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A new topical formulation for psoriasis: Development of methotrexate-loaded nanostructured lipid carriers

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ABSTRACT

The aim of the present work was to develop and assess the potential of nanostructured lipid carriers (NLCs) loaded with methotrexate as a new approach for topical therapy of psoriasis. Methotrexate-loaded NLCs were prepared via a modified hot homogenization combined with ultrasonication techniques using either polysorbate 60 (P60) or 80 (P80) as surfactants. The produced NLCs were within the nanosized range (274–298 nm) with relatively low polydispersity index (<0.25) and zeta potential values around −40 mV. NLCs demonstrated storage stability at 25 °C up to 28 days. The entrapment efficiency of methotrexate in NLC-P60 and -P80 was ~65%. Cryo-SEM images showed the spherical shape of the empty and methotrexate-loaded NLCs. FT-IR confirmed methotrexate presence within the NLCs. The *in vitro* release of methotrexate from the NLCs followed a fast release pattern reaching ~70% in 2 h. *In vitro* skin penetration study demonstrated that methotrexate-loaded NLCs-P60 had higher skin penetration when compared to free methotrexate, suggesting a significant role of drug-nanocarriers on topical administration. Methotrexate-loaded NLC-P60 provided drug fluxes of 0.88 μg/cm^2/h, higher ($P < 0.001$) than with the free drug (control, 0.59 μg/cm^2/h). The results indicate the potential of NLCs for the delivery of methotrexate to topical therapy of psoriasis.

1. Introduction

Psoriasis is a chronic inflammatory skin disease that affects 1 to 3% of the world population, with equal gender distribution (O'Daly, 2011; Raho et al., 2012). Incidence rates vary from 50 to 140 new cases per 100,000 people per year (Raho et al., 2012). It is associated with high levels of distress and morbidity, as well as a general decrease in the quality of life of the patient, even though it is not usually life-threatening. Nonetheless, severe psoriasis increases the risk of mortality, in comparison to the general population (Chandran and Raychaudhuri, 2010).

Psoriasis is a life-long disease and the management and treatment of psoriasis are different depending on the severity of the disease. The first line of active treatments for psoriasis is the use of topical agents (Chong et al., 2013). This type of therapy is typically sufficient in the management of mild to moderate psoriasis (Murphy and Reich, 2011), namely when this disease affects less than 10% of the body surface area (Mitra and Wu, 2010). Topical agents comprise coal tar (Bhatia et al., 2011) and dithranol (Rahman et al., 2012), corticosteroids (Horn et al., 2010), vitamin D analogs (Kamangar et al., 2013) and retinoids (Murphy and Reich, 2011), as well as keratolytic agents such as salicylic acid (Paul et al., 2012).

When the effects arising from topical therapies strategies are suboptimal or when the extent of the disease makes it unfeasible for the use of topical therapy, phototherapy and systemic therapy may need to be considered (Laws and Young, 2012). Currently available systemic therapies for psoriasis include non-biological and biological therapies. As previously stated, these are commonly used as monotherapies or in combination with other modalities of treatment in patients with moderate to severe psoriasis (body surface area higher than 10%) (Chong et al., 2013). The most prominently used non-biological systemic agents are methotrexate (MTX), cyclosporine and orally administrated retinoids (such as acitretin) (Chong et al., 2013; Laws and Young, 2012). Current therapeutic strategies for the treatment of psoriasis generally employ oral and parenteral administration routes for MTX as it inhibits epidermal cell proliferation (Shen et al., 2012) and has anti-inflammatory action at low doses (Micha et al., 2011; Shen et al., 2012). It should be noted that there is a large number of adverse effects (such as liver toxicity, gastrointestinal side-effects, including nausea, vomiting, diarrhea and stomatitis) associated to

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systemic administration of MTX (Micha et al., 2011). Nevertheless, some side-effects can be minimized by the concomitant supplementation of folate to the patient (Micha et al., 2011; Montaudié et al., 2011).

In the scope of the management and treatment of psoriasis, nano-dermatology and the development of nanoparticles for dermatological applications is without a doubt an area of increasing magnitude and interest (Saraceno et al., 2013). Drug carriers can provide a sustained drug release over a prolonged period of time, (Papakostas et al., 2011) and shields it from degradation. Hence, therapeutic effect can be maximized and toxicological concerns related to drug overdose and clearance can be minimized (Gupta et al., 2012). Additionally, patient compliance is higher, as these therapeutical strategies enable a reduction in the frequency of drug administration (Papakostas et al., 2011).

Lipid nanoparticles, as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are frequently used to incorporate and deliver lipophilic drugs (Kumar and Randhawa, 2013). Lipid nanoparticles are produced with generally regarded as safe (GRAS) lipids and surfactants. Other advantages include low production cost, easy to scale up and additionally low toxicity, as organic solvents are not required for the synthesis of these nanoparticles. NLCs are composed of solid and liquid lipids which allows the formation of an overall amorphous nanostructure with many imperfections within its matrix, providing NLCs with higher drug capacity and a lesser degree of drug expulsion during storage then SLNs (Wang et al., 2013). In the scope of topical administration, formulations of NLCs are characterized by their occlusive ability of creating a mono-layered lipid film onto the skin, thereby avoiding water evaporation and increasing skin moisture and hydration and, consequently, drug permeation (Gupta et al., 2012; Kumar and Randhawa, 2013). Indeed, literature concerning the topical administration of drug-carrying nano-systems as therapeutic strategies for psoriasis presents interesting examples of the application of lipid nanoparticles (Agrawal et al., 2013, 2010; Lin et al., 2010; Pradhan et al., 2013; Raza et al., 2013). Recently novel strategies for preparing SLNs *in situ* using electrospraying have been described, to overcome resolving problems associated with the formulation of poorly water-soluble drugs (Yu et al., 2011a,b).

Overall, the present work intends to present NLCs formulations that would be pertinent and efficacious for the dermal treatment of psoriasis. The success associated with the use of MTX by dermal application would also be mirrored in increased patient compliance, as topical administration of therapeutic substances constitutes a much less invasive and more comfortable and convenient route of administration.

2. Materials and methods

2.1. Materials

Witepsol® S51 was kindly provided by Cremer Oleo (Hamburg, Germany), oleic acid was purchased from May & Baker Ltd. (Dagenham, England) and polysorbates 60 (Tween 60) and 80 (Tween 80) were obtained from Merck (Darmstadt, Germany). All chemicals and solvents were of analytical grade. Aqueous solutions were prepared with double-deionized water (Arium Pro Sartorius AG, Göttingen, Germany, conductivity less than 0.1 $\mu S\,cm^{-1}$). MTX was kindly provided by Excella (Feucht, Germany) as a gift.

2.2. Preparation of NLCs

MTX-loaded NLCs were prepared by high-shear homogenization followed by the ultrasound method. Briefly, the lipid phase was prepared by melting Witepsol® S51 at 50 °C, then adding oleic acid, the surfactant (polysorbate 60, P60 or polysorbate 80, P80), and finally 1 mg of MTX was added to the hot solution. The melted lipid phase was dispersed in pre-warmed 50 °C double-deionized water to obtain a microemulsion by high-shear homogenizer (YSTRAL GMBH X10/20-E3, Ballrechten-Dottingen, Germany) at 12,000 rpm/min for 2 min. This microemulsion was then homogenized with a probe-type sonicator (VCX130, Sonics & Materials, Newtown, CT, USA) for 15 min with frequency amplitude of 70% in order to obtain a nanoemulsion. It was then cooled at room temperature, allowing the inner oil phase to solidify forming NLCs dispersed in the aqueous phase. As a control, drug-free NLCs were prepared in a similar manner without the addition of MTX to the lipid phase. The compositions of the NLCs are given in Table 1. The produced NLCs were transferred into glass vials and then freeze-dried using a Modulyo 4K freeze-dryer from Edwards (Crawley, West Sussex, U.K.) at 0.09 mbar for 72 h, with a condenser surface temperature of -60 ± 5 °C.

2.3. Determination of entrapment efficiency

Entrapment efficiency (%EE) of MTX in NLCs was determined by UV spectrophotometry. A 1:50 dilution of NLC formulations in double-deionized water was subsequently centrifuged (Heraeus™ Multifuge™ ×1R Centrifuge, USA) through centrifugal filter units (Amicon® Ultra Centrifugal Filters, Ultracel – 50 KDa, Darmstadt, Germany) at $2260 \times g$, 20 °C during 30 min or until complete separation between the NLCs retained in the filter unit and the aqueous phase corresponding to the filtrate. The filtrate was used to quantify the amount of non-incorporated MTX by UV–vis spectrophotometry (Jasco V-660 Spectrophotometer, USA) at λ_{max} 303 nm, which is the maximum absorption of MTX in aqueous solution (Lin et al., 2010). A standard curve of MTX in water was used to determine the concentration of MTX and the results are expressed as mean ± standard deviation ($n = 3$).

The results were compared to drug-free NLCs used as control. Taking into account the drug initially added to the NLCs formulation and subtracting the free MTX remaining in the filtrate, it was possible to determine the amount of drug incorporated in the NLCs and thus the entrapment efficiency by the following equation:

$$\%\text{EntrapementEfficiency} = \frac{\text{Total amount of MTX} - \text{free MTX in the filtrate}}{\text{Total amount of MTX}} \times 100$$

Table 1
Composition of the developed NLCs formulations.

Formulation code	Witepsol S51	Oleic acid	Polysorbate 60	Polysorbate 80	MTX
NLC-P60	700	300	200	–	–
MTX_NLC-P60	694	300	200	–	6
NLC-P80	700	300	–	200	–
MTX_NLC-P80	694	300	–	200	6

Amounts expressed in mg and all formulations were prepared in 8.8 mL of Milli-Q water.

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