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Different physicochemical properties of antimycotic agents are relevant for penetration into and through human nails

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This article reports the characterization of the physicochemical properties of two important antifungal topical drugs, amorolfine and ciclopirox. Furthermore, the release of the drugs from commercial lacquer formulations for treatment of onychomycosis was studied using the online FTIR-ATR technique. Based on the physicochemical background of these two drugs and their release from commercial lacquer formulations for treatment of onychomycosis, the suitability of these drugs for optimized local antifungal therapy to human nails is discussed. Amorolfine appears to be more suitable for drug delivery to human nails because it penetrates into the nails via the hydrophilic pathway. Furthermore amorolfine penetrates very well into fungal cells, due to the pH value of the nail, as well as the pK_a value of this antimycotic agent and the lipophilic properties of its base form.

1. Introduction

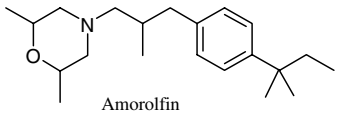
Onychomycosis, the most common of all nail infections, is reported to affect up to 18% of the general population, representing around 50% of all nail disorders (Roberts 1999; Scher 1999). Systemic and topically applied antimycotic drugs are used in mono- and combination (topical plus systemic) therapy of this disease. Topical antifungal drugs have an important role in the modern management of onychomycosis (Baran and Kaoukhov 2005). Modern nail lacquers containing active antimycotic agents are relatively new galenic formulations and have been termed transfungual delivery systems (Baran 2000). Currently available commercial lacquer formulations include those containing amorolfine 5% as hydrochloride (Loceryl® Nagellack; Galderma Laboratorium GmbH) and ciclopirox 8% (Nagel Batrafen®; Aventis Pharma). The main challenge of topical therapy is to achieve biologically active antimycotic concentrations of agents liberated from the vehicle into the nail plate and at the nail bed for a sustained period of time.

The physicochemical properties of pharmacologically active substances are critical for their therapeutic activity. Therefore, knowledge of the physicochemical background of drugs is very important for optimal drug delivery to the human nails and for therapy of nail diseases (e.g. onychomycosis). Furthermore, the physiological conditions in the human nail plates have to be taken into consideration. The nail plate has a pH value of about 7.4, containing few lipids (0.5–1.5%) and a relatively high amount of water (7–30%) (Baden et al. 1973; Finlay et al. 1980; Murdan 2002).

In the field of topical antimycotic drugs for onychomycosis, there have been some articles dealing with their pharmacokinetics in the nail (e.g. Franz 1992; Polak 1993; Ceschin-Roques et al. 1991), with the biological activity of the drugs (Mensing et al. 1992), and their biological activity due to sublimation (Polak et al. 2003). Furthermore, there are some reports focusing on physicochemical aspects of the penetration of antimycotic agents into and through human nails *in vitro* (Walters et al. 1985; Mertin and Lippold 1997; Kim et al. 2001).

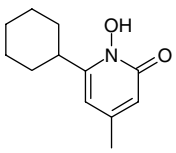
Therefore in this article, the physicochemical background of amorolfine (hydrochloride) and ciclopirox was studied and the release of these drugs from commercially available lacquer formulations was measured online using the Fourier transform infrared (FTIR) attenuated total reflection (ATR)-technique in direct comparison.

A



Amorolfin

B



Ciclopirox

Table: Physicochemical properties of amorolfine and ciclopirox

	Amorolfine HCl	Ciclopirox
Water solubility (mg/ml) Water	9.2 ± 0.06	8.6*
		32.8 ± 0.6 (Olamine)
Buffer	6.7 ± 0.2**	1.0***
pK_a	6.6****	8.07****
Partition coefficient n-octanol/buffer:		
pH 7.4	17.4 ± 1.9	27.7 ± 4.4
pH 4.0	0.035 ± 0.003	

N = 3, mean ± SD; *water at 32 °C, ** 7.6 pH, *** 7.4 pH (according to Mertin and Lippold (1997)), **** according to (Mertin and Lippold 1997)

2. Investigations, results and discussion

As shown in the Table, amorolfine is quite water soluble when it is used as the salt (9.2 mg/ml). This result is in agreement with the water solubility (9.9 mg/ml) reported by Mertin and Lippold (1997). Ciclopirox is very water soluble when it is used as ciclopirox olamine (32.8 mg/ml). The water solubility of ciclopirox was 8.6 mg/ml. However, the solubility of ciclopirox in buffer (pH 7.4) was 1.0 mg/ml compared to 6.6 mg/ml amorolfine in buffer (pH 7.6). The n-octanol/buffer partition coefficient was measured in order to characterize the lipophilicity of the drugs studied. As shown in the Table, amorolfine has a very low n-octanol/buffer partition coefficient (0.035) when an aqueous phase with a pH of 4.0 is used. Amorolfine is a base with a pK_a of 6.6. Therefore, amorolfine is highly ionized at a pH of 4.0. On the other hand, amorolfine has a very high n-octanol/buffer partition coefficient (17.4) when an aqueous phase with a pH of 7.4 is used because amorolfine is present 91% as the base at pH 7.4. The results show that the amorolfine base is very lipophilic in contrast to its ionized form.

Ciclopirox has a high n-octanol/buffer partition coefficient (27.7) at pH 7.4, which means that it is a very lipophilic drug.

The release of the two drugs was studied using both a lipophilic and a hydrophilic acceptor system. It can be seen that amorolfine was very rapidly (half life $t_{1/2}$ of about 4 min) released from the Loceryl® lacquer formulation (see Fig. 1) when water was used as the acceptor system: after 15 min 90% of amorolfine was released.

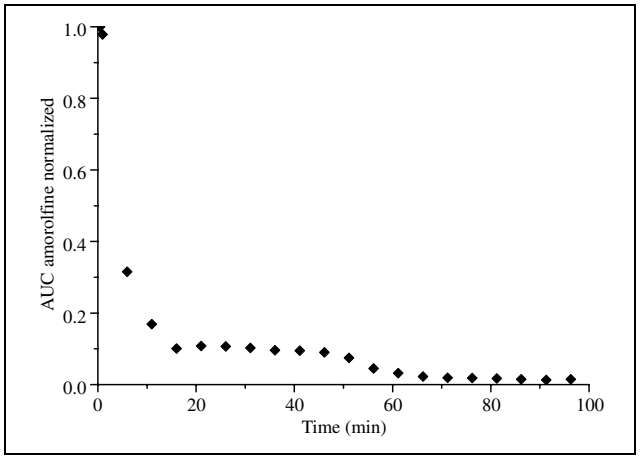


Fig. 1: Release of amorolfine HCl from Loceryl® nail laquer into water

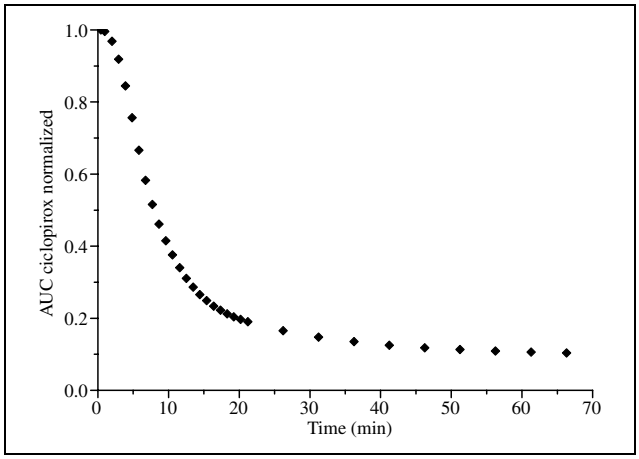


Fig. 2: Release of ciclopirox from Nagel Batrafen® into a dodecanol collodion membrane

In contrast, no release of ciclopirox from the Batrafen® lacquer formulation could be detected after 120 min when water was used as the acceptor phase (data not shown).

On the other hand, a very fast release (half life $t_{1/2}$ of about 8 min) of ciclopirox from the Batrafen® formulation was observed when a lipophilic acceptor system (dodecanol-collodion-membrane) was used (see Fig. 2).

In contrast, no release of amorolfine from the Loceryl® lacquer formulation was found after 120 min when the lipophilic acceptor was used (data not shown).

The results show that ciclopirox appears to penetrate into human nails via the lipophilic pathway. In contrast, because of the hydrophilicity of its ionized form (= transport form) amorolfine is capable of diffusing into the human nail plate via the hydrophilic pathway. The pH value in the nail is about 7.4, and therefore basic drugs with a pK_a of about 6.5 such as amorolfine are present in their unionized form (= active form) having a higher lipophilicity, which is necessary to enter the fungal cells.

As shown in the literature, the hydrophilic pathway and also the degree of hydration (Marty 1995) appear to be the most important prerequisites for drug penetration into human nails because in the human nails there are only 0.5–1.5% lipids, but a 7–30% water content. Therefore, the human nail plate behaves physicochemically more like a hydrophilic gel membrane as opposed to a lipohilic membrane, such as stratum corneum (Mertin and Lippold 1997; Walters et al. 1983). For further characterisation of different physicochemical properties of amorolfine and ciclopirox, investigations on alternative biological membranes (bovine hoofs and human nails) have been initiated.

Taking all the results together, we can conclude, that amorolfine is physicochemically more suitable for delivery to the human nails because it penetrates via the hydrophilic pathway. Due to the optimal pH value of the nail plate, as well as the pK_a value and lipophilic properties of amorolfine, the active agent penetrates very efficiently into fungal cells. These physicochemical data confirm other experimental results published earlier, which demonstrated that amorolfine penetrates very well not only into, but also through human nail plates into subungal debris to maintain effective antifungal drug concentrations even after termination of local treatment in patients with onychomycosis (Mensing et al. 1992).

These experimental data should at least partially explain clinical results published recently, in which amorolfine 5% nail laquer had higher clinical and mycological cure rates

than ciclopirox 8% nail laquer in mono- or combination therapy of onychomycosis (Halmy 2004; Baran and Kaoukhov 2005).

3. Experimental

3.1. Materials

The model antifungal drugs, amorolfine HCl and ciclopirox olamine, were obtained from Galderma Laboratorium GmbH (Düsseldorf, Germany) and Sigma (Taufkirchen, Germany), respectively. Ethanol was supplied by Bundesmonopolverwaltung für Branntwein (Offenbach, Germany), ether by Kraemer & Martin (Sankt Augustin, Germany), and dodecanol by Merck-Schuchardt, (Hohenbrunn, Germany). Collodium (solution 4%, DAC 99) was supplied by Caelo (Hilden, Germany); all the other chemicals required were purchased from Sigma (Taufkirchen, Germany).

3.2. Analytical assays

3.2.1. Partition coefficient and water solubility

The concentrations of amorolfine and ciclopirox in the aqueous phase were determined after the experiments for determination of the partition coefficient. The measurements were carried out photometrically at $\lambda = 214$ nm (amorolfine) and at $\lambda = 220$ nm (ciclopirox) using a HP Diode Array Photometer (Waldkirchen, Germany).

3.2.2. Release studies

FTIR-ATR-spectroscopy (see Wartewig et al. 2002) was used to determine the release of the drugs studied from commercial lacquer formulations. The experiments were carried out with an IFS 28 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a SpeculATR ATR-attachment (Thermo Spectra-Tech Inc., Shelton, USA). The sampling compartment is a Fresnel ATR accessory that uses a ZnSe crystal with an angle of incidence of 45° in a horizontal orientation. The diameter of the top of the crystal is 20 mm. Each IR spectrum was collected at room temperature with 32 scans and a spectral resolution of 2 cm^{-1} in the wave number range from 680 cm^{-1} to 4000 cm^{-1}. OPUS software (Bruker Optics) was used for data treatment. The FTIR-ATR spectra of the nail lacquers, Loceryl® and Batrafen®, are presented in Fig. 3. The decrease over time of the integral intensity of the IR band between 2420 cm^{-1} and 2580 cm^{-1} was used to investigate the release of amorolfine from the lacquer. In the case of ciclopirox, the evolution over time of the sharp IR band at 1560 and 1640 cm^{-1}, both associated with the drug, were evaluated.

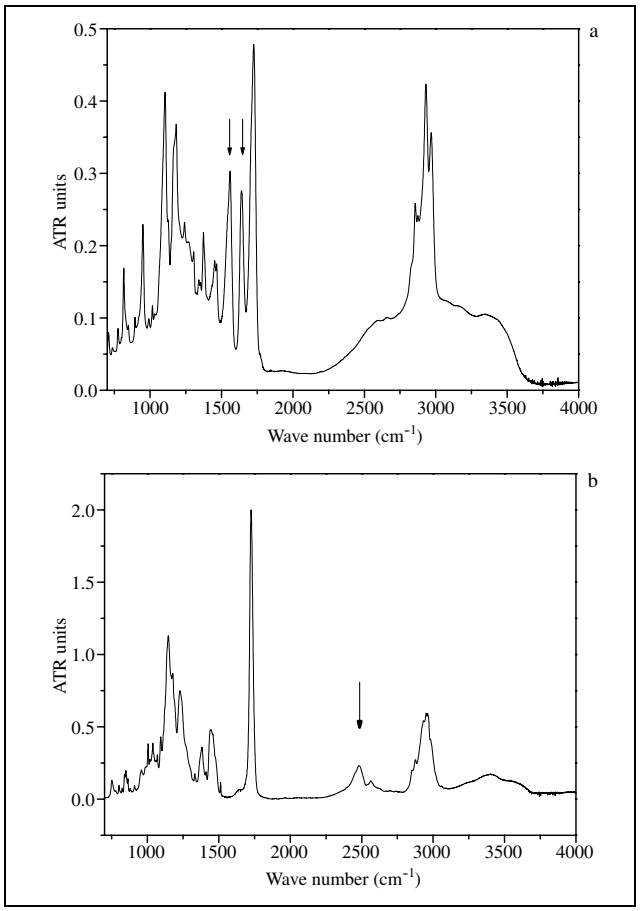


Fig. 3: FTIR-ATR spectra of nail lacquers: (a) Nagel Batrafen® and (b) Loceryl® nail lacquer. The drug IR bands evaluated for release are marked with arrows.

3.3. Lacquer formulations used

Two commercial lacquer formulations were used: Nagel Batrafen® (containing ciclopirox) and Loceryl® nail lacquer (containing amorolfine · HCl).

3.4. Determination of partition coefficients

The partition coefficients of the above compounds were determined for n-octanol/phosphate buffer pH 5.7 and 7.4 at 25 °C. Solutions of the above drugs (4 ml) in the buffer at a concentration of 100 µg/ml and 4 ml of n-octanol were decanted into screwed cap tubes. The tubes were placed in a shaking water bath at 25 °C. At the equilibrium time (5 h) the tubes were centrifuged. The n-octanol layer was separated and discarded and the absorbances were read for the aqueous phase at a suitable λ_{max} against a blank of buffer of the pH used. The partition coefficients were calculated from three determinations each.

3.5. Release studies

The nail lacquer was directly prepared on the top of the ATR-crystal by spreading a defined amount of liquid formulation on the surface of the ZnSe crystal. The acceptor media, water and a dodecanol collodion membrane, respectively, were located at the surface of the lacquer formulation. The preparation of the dodecanol collodion membranes was described by Bendas et al. (1992) and Neubert et al. (1993).

3.6. Determination of water solubility

Solubilities of the drugs were measured in water and in buffer at 25 °C as a function of the equilibrium time at 5 h and 24 h. Ten milligrams of compound were weighed into screwed cap tubes and 5 ml of the above phosphate buffer were added. The tubes were placed in a shaking water bath at 25 °C. The tubes were centrifuged 5 h and 24 h later at 3000 g for 5 min and a suitable volume of supernatant was withdrawn, diluted with water or the buffer, respectively, and the absorbances were recorded at λ_{max} of the appropriate compound against a suitable phosphate buffer blank. The concentrations of the compounds were calculated from the Beer's law equation. The solubilities were calculated in µg/ml from an average of three experiments with each drug.

References

Bendas B, Göpfrich A, Lee G, Neubert RHH (1993) Study of *in vitro*-penetration of betamethasone-17-valerate from solution type gels into the multilayer membrane system. Pharmazie 48: 199–201.

Baden HP, Goldsmith LA, Fleming B (1973) A comparative study of the physicochemical properties of human keratinized tissues. Biochim Biophys Acta 322: 269–278.

Baran R (2000) Dermatolopharmacology of topical anti-fungal preparations in nail tissue. In: Gabard B, Elsner P, Surber C, Treffel P (Eds.) Dermatology of Topical Preparations. Springer, London, pp. 281–295.

Baran R, Kaoukhov A. (2005) Topical antifungal drugs for the treatment of onychomycosis: an overview of current strategies for monotherapy and combination therapy. J Eur Acad Dermatol Venerol 19: 21–29.

Ceschin-Roques CG, Hanel H, Pruja-Bougaret SM, Luc J, Vandermander J, Michel G. (1991) Ciclopirox nail lacquer 8%: *in vivo* penetration into and through nails and *in vitro* effect on pig skin. Skin Pharmacol 4: 89–94.

Finlay AY, Frost P, Keith AD, Snipes W (1980) An assessment of factors influencing flexibility of human fingernails. J Dermatol 103: 357–365.

Franz TJ (1992) Absorption of amorolfine through human nails. Dermatol 184 (Suppl. 1): 18–20.

Halmy K (2004) Clinical experiences with nail laquers containing amorolfine 5% or ciclopirox 8% in subjects with onychomycosis. Poster 13th Congress of the EADV, Florence Nov. 2004; J Eur Acad Dermatol Venereol 18 (Suppl. 2): 242.

Kim JH, Lee CH, Choi HK (2001) A method to measure the amount of drug penetrated across the nail plate. Pharm Res 18: 1468–1471.

Mensing H, Polak-Wyss A, Splanemann V (1992) Determination of the subungual antifungal activity of amorolfine after 1 month's treatment in patients with onychomycosis: comparison of two nail lacquer formulations. Clin Experim Dermatol 17 (Suppl. 1) 29–32.

Marty J-PL (1995) Amorolfine nail lacquer: a novel formulation. J Eur Acad Dermatol Venerol 4 (Suppl. 1) S17–S21.

Mertin D, Lippold BC (1997) *In-vitro* permeability of the human nail and of keratin membrane from bovine hooves: Prediction of the penetration rate of antimycotics through the nail plate and their efficacy. J Pharm Pharmacol 49: 866–872.

Murdan S (2002) Drug delivery to the nail following topical application. Int J Pharm 236: 1–26.
Neubert RHH, Bendas B, Wohlrab W (1991) Use of a multilayer membrane system and excised human skin for studying topical availability of glucocorticoids. Eur J Pharm Biopharm 38: 11–16.
Polak A (1993) Kinetics of amorolfine in human nails. Mycoses 36: 101–103.
Polak A, Jäckel A, Noak A, Kappe R (2003) Agar sublimation test for the *in vitro* determination of the antifungal activity of morpholine dervatives. Mycoses 47: 184–192.
Roberts DT (1999) Onychomycosis: Current treatment and future challenges. Br J Derm 141 (Suppl. 56): 1–4.
Scher RK (1999) Onychomycosis: Therapeutic update. J Am Acad Dermatol 40: S21–S26.
Walters KA, Flynn GL, Marvel JR (1983) Physicochemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. J Pharm Pharmacol 35: 28–33.
Walters KA, Flynn GL, Marvel JR (1985) Penetration of the human nail plate: the effects of vehicle pH on the permeation of miconazole. J Pharm Pharmacol 37: 498–499.
Wartewig S, Hartmann M, Neubert R (2002) Infrarotspektroskopie – Neuere Entwicklungen und deren Anwendung in der Dermatopharmazie. PZ Prisma 9: 5–13.