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Research paper

Dermal targeting using colloidal carrier systems with linoleic acid

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ABSTRACT

In the basic therapy of chronic skin diseases characterized by xerosis, the local treatment is an essential strategy to reach ideal therapeutic effects. Suitable active ingredients for this aim are fatty acids, in particular linoleic acid, which is an essential component for the organization and perpetuation of the skin barrier. In the present work, the development of a well-tolerated colloidal carrier system (microemulsion) containing linoleic acid as active ingredient is described. A comprehensive physiochemical characterization of the novel microemulsion system was performed using different techniques. The potential of the developed microemulsion system compared to a cream as suitable carrier for the dermal delivery of linoleic acid was determined. Penetration studies showed higher linoleic acids concentrations after administration of the colloidal carrier system in all skin layers independent of the time of incubation. Up to 23% of applied dose reached the skin from the colloidal carrier system whereas at most 8% of the active ingredient could be detected after applying the cream. Particularly, the percentage of the linoleic acids penetrated through the microemulsion in the stratum corneum and the viable epidermis differed significantly ($p < 0.01$) when compared to that through a standard cream. Furthermore, linoleic acids accumulated in the epidermis at longer incubation times. Using the microemulsion, the penetration of linoleic acids was enhanced significantly ($p < 0.01$). Hence, the microemulsion might be an innovative vehicle for the delivery of linoleic acids to the epidermis improving its use as their barrier regeneration and providing possible anti-inflammatory effects.

1. Introduction

Since the skin is the largest organ of the body, it is an important target site for the application of drugs aside from its physiological functions. In the treatment of local diseases, topical drug delivery is a well-suited strategy to restrict the therapeutic effect to the affected area and to reduce systemic side effects. Additionally, transdermal delivery of drugs is an appropriate possibility to minimize the first-pass-effect or avoid gastrointestinal side effects. However, in order to reach therapeutic concentrations in the epidermis and/or in the dermis or even in the systemic circulation, the skin barrier has to be overcome.

In the basic therapy of chronic skin diseases characterized by xerosis, the local treatment with emollients is essential to prevent exacerbation of the disease. Hereby, the preservation and regeneration of the skin barrier is an important factor to increase hydration, to reduce transepidermal water loss and to avoid penetration of pathogens, e.g. bacteria or allergens. Well-established active pharmaceutical ingredients to achieve this are unsaturated fatty acids, components of the intercellular lipid matrix of the stratum corneum and thus contributing to the arrangement of its bilayers. Moreover, in particular, linoleic acid is an essential component for the organization and perpetuation of the skin barrier [1–3]. Aside from the possible integration of free linoleic acid in the lamellar bilayers, it is an essential part of the synthesis of ceramide [EOH], ceramide [EOP] and ceramide [EOS]. Ceramide [EOS] is known to play a key role in the organization of the bilayer structure and barrier function of the stratum corneum [4–7]. The synthesis of linoleic acid containing ceramides takes place in the odland bodies, located in the viable epidermis [8]. In addition to the simple substitution of linoleic acid, due to its chemical structure linoleic acid exerts multiple effects on cell membranes, epidermal barrier and even inflammatory processes [9–11]. Regarding the anti-inflammatory effects, evidence suggests that linoleic acid interferes with the prostaglandin and leucotriene synthesis and also binds to peroxisome proliferator activator receptors by unsaturated fatty acids or its metabolites [9,12,13].

The physicochemical properties of linoleic acid are not optimal for epidermal administration. It represents a highly lipophilic molecule with a very high partition coefficient ($\log P = 7.180 \pm 0.256$). The molar mass is 280.45, and its calculated p*K*a value is

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4.78 ± 0.10 [14]. A novel approach to deliver high contents of linoleic acid into the stratum corneum as well as into the viable epidermis is the application of colloidal carrier systems such as microemulsions (ME). These colloidal carrier systems are well described in literature as suitable vehicle for topical administration of drugs. Microemulsions are thermodynamically stable colloidal carriers of oil and water, stabilized by surfactants and in some cases additionally by co-surfactants. They are single, optically isotropic, transparent or slightly opalescent solutions of low viscosity with Newtonian behavior and can almost form spontaneously without any energy input. Microemulsion systems provide small particle sizes of the colloidal phase (5–100 nm) and due to their fluctuating interface various resulting microstructures [15–17]. The excellent drug delivery potential as well as the solubilization capacity is attributable to a variety of factors depending on the composition and the resulting microstructure, but in general, continuously and spontaneously fluctuating interfaces of microemulsions enable high drug mobility and might enhance the drug diffusion process [18,19]. Hereby, the application of microemulsion systems that feature several advantages like renewable raw materials, high skin tolerability and environmental compatibility is an innovative concept for the development of customized drug carrier especially in the treatment of diseased skin [20].

In the present work, the development of a well-tolerated colloidal carrier system containing linoleic acids as the active ingredient, alkylpolyglucosides (APG) acting as very mild surfactant system and 1,2-pentylene glycol as co-solvent is described. A comprehensive characterization of the novel microemulsion system was performed by constructing the pseudoternary phase diagram and regarding dynamic viscosity, electric conductivity as well as performing differential scanning calorimetry investigations. Additionally, the size of the colloidal phase was determined by dynamic light scattering. HET CAM was carried out to evaluate the irritating potential of the system. With regard to the linoleic acids, their stability was evaluated in the microemulsion. In this study, the potential of the developed microemulsion system as suitable vehicle for the dermal delivery of linoleic acid and thereby for an effective and cosmetically improved therapy of dry skin was determined. Therefore, penetration profiles on full thickness human skin of linoleic acids were achieved after applying the microemulsion compared to the penetration profile following the application of a standard vehicle containing the same active pharmaceutical ingredient.

2. Experimental

2.1. Materials

Linoleic acids, containing 25% 9,12-octadecadienoic acid and 65% 9,11-octadecadienoic acid, with a purity of 90% were purchased from Oelon GmbH (Emmerich am Rhein, Germany). Cognis GmbH (Duesseldorf, Germany) donated Plantacare 1200 UP, and Evonik Goldschmidt GmbH (Essen, Germany) kindly offered Tegocare CG 90. 1,2-Pentylene glycol was obtained by courtesy of Symrise GmbH & Co. KG (Holzminden, Germany). Dr. Straetmans GmbH (Hamburg, Germany) kindly offered the antioxidant ascorbyl palmitate. Butylated hydroxytoluene and trifluoroacetic acid were purchased from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany). The standard vehicle containing the same ingredient as the microemulsion was a water-in-oil emulsion. The internal standard mometasone furoate was purchased from Almirall Hermal GmbH (Reinbek, Germany). HPLC grade acetonitrile and methanol were obtained from J.T. Baker, Mallinckrodt Baker B.V. (Deventer, The Netherlands). Merck KgaA (Darmstadt, Germany) supplied buffer substances. Octanol was supplied by Gruessing GmbH (Filsum, Germany). Water was of bidistilled quality. Human breast skin was kindly offered by Department of Dermatology with approval of the independent ethics committee of Faculty of Medicine at the Martin Luther University Halle-Wittenberg.

2.2. Construction of the phase diagram

The phase diagram was constructed at a fixed surfactant/co-surfactant mass ratio of Plantacare 1200 UP and Tegocare CG 90 of 3:1. Since Plantacare 1200 UP contains 51.5% active substance and 48.5% water, the mass ratio was corrected only considering the active substance to 1.545:1. Different systems were prepared by shaking the mixtures of 1,2-pentylene glycol in a fixed concentration of 20%, the surfactant system, the linoleic acids and water in appropriate ratios. Increments of 2.5–5% were chosen to determine the phase borders. All mixtures were stored at room temperature for 6 months for equilibration. Afterwards, the samples were investigated visually and by polarization light microscopy (Zeiss Axiolab Pol, Carl Zeiss MicroImaging GmbH, Jena, Germany). Microemulsions were identified as transparent, low-viscous and optical isotropic systems.

2.3. Viscosity measurements

Dynamic viscosity of the microemulsions was investigated using a rotational rheometer equipped with a cylindrical measuring cell and double slit (Anton Paar GmbH, Graz, Austria). Equalization of temperature was performed with a peltier cell (Anton Paar GmbH, Graz, Austria). Measurements were accomplished at a temperature of 25 °C ± 0.2 °C and shear rates ranging from 0.1 s^{-1} to 100 s^{-1}.

2.4. Conductivity measurements

Electrical conductivity measurements were performed at room temperature using Cyberscan CON 11 instrument (Eutech Instruments Europe B.V., Nijkerk, The Netherlands) with a cell constant of 1.0 cm^{-1}. Values, which remained stable for 1 min, were noted.

2.5. Differential scanning calorimetry

DSC investigations were carried out using a DSC 200 (Netzsch-Gerätebau GmbH, Selb, Germany). The samples (approx. 15 mg) were accurately weighed in and filled in aluminum pans. These were hermetically sealed to avoid water evaporation. DSC curves were achieved by cooling the samples from 40 °C to −60 °C with a cooling rate of 10 K min^{-1}. After equilibration of 5 min at −60 °C, the samples were heated to 120 °C with a heating rate of 10 K min^{-1}. An empty pan was used as reference. Nitrogen with a flow of 10 ml min^{-1} was applied as purge gas.

2.6. Dynamic light scattering

In advance of the DLS investigations, the samples were filtrated through a syringe filter (pore size 0.45 μm) (Rotilabo Nylon-Spritzenfilter, Carl Roth GmbH & Co. KG Karlsruhe, Germany). The measurements were realized at a temperature of 25.0 °C using a compact-goniometer ALV/SP 86 (ALV-Laser Vertriebsgesellschaft mbH, Langen, Germany) equipped with a Nd:YAG-Laser (ADLAS GmbH, Weil im Schönbuch, Germany) and a wavelength of 532 nm and an output of 140 mW.

All measurements were made at thirteen different scattering angles between 30° and 140°. The cylindrical sample cells consisted of Suprasil quartz glass by Hellma (Muellheim, Germany) and had a diameter of 10 mm. The necessary refractive index of the main phase for size calculation of the colloidal phase was ob-