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In vitro permeation behaviour and antimycotic efficacy of the antimycotic agent ciclopirox olamine incorporated into a variety of poloxamer 407-based formulations

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Introduction: Fungal nail infection (onychomycosis) is the most common nail disorder in adults and mostly induced by dermatophytes such as Trichophyton rubrum and Trichophyton mentagrophytes. Due to slow nail growth and depending on nail size and infected area, the treatment takes 3 – 9 months (1). To ensure a successful treatment, a highly effective active pharmaceutical ingredient (API) and a high penetration into and permeation through the nail is required in local therapy to maintain the drug concentration above the minimum inhibitory concentration (MIC).

In this study, the antimycotic agent ciclopirox olamine (CPX) was incorporated in concentrations up to 4 % (w/w) into poloxamer 407-based formulations and studied regarding its permeation behaviour through a keratin film as an artificial nail model. The poloxamer 407-based hydrogels consisted of poloxamer 407 (POX), double distilled water, propylene glycol (PG), isopropyl alcohol (IPA) and medium chain triglycerides (MKT). The marketed nail lacquer Ciclopoli®, containing 8 % ciclopirox as API, served as a reference.

Methods: The poloxamer 407-based formulations were weighed in an Unguator® jar and automatically stirred at 1440 rpm for 1.5 min with an Unguator® e/s GAKO Konietzko GmbH (Bamberg, Germany). To ensure sufficient equilibration, subsequent storage was done for 24 h at 20 °C. All the formulations were given codes reflecting their quantitative composition, e.g. 1P2525 represented a formulation loaded with 1 % CPX, while the vehicle itself contained 25 % POX/MKT (4:1), 25 % IPA/PG (1:1) and 50 % double distilled water (all w/w).

In vitro permeation studies (infinite dose technique) were carried out in modified Franz cells at 32 °C with 120 µm thick keratin films (KF) as permeation barrier. The receiver solution consisted of phosphate buffered saline (PBS) of pH 7.4. The quantification of the permeated drug amount was done with high performance liquid chromatography (HPLC) (Waters, Eschborn, Germany) by using a Grom-Sil 120 ODS 3-CP, 5 µm, 125 x 4 mm column (Grom, Herrenberg-Kayh, Germany) with acetonitrile/ethylenediamine tetraacetic acid disodium salt dihydrate solution (0.96 g/L)/acetic acid (600:400:0.1 (v/v/v)). The flow rate was set at 1.0 mL/

min; UV detection was done at 298 nm. A calibration curve ranging from 0.25 µg/mL to 200 µg/mL was recorded (r2 > 0.999) with a limit of detection (LOD) of 0.1255 µg/mL and a limit of quantification (LOQ) of 0.2510 µg/ml, respectively.

Infected nail plate studies analysing the antifungal activity were performed according to Lusiana et al (2).

Results: A variety of poloxamer 407-based formulations were analysed regarding the permeation behaviour as well as the antimycotic activity.

The vehicle P4030 showed a gel-like appearance with a ringing effect when the jar was knocked onto a hard surface. The saturation solubility was 5 % CPX. Saturation solubility was defined as the concentration with first appearance of API crystals under a polarising microscope Leica DM LM (Leica Microsystems GmbH, Wetzlar, Germany). With increasing drug concentrations, the poloxamer 407-based formulations became softer. Permeation studies through a keratin film as an artificial nail model showed that fluxes J (amount of permeated drug per area versus time) of the 2-4P4030 formulations were slightly higher (J = 1.4-3.5 $\cdot 10^{-8}$ g/(cm^2·s) than the reference Ciclopoli® (J = 1.1$\cdot 10^{-8}$ g/(cm^2·s). The permeation coefficient (P = flux/drug concentration) was 4.6 – 6.0 times higher than the one determined for Ciclopoli®. Moreover, microbiological studies using the infected nail plate model with the fungus Trichophyton rubrum showed an entire fungal growth inhibition by applying the poloxamer 407-based formulation 1P4030 onto KF and hoof plates (scores 0, n = 8). Due to a high water content of 30 % in the vehicle P4030, swelling of the nail bed and thus an increase in drug permeation is likely to occur. IPA content of 15 % may act as permeation enhancer. Furthermore, as a lipophilic drug of low molecular weight (207.27 g/mol) (3), CPX may be released quickly from a hydrophilic vehicle such as the poloxamer 407-based formulation.

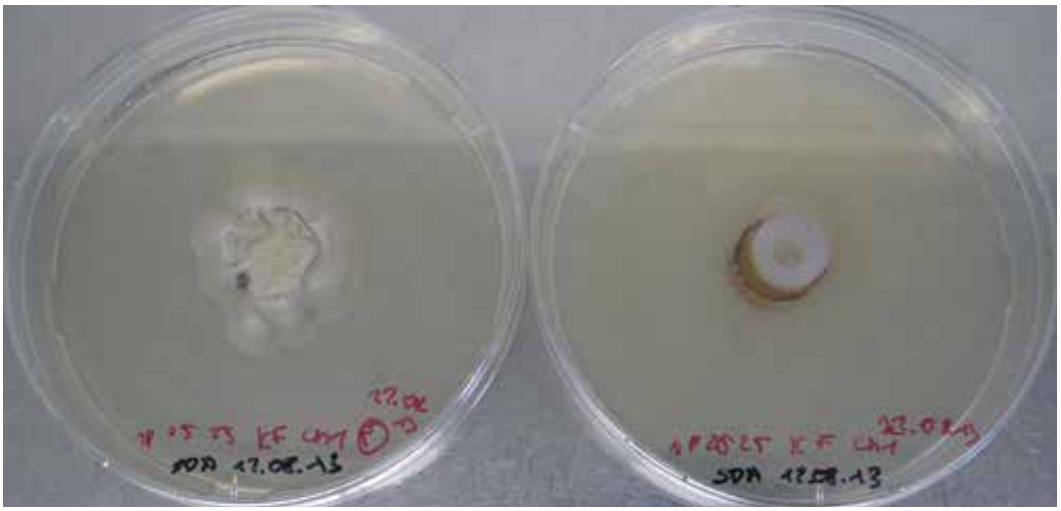

Fig. 1: A: positive control (KF) without treatment (score: 10); B: KF treated with 1P2525 (score: 0)

The vehicle P2525 had a cream-like appearance with a water content of 50 % and an IPA content of 12.5 %, respectively. A maximum of 4 % CPX was dissolved in the vehicle leading to a liquid and translucent macroscopical appearance. Permeation studies through a keratin film indicated a 2.9 – 5.4 times higher flux and an approximately 10 times higher permeation

coefficient in comparison to Ciclopoli®. Regarding the infected nail plate studies, the formulation 1P2525 showed an almost complete fungal inhibition on KF (score 0.125, n = 4) and on hoof plates (score 0.188, n = 8).

Conclusion: The present contribution shows the influence of the vehicle on the in vitro drug permeation. Poloxamer 407-based formulations with low drug content, but higher fluxes and permeation coefficients than the marketed nail lacquer Ciclopoli® were successfully developed. Moreover, microbiological studies showed that P4030 formulations with only 1 % ciclopirox olamine as API were as effective as Ciclopoli® and P2525 with 1 % API were slightly less effective than Ciclopoli® on KF.

In conclusion, we have developed poloxamer 407-based formulations with acceptable permeation behaviour and antifungal activity despite low drug content.

References:

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