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Research paper

Dermal miconazole nitrate nanocrystals – formulation development, increased antifungal efficacy & skin penetration

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ABSTRACT

Miconazole nitrate nanosuspension was developed to increase its antifungal activity and dermal penetration. In addition, the nanosuspension was combined with the synergistic additive chlorhexidine digluconate. The production was performed by wet bead milling and both production and formulation parameters were optimized. A stabilizer screening revealed poloxamer 407 and Tween 80 both at 0.15% as the most effective stabilizers for miconazole nanosuspensions at 1.0%. The nanocrystals were incorporated into a hydroxypropyl cellulose gel base. Short-term stability (3 months) of the nanocrystal bulk population could be shown at room temperature and fridge. Besides the stable bulk nanocrystals, some longitudinal crystal growth to needle like crystals occurred. The addition of ionic compounds as the chlorhexidine digluconate often destabilizes suspensions. Surprisingly here, the addition minimized the crystal growth. An underlying mechanism is proposed. An inhibition zone assay was performed using Candida albicans (ATCC® 10231™). When comparing the nanocrystals in suspension and in gel to µm-sized miconazole nitrate formulations and two market products, the increase in inhibition zone diameter for the nanosuspension formulations was most pronounced in the chlorhexidine digluconate free formulations. These nanocrystal formulations were closely or similarly effective as the microsuspensions and the market products containing the synergistic chlorhexidine digluconate, showing the potential of the nanosuspension formulation. Nanosuspension performance was even further increased when chlorhexidine digluconate was added. *Ex-vivo* skin penetration studies on porcine ears revealed distinctly less remaining miconazole nitrate on the skin surface for nanocrystals (*e.g.*, 76–86%) compared to market products (*e.g.* 94%). Also, penetration was increased *e.g.* in skin depth of 5–10 µm from < 1.0/1.7% to *e.g.* 3.3–6.2% for nanocrystals.

1. Introduction

Nanocrystals are one of the most successful nanotechnologies of the last 20 years in pharma (Patel and Shah, 2015; Müller et al., 2011). Invented in 1991 by Liversidge et al. (1992), the first products appeared on the market in 2000, *i.e.* in less than 10 years from invention to the market. Most of the products are for oral administration, an injectable is for example paliperidone palmitate (INVEGA SUSTENNA® from Janssen Pharmaceuticals). Meanwhile the technology was also transferred to the dermal application route with the first cosmetic product appeared on the market in 2007 containing rutin nanocrystals (line JUVEDICAL® from JUVENA) (Petersen, 2015; Mauludin et al., 2009). The same principles of the nanocrystal technology can be also applied to other administration routes, *e.g.* for dermal delivery.

Skin penetration of nanocrystalline actives is increased due to their increased saturation solubility Cs compared to µm-sized crystals (Müller and Keck, 2012), resulting in an increased concentration gradient Cs-Cskin between dermal formulation and skin, subsequently leading to a higher diffusive flux. In addition nanomaterials are highly adhesive leading to an increased residence time on skin (Kocbek et al., 2006). Penetration into the skin is longer lasting, especially important for fungal infections. To benefit from these advantages, miconazole nitrate was formulated as nanosuspension in this study and incorporated into gels for dermal application. For good tolerability, the focus was on skin-friendly stabilizers.

Antifungal therapy with miconazole nitrate is described being much more efficient when combining with chlorhexidine digluconate (Perrins et al., 2005; Perrins and Bond, 2003). In different publications, an up to 4 times increased antifungal effect was measured proving the strong synergy between both compounds (Codd and Deasy, 1998). Thus in this study, nanosuspensions were produced with additive chlorhexidine digluconate. However, the addition of salts can impair the physical stability of suspensions due to zeta potential reduction (Hunter, 2013), especially of highly dispersed nanosuspensions. Therefore, attention

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had to be given to the physical stability of these formulations.

To predict the potential improvement in performance, the nanocrystal formulations (without and with chlorhexidine digluconate) were compared to µm-sized crystal formulations (microsuspensions) and to two market products (here again one without and one with chlorhexidine digluconate). For this comparison *in-vitro* inhibition zone assays (Hultmark, 1998) were performed using Candida albicans (ATCC® 10231™), as well as *ex-vivo* penetration studies by tape stripping test (Weigmann et al., 2001) on porcine ear skin.

2. Materials and methods

2.1. Materials

The two actives miconazole nitrate and 20% aqueous chlorhexidine digluconate solution as well as hydroxypropyl cellulose and glycerol at 85% were purchased from Caesar & Loretz GmbH (Germany). Plantacare® 810 UP and Plantacare® 2000 UP were kindly provided from BASF SE (Germany). The poloxamers 188 and 407 were gifts from BASF SE (Germany). Tween® 80 was bought from Sigma-Aldrich (USA) and Miranol® Ultra C32 from Solvay Novecare (Belgium). Purified water was obtained from a Milli-Q system of Merck KGaA (Germany).

2.2. Production of miconazole nitrate nanosuspension

Miconazole nitrate nanosuspension was produced by wet bead milling (Merisko-Liversidge et al., 2003; Kwade, 1999). First, 100 g of pre-suspension was prepared by dispersing 1.0% miconazole nitrate raw drug powder in a 0.15% aqueous surfactant solution at 20 ± 5 °C, using a magnetic stirrer for 30 min. Subsequently, wet bead milling was processed using a PML-2 equipped with the small milling chamber (Bühler AG, Switzerland) and 570 g of yttria stabilized zirconium oxide beads (Hosokawa Alpine, Germany) with diameters of 0.2 mm. As production parameters 5 ± 2 °C, 2000 rpm of the agitator and 60 min running time were set. After every 5 min 1.5 mL sample was taken for the analysis of the mean particle size and polydispersity index as a function of milling time. All samples were produced again at optimized production parameters and were stored at 4 ± 3 °C, 25 ± 2 °C and 40 ± 3 °C for physical storage stability investigations. The variation behind the desired storage temperature represents the maximum deviation up- and downwards of the true temperature measured every 3rd day.

Particle size was measured directly after production, after 1 week, 1 month and 3 months.

2.2.1. Production of miconazole nitrate nanosuspension with chlorhexidine digluconate

The influence of chlorhexidine digluconate addition at different steps of the production process on the particle size of miconazole nitrate nanocrystals was investigated. Therefore, one sample was prepared by adding the chlorhexidine digluconate solution before bead milling (directly into the pre-suspension), the other sample by preparing a miconazole nitrate nanosuspension concentrate, which was diluted afterwards with a concentrated chlorhexidine digluconate solution. Bead milling, before or after addition of chlorhexidine digluconate solution was performed applying the production parameters described in Section 2.2. Both samples were stored for 3 months at room temperature and particle size analysis was performed by photon correlation spectroscopy (Section 2.4.1) and light microscopy (Section 2.4.2).

2.3. Production of miconazole nitrate nanocrystal hydrogel

The hydrogels were prepared by admixing freshly produced miconazole nitrate nanosuspension (with or without chlorhexidine digluconate) to the gel base using a beaker and magnetic stirrer for 3 h at room temperature. The gel base itself was prepared by dispersing 5% hydroxypropyl cellulose into 70 °C hot purified water. Under stirring with a pestle in an ointment bowl the mixture was cooled down to room temperature. The amount of evaporated water was replaced and as last step 5% glycerol (85% v/v) was incorporated by gently stirring.

To investigate the storage stability of miconazole nitrate nanocrystals in hydrogel, fresh miconazole nitrate nanosuspensions without and with chlorhexidine digluconate were produced applying optimized production conditions (Section 2.2.1). These nanosuspensions consisted of 2.0% miconazole nitrate, 0.3% surfactant and no or 2.0% chlorhexidine digluconate. They were admixed to the above mentioned gel base at a ratio of 1:1. The so prepared hydrogel consisted of 1.0% miconazole nitrate, 0.15% surfactant and if present 1.0% chlorhexidine digluconate. The nanocrystal hydrogels were all stored at 4 ± 3 °C, 25 ± 2 °C and 40 ± 3 °C and particle size investigation was carried out directly after production, after 1 and 3 months.

For both the *in-vitro* inhibition zone assay (Section 2.5) and *ex-vivo* penetration study (Section 2.6) dermally applicable hydrogel formulations had to be produced with identical concentration of miconazole nitrate and chlorhexidine digluconate (if present) as reference products on the market, to be comparable. Therefore, a miconazole nitrate nanocrystal suspension concentrate of 10.0% was prepared stabilized with 1.5% surfactant, and if required 10.0% chlorhexidine digluconate. This concentrate was always prepared freshly and diluted to the required concentration before incorporating into the gel base. For the *in-vitro* inhibition zone assay (Section 2.5) hydrogels with µm-sized miconazole nitrate raw drug powder were additionally prepared by admixing the pre-suspension directly into the gel base, skipping the bead milling process. In addition two market products were used as reference, one with 2.0% miconazole nitrate as µm-sized powder incorporated into an ointment base and another with additional chlorhexidine digluconate at 2.0%.

2.4. Characterization of miconazole nitrate nanocrystals and hydrogel

2.4.1. Photon correlation spectroscopy (PCS)

The intensity weighted mean diameter of the nanocrystals (z-average) and the related width of their size distribution (polydispersity index, PdI) were analyzed by PCS using a Zetasizer Nano ZS (Mavern Instruments, UK). For the measurement, samples were prepared by diluting the miconazole nitrate suspensions with purified water at 25 °C. Measurement was performed 10 times and the average calculated as result.

2.4.2. Light microscopy (LM)

To investigate the shape and size distribution of miconazole nitrate nanocrystals, microcrystals and potential aggregates in suspensions and hydrogels, LM was performed at 100, 400 and 1000 fold magnification using a Motic BA210 LED equipped with a digital camera Moticam 3.0 MP (both from Motic Deutschland GmbH, Germany). The mean Feret diameter as well as the frequency of crystals and aggregates larger than 1 µm were evaluated by the software ImageJ (Collins, 2007).

2.4.3. Zeta potential (ZP)

For the prediction of the physical long-term stability of produced miconazole nitrate nanocrystal suspensions, ZP was measured using a Zetasizer Nano ZS (Malvern Instruments, UK) with single-use cuvettes. The electrophoretic mobility of the suspensions was investigated in two different media: original dispersion medium of the suspensions and conductivity adjusted water to 50 µS/cm and pH 5.5. For the measurement 80 µL of each suspension was diluted in 2.0 mL of media. To obtain the ZP from the measured electrophoretic mobility the Helmholtz-Smoluchowski equation was used.

2.5. In-vitro inhibition zone assay

To investigate the antifungal efficacy in dependence on miconazole

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