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Sublimation of antimycotic agents as proved by various analytical methods

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Qualitative and quantitative results demonstrate that the pure substances amorolfine base, amorolfine hydrochloride, two selected morpholine derivatives and terbinafine hydrochloride are clearly able to sublimate. As amorolfine hydrochloride is also capable to sublimate from galencial forms laquer and cream in this experimental setup, a clinical relevance of sublimation phenomenon at least for topical treatment of onychomycosis has to be considered. This phenomenon could be one reason for advantageous clinical and mycological cure rates of amorolfine nail laquer to comparable topical products reported in the literature.

Topical antifungal drugs have an important role in the modern management of onychomycosis in mono- or combination therapy with systemic drugs (Baran and Kaoukhov 2005; Lecha et al. 2005). Modern nail lacquers containing active antimycotic agents are relatively new galenical formulations and have been termed transungual delivery systems (Baran 2000).

Dermatophytoma is an air rich thick mass of fungal hyphae and arthrospores as well as necrotic keratin between the nail plate and the nail bed (Roberts and Evans 1998). This subungual keratosis could be responsible for therapeutic failures of local and/or systemic therapy approaches. Therefore antifungals being capable of sublimating to overcome air cavities in mycotic lesions in concentrations which are still microbiologically active should clinically have an advantage over other compounds in topical therapy of onychomycosis. Recently, Polak et al. (2006) reported in a microbiological model that several antimycotic agents are able to exert their fungistatic and fungicidal activities over a certain distance, thus spanning or bridging air-filled space, without direct contact to the target fungal cells. They suggested that this effect may be related to sublimation. In order to provide complementary evidence for the sublimation of these agents, we performed systematic investigations using Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy, mass spectrometry (MS), and gravimetry.

The FTIR-ATR spectra of amorolfine hydrochloride, amorolfine base, and the accompanying sublimates are presented in Fig. 1 a and b. These findings clearly demonstrate that both substances are able to sublimate. Obviously, the IR spectra of amorolfine base and its sublimate are almost identical. On the other hand, the spectrum of the sublimate of amorolfine hydrochloride differs from that of the source agent. In particular, this is evident in the disappearance of the characteristic bands at about 2480 and 2560 cm^{-1}, which belongs to the NH^+ stretching vibration located at the nitrogen atom of the morpholine ring forming a quaternary ammonium salt with Cl^- as counter ion (Günzler and Gremlich 2002). It is surprising that the spectrum of the sublimate of amorolfine hydrochloride does not match the spectrum of the amorolfine base.

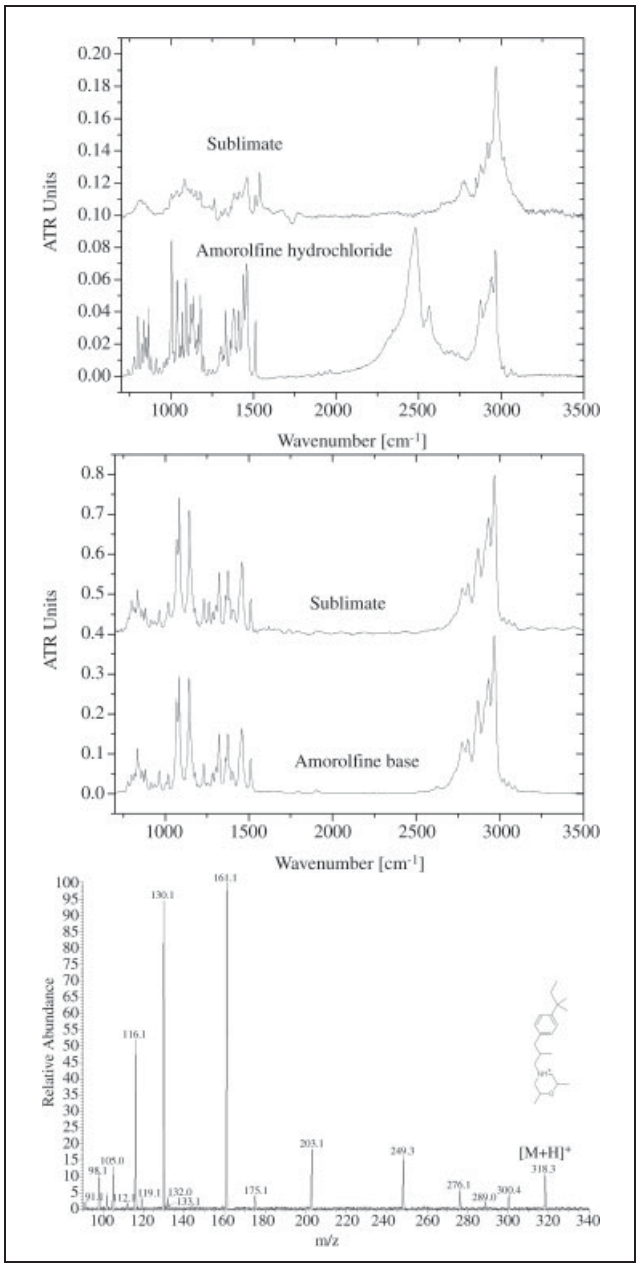

Fig. 1: FTIR-ATR spectra of (a) amorolfine hydrochloride and its sublimate (top), (b) amorolfine base and its sublimate (top), and (c) positive ion trap tandem mass spectrum of amorolfine $[M + H]^+$ $m/z = 318.3$ at a relative collision energy of 19%

Qualitative experiments performed with all pure antimycotic substances and galenical forms (Loceryl® nail laquer and Loceryl cream) at room temperatures (23–25 °C, 13 days) demonstrate comparable results for the sublimation phenomenon.

In order to verify the sublimation, MS has been applied, too. For that purpose the pure antimycotic substances as well as the respective sublimates were dissolved in methanol and analyzed by LC/ESI-MS on a single quadrupole instrument or tandem MS on an ion trap mass spectrometer. Thus, the existence of the amorolfine ion $[M + H]^+ = 318.3$ could be confirmed unambiguously (limit of detection 500 pg mL^{-1}).

An exemplary tandem mass spectrum of the amorolfine base is shown in Fig. 1c. No differences in the fragment pattern of the sublimates with respect to its original substances could be found. This can be explained by the fact that for MS investigation the substance must be dissolved in an organic solvent such as methanol and, thus, amorolfine and not its salt was detected.

The quantitative gravimetric studies have shown that about 5% of amorolfine base sublimate at 37 °C over a period of 5 days, whereas the rate of sublimation of amorolfine hydrochloride is 10 times lower under the same experimental conditions, namely about 0.5%. The lower rate of amorolfine hydrochloride emphasizes the higher capability of base form to sublimate. It is a remarkable finding that even very small proportional amounts of amorolfine hydrochloride sublimate found are able to perform high fungicidal activities against fungal cells of different strains as clearly demonstrated in a microbiological model (Polak et al. 2004).

We have also proved qualitatively that terbinafine hydrochloride and the morpholine derivatives #1 and #2 as pure substances exhibit sublimation at 37 °C and 23–25 °C. Terbinafin hydrochloride is the active substance in a wide range of original and generic antimycotic drugs in tablet form.

Amorolfine hydrochloride is also capable to sublimate from galencial forms (Loceryl® nail laquer, Loceryl® cream) without obvious qualitative differences in comparison to pure substance in both temperature ranges. These results confirm findings from the microbiological model (Polak et al. 2004) and demonstrate clinical relevance of sublimation for topical treatment of onychomycosis.

The property of amorolfine hydrochloride to sublimate confirms other data, which demonstrated that this agent penetrates very well into and through human nail plates via hydrophilic pathways to reach effective antifungal drug concentrations in all relevant compartments of anatomic nail structure (Mensing et al. 1992; Baran 2000; Baran and Kaoukhov 2005; Neubert et al. 2006). Due to the ability to pass through air filled cavities (e.g. in and under diseased nail plate) amorolfine can reach tissue layers on the other side of cavities and could be effective against dormant fungal cells sometimes remaining viable into cavities.

This effect may contribute additionally to relevant advantageous clinical efficacy in onychomycosis in comparison to drugs with agents not able to sublimate, e.g. as recently published for clinical and mycological cure rates of amorolfine 5% nail laquer in mono- or combination therapy than ciclopirox 8% nail laquer (Halmy 2003 and 2004; Baran and Kaoukhov 2005).

Using FTIR-ATR spectroscopy, mass spectrometry, and gravimetry we have demonstrated that the antimycotic drug amorolfine hydrochloride exhibits sublimation at physiological temperatures both as pure substance and from two galenical forms. Terbinafine hydrochloride and two morpholine derivatives show comparable effects.

The experimental data presented here demonstrate an outstanding property of the drug amarolfine with clinical relevance for the topical treatment of onychomycosis.

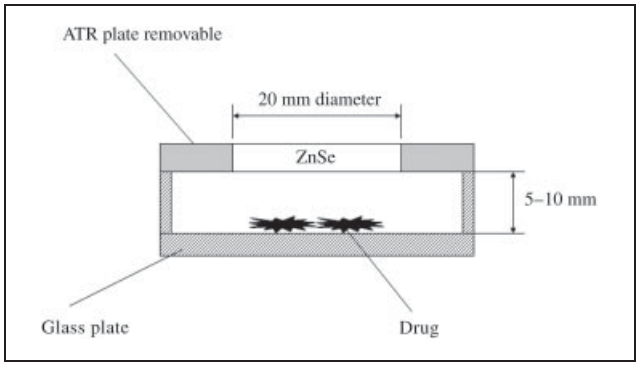

Fig. 2: Scheme of the setup for sublimation experiments

Experimental

1. Materials

A sample of amorolfine base ($C_{21}H_{35}NO$) was kindly provided by Dr. R. Fitzi, Siegfried Exclusives, Siegfried Ltd. (Zofingen, Switzerland). Amorolfine hydrochloride ($C_{21}H_{35}NO \cdot HCl$) was obtained from Galderma Laboratorium, GmbH (Düsseldorf, Germany). Terbinafine hydrochloride ($C_{21}H_{25}N \cdot HCl$) was a gift from Novartis Pharma AG (Basel, Switzerland). Two morpholine derivatives #1 ($C_{25}H_{35}NO$ cis) and #2 ($C_{20}H_{39}NO$) were synthesized at Dr. R. Maag GmbH or Hoffmann La Roche (Basel, Switzerland). Loceryl® nail lacquer and Loceryl® cream were obtained by Galderma Laboratorium GmbH, Düsseldorf, Germany.

2. Experimental setup for sublimation

The sublimation experiments were performed using a simple setup (see Fig. 2). Experimental conditions were: Incubation time of 5 days at 37 °C or 13 days at 23–25 °C, small amount of pure antimycotic substances or topical galencial forms (200 and 400 µl of nail lacquer, drop of cream), respectively, detection at ATR crystal.

3. FTIR-ATR spectroscopy

The IR-ATR spectra were acquired by using a Bruker FTIR spectrometer IFS 28 (Bruker Optics, Ettlingen, Germany) equipped with a Spectra-Tech Foundation SpeculATR attachment (Thermo Electron Corporation, Madison, WI, USA).

4. HPLC/Electrospray ionization (ESI) mass spectrometry

The system used for reversed-phase HPLC/ESI-MS consisted of a Waters 600E pump and a WISP 712 auto sampler (Waters, Eschborn, Germany). The HPLC system was coupled to a single quadrupole mass spectrometer Finnigan SSQ 710C (Thermo Electron, San José, CA, USA) with electrospray ionization (ESI) interface equipped with metal needle and operated in the positive ion mode.

5. Gravimetry

The amount of sublimated amorolfine hydrochloride and amorolfine base, respectively, was determined quantitatively by gravimetrical method at 37 °C, in which the loss of drug due to sublimation was evaluated.

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Properties of colour reference solutions of the European Pharmacopoea in CIE $L^*a^*b^*$ colour space

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The coordinates of CIE $L^*a^*b^*$ uniform colour space have been acquired from the transmitance spectra of colour reference solutions of European Pharmacopoeia (Ph.Eur.). Calculation of colour differences of these solutions from purified water ΔE^* gave their values in the range between 0.7 (B_9 solution) to 36 (Y_1 solution) CIE units. Excluding red colour reference soulutions, ΔE^* values did not depend on concentrations of colour compounds linearly. Small ΔE^* values founded by the brown and brownish-yellow colour reference solutions of the lowest concentrations can possibly cause some problems of visual examination of the degree of coloration of liquids according to Ph.Eur.

Colour reference solutions are used by Ph.Eur. for visual examination of the degree of coloration of liquids in the range brown-yellow-red. Colorimetric properties of these colour reference solutions have only rarely been reffered to (Ali and Castle 2003). An older paper deals with colour reference solutions of the Hungarian Pharmacopoeia (Stampf and Jelinekné Nikolics 1989). There are colour reference solutions mixed in eight concentrations from four primary colour solutions to four colour series (yellow, pink, green, brown) described here, while Ph.Eur. uses different solutions mixed to five colour series from three primary colour solutions in seven (yellow, Y, red, R, greenish-yellow, GY, brownish-yellow, BY) or nine respectively (brown, B) concentrations. The aim of this study was to acquire information about properties of colour reference solutions Ph.Eur. in CIE $L^*a^*b^*$ colour space. Spatial colour difference in this space can be expressed as a single numerical value ΔE^* calculated according to the formula $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Wyszecki and Stiles 2000; Krishna Prasad et al. 1996). Colour differences in CIE units in this paper were calculated against purified water and are summarized in the Table.

It is apparent from the data in the Table that the range of colour changes ΔE^* in the series of red colour reference solutions Ph.Eur. is 24 units, while in other colour reference solution series it is greater and ranges from 31 to 36 CIE units. A change of colour corresponding to a value of $\Delta E^* > 1.5$ can be perceived by the human eye (Stark et al. 1996). The values determined in colour reference solutions