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Development of microemulsions of suitable viscosity for cyclosporine skin delivery

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ABSTRACT

Psoriasis is a widespread chronic disease affecting 2–4% of the population in Western countries. Its mild-to-moderate form, representing approximately 80% of the total cases, is treated by topical application, with corticosteroid being the standard treatment. However, in case of psoriasis, no single treatment works for every patient and optimizing topical therapy is a key aspect. A possible alternative is represented by cyclosporine, an immunosuppressant cyclic peptide administered orally in the treatment of the severe form. Its topical application could avoid the problems related to systemic immunosuppression, but the unfavourable physico-chemical properties (MW: 1202 Da; LogP ≈ 3) hinder its permeation across the stratum corneum. The aim of the paper was the preparation, characterization and *ex-vivo* evaluation of cyclosporine loaded microemulsions using oleic acid as oil phase, either Tween®80 or a soluble derivative of vitamin E (TPGS) as surfactants and either Transcutol®, propylene glycol or 1,3 propanediol as co-surfactants. The issue of formulation viscosity was also addressed 1) by evaluating the thickening of Tween®80-based microemulsions by direct addition of different rheological modifiers, 2) by building pseudo-ternary phase diagrams using TPGS, to identify the water/oil/surfactants proportions resulting in viscous self-gelifying systems. Nine formulations (five Tween®80-based and four TPGS-based) were selected, characterized in terms of droplets size (low viscosity systems) or rheological properties (high viscosity systems), loaded with 6 mg/g cyclosporine and applied *ex-vivo* on porcine skin for 22 h. A relevant skin accumulation was obtained either with a low-viscosity Tween®80-based microemulsion (9.78 ± 3.86 μg/cm^2), or with a high viscosity TPGS-based microemulsion (18.3 ± 5.69 μg/cm^2), with an increase of about 3 and 6 times respectively for comparison with a control cyclosporine solution in propylene glycol. The role of water content, surfactant, co-surfactant and viscosity was also addressed and discussed. The kinetic of skin uptake from the best performing formulation was finally evaluated, highlighting a relatively quick skin uptake and the achievement, after 2 h of contact, of potentially therapeutic cyclosporine skin concentrations.

1. Introduction

Psoriasis is a chronic inflammatory and autoimmune skin condition affecting 2–4% of the population in Western countries. It is characterized by the presence of plaques of thickened and erythematous skin with white scales, due to the hyperproliferation of epidermal keratinocytes. This excessive proliferation is triggered by inflammatory mediators such as interferon-γ, interleukin (IL)-17 and IL-22, secreted by T lymphocytes (Di Meglio et al., 2014). The treatment of this condition differs depending on disease severity, assessed by the appearance and extension of the plaques. For severe psoriasis, the systemic treatment is necessary; together with the classic immunosuppressive agents, such as cyclosporine, retinoids, and methotrexate, new therapeutic options are now available, and include both “biologics” (anti T-cells and anticytokine) and small drugs (tofacitinib, apremilast). In the case of mild-to-moderate psoriasis, representing approximately 80% of the patients affected by this disease, topical treatment is recommended (Menter et al., 2009). The main therapeutic options include corticosteroids, Vitamin D analogues, tazarotene, tacrolimus and picrolimus, with corticosteroid being the standard treatment for most patients. Indeed, corticosteroids are versatile drugs, being available on the market in different potency, strength and formulations. However, they have important local and systemic side effects and are not always efficacious for psoriasis. Indeed, no single treatment works for every patient and optimizing topical therapy is a key aspect of psoriasis treatment as witnessed by the development of new drugs for topical application

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(Papp et al., 2016; Chiricozzi et al., 2014) and by the combinations used for added efficacy (Koo et al., 2017).

A considerable literature has grown up around the possibility of administering cyclosporine by topical application, as an alternative therapeutic tool. This administration route could avoid the problems related to systemic immunosuppression, but the unfavourable physico-chemical properties of cyclosporine (MW: 1202 Da; LogP ≈ 3; low water solubility) hinder its permeation across the stratum corneum. Indeed, up to now, topical cyclosporine for psoriasis treatment has been limited by the lack of formulations providing sufficient skin absorption (Burns et al., 1992; Hermann et al., 1988). Recently, a small therapeutic exploratory study highlighted the possibility of an efficient skin delivery: Kumar et al. (2016) demonstrated that 2% topical cyclosporine liposomal formulation included in a Carbopol® gel was effective in the treatment of limited chronic plaque psoriasis, contrarily to a traditional o/w emulsion, supporting the importance of the formulation on cyclosporine skin delivery.

Given the interest for the topical application of this molecule, several approaches have been evaluated by different researchers worldwide. Chemical enhancers were investigated such as ethanol, ethyl oleate, Transcutol®, propyleneglycol, azone (Liu et al., 2006), decylmethylsulfoxide (Choi et al., 1995), monoolein (Lopes et al., 2005) and skin penetrating peptides (Chen et al., 2015; Kumar et al., 2015). Also physical enhancing technique were evaluated, in particular electroporation (Wang et al., 1998), and ultrasounds (Liu et al., 2006). Among nanocarriers, nanoparticles (Romero et al., 2016; Frusic-Zlotkin et al., 2012; Musa et al., 2017), liposomes (Niemiec et al., 1995; Verma and Fahr, 2004; Guo et al., 2000) and micelles (Lapteva et al., 2014) were investigated. Other authors have evaluated the possibility to deliver cyclosporine A by using nanoemulsions (Musa et al., 2017), microemulsions (Liu et al., 2009, 2007) and/or liquid crystalline systems (Lopes et al., 2006a,b). Indeed microemulsions (ME) (i.e. transparent single-phase systems generally composed of a blend of oil, water a surfactant and a co-surfactant) could be particularly advantageous for cyclosporine delivery, due to their capability to solubilize highly hydrophobic drugs. Furthermore, ME offer the advantage of spontaneous formation and thermodynamic stability and have widely demonstrated the capability to enhance drug uptake into the skin. However, a limitation in the use of ME for skin delivery is represented by the very low viscosity, that makes skin application difficult. It is worth underlying that rheological properties play a crucial role in dermal administration, since viscosity not only favours the retention of the formulation on the skin surface but can also slow down water evaporation maintaining for a longer period of time the ME structure and thus its peculiar enhancing properties. Additionally, the rheological properties of a dermal formulation have an impact on patient's acceptability and adherence, a very relevant issue in psoriasis treatment (Devaux et al., 2012; Marty et al., 2005; Alisa et al., 2017).

Different approaches can be followed to increase the viscosity of a microemulsion: 1. mix of the ME with an already prepared gel (Zhao et al., 2014; Lopes, 2014) 2. direct addition of a thickening agent to the ME (Sabale and Vora, 2012; Chen et al., 2006) 3. use of specific excipients able to give high viscosity systems, often associated to the formation of lamellar, cylindrical or worm-like structures (Hosmer et al., 2011; Telo et al., 2017; Hosmer et al., 2013; Milak and Zimmer, 2015; Lawrence and Rees, 2000).

The aim of this work was the development of ME of suitable viscosity for cyclosporine skin delivery using oleic acid as oil phase and either Tween®80 or TPGS (D-α-Tocopheryl polyethylene glycol 1000 succinate) as surfactants. Tween®80-based microemulsion, prepared using Transcutol® as co-surfactant, were simply added of different rheological agents. In case of TPGS-based systems, pseudo-ternary diagrams were built to assess the ratio between water, oil phase and surfactant – co-surfactant mixture (Smix), necessary to obtain self-gelifying formulation. In this case, three different co-surfactants, namely Transcutol®, propylene glycol and 1,3 propanediol, were evaluated. The formulations were characterized and evaluated for cyclosporine skin uptake.

2. Materials and methods

2.1. Materials

Cyclosporine ($C_{62}H_{111}N_{11}O_{12}$, MW 1202.61 Da crystalline solid) was from ThermoFisher Scientific (Karlsruhe, Germany). D-α-Tocopheryl polyethylene glycol 1000 succinate (Kolliphor® TPGS, MW 1513 Da) and poloxamer 407 (Pluronic® F127, MW 12.6 kDa) were a kind gift from BASF (Ludwigshafen, Germany). Trifluoroacetic acid (TFA, MW 114.02 Da), albumin from bovine serum (≈66 kDa, ≥96%), 1,3-propanediol (MW 76 Da) and alginic acid sodium salt were purchased from Sigma Aldrich (St. Louis, MO, USA). Oleic acid was from Alfa Aesar (Karlsruhe, Germany), Transcutol® was a gift from Gattefossè (Lyon, France). Sodium hyaluronate (MW 1000 kDa) was a gift of IBSA Farmaceutici S.p.A (Lodi, Italy). 1,2-propanediol (MW 76 Da) was purchased from A.C.E.F. S.p.A. (Fiorenzuola d'Arda, Italy). Aerosil® 200 Pharma (fumed silica) was from Evonik Industries (Essen, Germany). Carbopol® 940 was purchased from The Lubrizol Corporation (Wiekliffe, OH, Usa). For HPLC analysis, pure water (Purelab® Pulse, Elga Veolia, UK) and HPLC grade acetonitrile were used. Phosphate-buffered saline (PBS) composition was 0.19 g/l KH_2PO_4, 5.98 g/l $Na_2HPO_4{\cdot}12H_2O$, 8.8 g/l NaCl, pH 7.4

2.2. Cyclosporine quantification method

Cyclosporine was quantified by HPLC-UV (Infinity 1260, Agilent Technologies, Santa Clara, CA, USA), with a reverse-phase Nova-Pack C_{18} cartridge (150 * 3.9 mm, 4 μm) (Waters, Milford, Massachusetts, USA) and a C_{18} guard column (3.2 * 0.8 mm, Security Guard™ Cartridge, Phenomenex, Torrance, USA) both thermostatted at 65 °C. The mobile phase, pumped at 1.6 ml/min, was a 65:35 (v/v) mixture CH_3CN:water with TFA 0.1%. The injection volume was 100 μl, and absorbance was monitored at 230 nm. In these conditions, cyclosporine retention time was about 5 min. The method was previously validated in the concentration interval 0.25–50 μg/ml (Grimaudo et al., 2018).

2.3. Pseudo-ternary phase diagram construction

Pseudo-ternary phase diagrams were built to identify the microemulsion region in multiphasic systems. Oleic acid was used as oil phase, and a 1/1 (w/v; g/ml) mixture of TPGS and co-surfactant (either Transcutol®, 1,2-propanediol or 1,3-propanediol) was used as surfactant system (Smix). The diagrams were built using the aqueous titration method: for fixed ratios oil/Smix (0.5/9.5, 1/9, 1.25/8.75, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2, 9/1) increasing amounts of water, between 5 and 95%, were added. After each addition, the mixture was vortexed and left 1 min to rest, then by visual observation the viscosity and clearness of the system were evaluated. In case of highly viscous mixtures, the system was heated in a thermostatted bath at 50 °C before each water addition in order to reduce the viscosity and favour the mixing by vortex to achieve homogeneity. The evaluation of the system was performed after cooling at room temperature. The formulation is clear and exhibits low viscosity in the microemulsion region, while it is clear and viscous in the microgel region where the formulation does not slide along the vial walls. The diagrams were built using OriginPro® 2016 (Originlab, Northampton, MA, USA).

2.4. Thickening of Tween®80-based microemulsions

Tw20T (composition in Table 1) was prepared by mixing the different components into a glass vial, under magnetic stirring, in the following order: oil phase, co-surfactant, surfactant and water. Then, the thickener was added and the mixture was slowly magnetically