as well as hard nails, so the total amount of amorolfine found in the nails is dependent not only on their thickness but also on their consistency. Also, when the diseased nail contains holes, the amount of amorolfine found is of course less than in a compact nail. Details of the nail structure are given in Table 2.

The typical kinetics of penetration were also in some cases altered by physical factors. A soft or 'floury' nail, or one with holes, is extremely difficult to slice. In addition, if the nails were too

Table 2. Structure of the nails

Methylene chloride
- A hard nail, easily sliced
- B soft nail, easily sliced, no holes, proper layers
- C soft nail, hard to slice
- D soft nail, easily sliced, no holes, proper layers
- E soft nails, holes, extremely bent in lower layers, part of surface included

Ethanol
- A hard nail, easily sliced
- B hard nail, no holes but extremely bent in lower layers, part of surface included
- C soft nail, hard to slice, 'floury'
- D soft nail, easily sliced
- E hard nail, hole in the middle, not properly sliced in lower layers, part of surface included

Table 1. Concentration of amorolfine in human nails after 24 h contact time (μg mg^{-1})

Solvent	Sample	Nail layers												Remainder	Total amount in nail (μg)
		1	2	3	4	5	6	7	8	9	10	11	12		
Methylene chloride	A	3.88	1.0	0.4	0.145		0.46	0.12						0.03	103.25
	B	5.7	5.09	4.5	3.6	1.04	1.11	1.27	1.22	0.42	0.66			0.25	787.05
	C	2.57	1.09	0.76	1.23	0.27	0.05							0.01	59.68
	D	2.43	1.86	1.56	0.76	0.83	0.9	0.97	0.85	1.86				0.24	112.6
	E	2.03	0.59	0.58	0.78	0.55	1.14							0.43	51.0
Ethanol	A	6.68	2.19	1.59	0.84	0.77	0.8	0.39						0.09	127.2
	B	1.36	0.93	0.38	0.36	0.42	0.33	0.41	0.55	0.43	0.55	0.35	0.66	0.44	333.17
	C	2.01	1.21		0.58	0.08								0.19	35.6
	D	1.66	1.05	0.73	0.48	0.63	0.6	0.41	0.16	0.06				0.16	37.78
	E	0.96	0.51	0.42	0.35	0.34	0.62	0.83						0.43	41.21

bent (because of the infection or just naturally) the layers could not be easily sliced and then the lower slices tend to contain parts of the surface of the nail, and the upper slices a portion of inner nail. This obviously introduces artefacts into the concentration gradient measured.

Some studies have been performed to measure the concentration of terbinafine and itraconazole in the nail after oral application [2, 3]. Terbinafine was measured in nail clippings, the first measurement being performed after 4 weeks of therapy with 250 mg daily. Terbinafine was detected in six out of 12 patient samples at 4 weeks, rising to 11 out of 12 at 12 weeks. The levels of terbinafine ranged from 0.1 to 2.89 ng mg^{-1} nail. According to the authors [3] these data suggest that terbinafine diffuses relatively rapidly into formed nail plates. After a single topical application of amorolfine lacquer we find levels of amorolfine orders of magnitude higher after only one day of contact.

Itraconazole was detected in the nail at 56 ng mg^{-1} on day 7 after therapy with 100 mg. The level increases to 128 ng mg^{-1} on day 28, but this is still less than the typical amorolfine concentration after 24 h [4].

The two studies mentioned above are not strictly comparable as the times of measurement are different, but it seems that itraconazole penetrates faster into the nail than terbinafine. However, higher concentrations per mg nail are reached after topical treatment with amorolfine than with either terbinafine or itraconazole. Furthermore, penetration after local application is much faster than after oral therapy.

Our studies on amorolfine as well as those with itraconazole and terbinafine measure levels of the antimycotic drug in the nails, but do not show whether the concentration measured is freely available to inhibit fungi or whether it is bound to keratinized tissue. It is known that amorolfine, terbinafine and itraconazole are all relatively strongly bound to serum, but no measurements have been performed to study their binding to keratinized tissue. With amorolfine, the levels reached are well in excess of the MIC values of most fungi causing nail infections, and although the percentage of unbound drug is not known, the high cure rate in onychomycosis points to the fact that there is sufficient amorolfine available to reach the target in the fungal cell and thus kill the fungus in the nail.

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