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Rapid communication

In vitro evaluation of the antifungal efficacy of poloxamer 407-based formulations in an infected nail plate model

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ABSTRACT

The *in vitro* efficacy of poloxamer 407-based formulations with antifungal ciclopirox olamine has been analysed in an infected nail plate model. As artificial nail plates, keratin films made of human hair keratin and slices from bovine hooves have been utilised. Several poloxamer 407-based formulations with 1 % active ingredient indicated complete growth inhibition of the dermatophyte fungus *Trichophyton rubrum* after 6 days of incubation.

Fungal infections of the nail plate and/or nail bed are called onychomycosis and lead to a stepwise damage of the nail plate (Grover and Khurana, 2012; Murdan, 2002). They account for up to half of all nail disorders (Ghannoum et al., 2000) with an increasing prevalence in the elderly (Gupta et al., 2000). According to a survey by Haneke and Roseeuw (1999), 23 % of the Europeans suffer from fungal nail diseases. Onychomycosis is mostly caused by the dermatophyte fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Ghannoum et al., 2000; Haneke and Roseeuw, 1999). It is not only a medical issue, but also a psychosocial one. Besides the fact that infected nails serve as a fungal reservoir, they likewise cause e.g. pain and embarrassment and thus affect patients' quality of life (Drake et al., 1998). Therefore, an appropriate onychomycosis therapy regime is highly important. However, it still remains a great challenge due to the low permeability of the nail plate, recurrence, fungal resistance and consequently low cure rates (Bseiso et al., 2015; Flores et al., 2016; Piérard et al., 1993).

In this contribution, the antifungal efficacy of various poloxamer 407 (P407)-based formulations has been analysed in an *in vitro* infected nail plate model. The P407-based formulations were 5-component systems consisting of P407, propylene glycol (PG), medium chain triglycerides (MCT) (all purchased from Fagron, Barsbüttel, Germany), double distilled water, isopropyl alcohol (IPA) (received from VWR International, Leuven, Belgium) in given ratios (Täuber and Müller-Goymann, 2015). Since the P407-based formulations are intended for a simultaneous antifungal skin and nail therapy, IPA, MCT and PG were included as skin penetration enhancers (PE). They fluidise the stratum corneum lipids and thus lead to an improved permeation across the membrane. Water served both as nail and skin PE due to hydration. Into these vehicles, antifungal ciclopirox olamine (CPX) (purchased from Fagron, Barsbüttel, Germany) was incorporated. Formulations were given codes, which reflected their quantitative composition, e.g. 1P1050 denoted a formulation containing 1 % CPX, while the vehicle was composed of 10 % P407/MCT (4:1), 50 % IPA/PG (1:1) and 40 % double distilled water (all w/w).

In vitro infected nail plate studies were performed according to Lusiana et al. (2013). The performance of this assay is illustrated in Fig. 1. In brief, bovine hoof plates (thickness: 100 μm) and keratin films (KF) (thickness: 120 μm, manufactured according to Lusiana et al. (2011)) were established as artificial nail plate models and infected with the dermatophyte fungus *T. rubrum* (strain DSM 19959, obtained from German Collection of Microorganisms and Cell Cultures, DSMZ, Braunschweig, Germany) on a potato glucose agar. After 7 days of incubation, the infected membranes were transferred onto a Sabouraud dextrose agar and a polyamide ring was glued with silicone paste (Baysilone-Paste, high viscous from GE Bayer Silicones, delivered by Carl Roth GmbH, Karlsruhe, Germany) onto the membrane. Into the polyamide ring, the formulation was applied. A PET-foil was glued onto this assembly to prevent evaporation of volatile compounds. Therefore, it could be assured that the composition of the applied formulation did not

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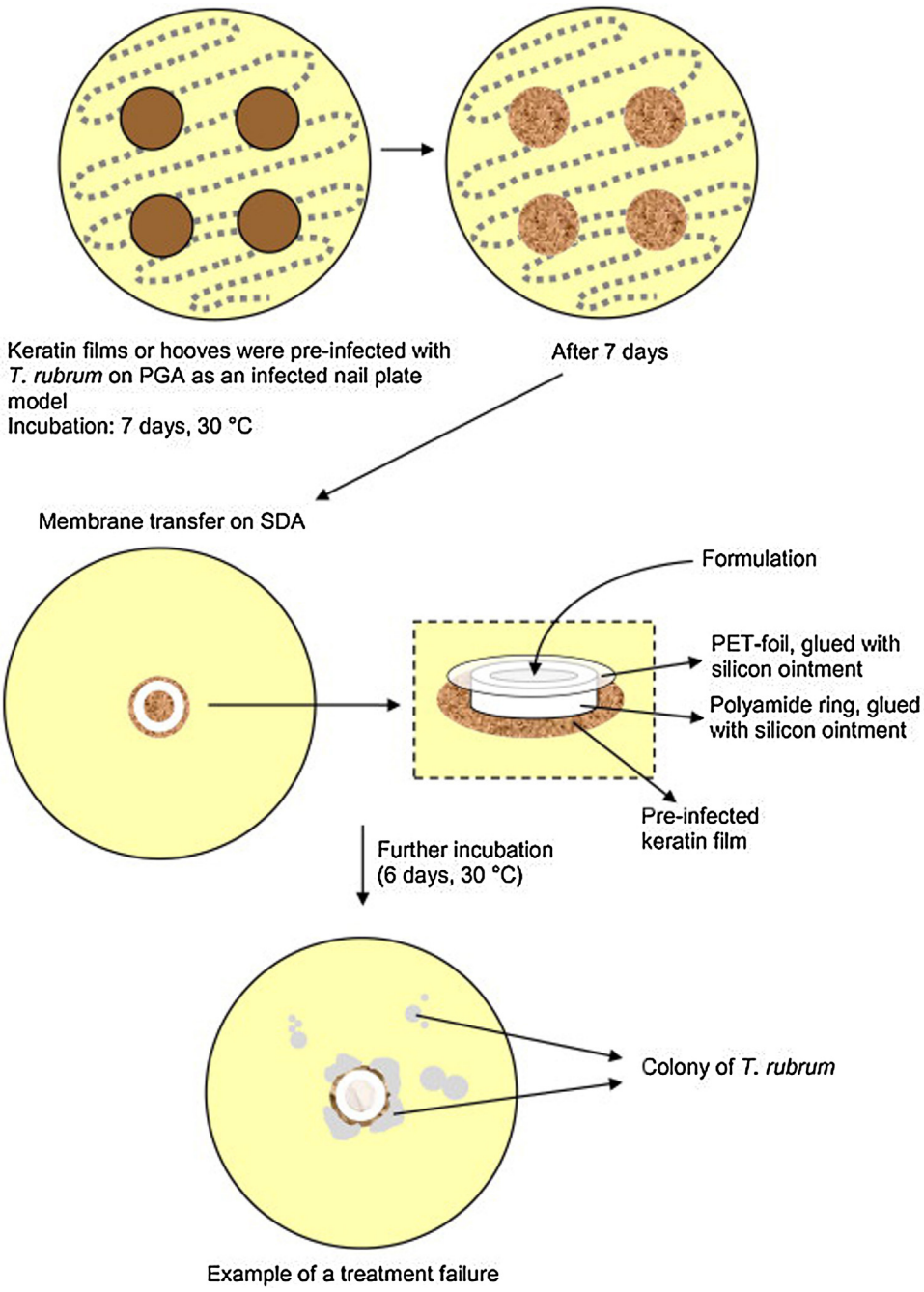

Fig. 1. Performance of the microbiological assay according to Lusiana et al., (2013).

change. After 6 days of incubation, the fungal growth inhibition was evaluated. Results were assessed with a score of 0–10, whereas 10 represented full fungal growth. Assemblies without fungi were scored with 0. Partial fungal growth was evaluated on a percentage basis. For instance, a score of 1 indicated that 10 % of the membrane border was infected. A score of 0.5 was used for fungal growth outside the assembly. All data were illustrated as means ± standard deviations (SD) and medians. Statistical analysis was done by using the software IBM® SPSS Statistics 21. To examine normal distribution for $n < 50$, the Shapiro-Wilk test was carried out. Due to not normally distributed scores, the non-parametrical Mann-Whitney *U* test was utilised. A p value <0.05 was considered statistically significant.

The results of the *in vitro* infected nail plate studies are presented in Tab. 1. Six vehicles without CPX (semi-solid P2525, P4030 and liquid P3040, P1050, P1070, P1070_21) were included in this assay to exclude that the incorporated IPA already inhibits fungal growth. The highest IPA content tested was 46.67 % in P1070_21 comprising an IPA/PG ratio of 2:1. The results of P1070_21 after 6 days of incubation are shown in Fig. 2C. As expected, complete fungal growth on KF and bovine hoof plates was detected for each vehicle (score: 10). In contrast, the liquid formulations 1P1050, 1P1060 and 1P1070 (Fig. 2B) comprising 1 % CPX completely inhibited fungal growth on both nail plate models (score: 0). Augmenting the CPX content to 5 % likewise led to a complete fungal growth inhibition. Moreover, the liquid transparent formulation 1P1050_w/oMCT, which did not contain MCT, was analysed. It also indicated good fungal growth inhibition with a median score of 0. Data on KF and bovine hoof plates were comparable ($p > 0.05$). Therefore, the usage of KF as artificial nail