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Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema—An in vivo study

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Abstract

Dry skin and other skin disorders such as atopic dermatitis are characterized by impaired stratum corneum (SC) barrier function and by an increase in transepidermal water loss (TEWL) leading to a decrease in skin hydration. The possibility that dermatological and cosmetic products containing SC lipids could play a part in the restoration of disturbed skin barrier function is of great interest in the field of dermatology and cosmetics. The aim of the present study was to evaluate the effect of positively charged oil/water nanoemulsions (PN) containing ceramide 3B and naturally found SC lipids (PNSC) such as ceramide 3, cholesterol, and palmitic acid on skin hydration, elasticity, and erythema. Creams of PNSC were compared to PN creams, to creams with negatively charged o/w nanoemulsion and SC lipids (NNSC) and to Physiogel® cream, a SC lipid containing formulation, which is already on the market. The formulations (PN, PNSC, and NNSC) were prepared by high-pressure homogenization. After adding Carbopol 940 as thickener, particle size and stability of the creams were not significantly changed compared to the nanoemulsions. The studies were carried out on three groups, each with 14 healthy female test subjects between 25 and 50 years of age, using Corneometer® 825, Cutometer® SEM 575 and Mexameter® 18 for measurements of skin hydration, elasticity, and erythema of the skin, respectively. The creams were applied regularly and well tolerated throughout the study. All formulations increased skin hydration and elasticity. There was no significant difference between PNSC and Physiogel®. However, PNSC was significantly more effective in increasing skin hydration and elasticity than PN and NNSC indicating that phytosphingosine inducing the positive charge, SC lipids and ceramide 3B are crucial for the enhanced effect on skin hydration and viscoelasticity.

Keywords: Ceramide; Phytosphingosine; Positively charged nanoemulsion; Skin elasticity; Skin hydration; Skin measurement in vivo

1. Introduction

Stratum corneum (SC) lipids such as cholesterol (Feingold et al., 1990), free fatty acids (Mao-Qiang et al., 1993a,b) and especially ceramides (Holleran et al., 1991) have been recognized as playing a major role in the skin barrier homeostasis. It is believed that one cause of dry skin is the reduction in the amount of ceramides within the intercellular lipid lamellae of the stratum corneum (Gaetani et al., 2003; Rawlings, 2003). Thus, it is desirable to be able to successfully replace these depleted SC lipids via the topical route. Ceramides are extremely insoluble compounds, a property directly linked to their intrinsic functionality, i.e. the formation of a water-impermeable barrier. In order to provide this function, ceramides must be able to penetrate the stratum corneum in order to reach the lipid lamellae. A potential problem with the topical application of skin products is finding a suitable dosage form to deliver the active ingredients such as ceramides in sufficient amounts to the active site. The complete dissolution of drug in the formulation and skin permeability enhancement are important considerations for the effective delivery of ceramides via the topical route (De Paepe et al., 2000, 2002).

Oil/water (o/w) nanoemulsions are promising colloidal drug carrier systems for diverse therapeutic applications. Successfully developed, intravenous, oral and ocular delivery systems showed reduced side effects of various potent drugs and prolonged pharmacological effect of drugs in these nanoemulsion formulations (Gershanik and Benita, 1996; Klang and Benita, 1998; Abdulrazik et al., 2001). Since a positively charged delivery system might enhance the permeability of a poorly soluble drug (Piemi et al., 1999), due to the strong interaction between epithelial membranes with positively charged

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solutes (Rojanasakul et al., 1992), the development of positively charged nanoemulsion for the skin penetration enhancement of low soluble biological active compounds such as ceramides is interesting.

Recently, a 6-months stable, positively charged o/w nanoemulsion incorporating ceramides was successfully developed in our labs (Yilmaz and Borchert, 2005). The droplets of the nanoemulsion exhibited their positive charge upon the physiological compound phytosphingosine (PS). PS, a free amphiphilic sphingoid base, is naturally found in the human body and is present at high levels in the SC. Topical application of PS and its derivatives has been shown to increase stratum corneum ceramide levels and barrier function (Rawlings, 2003). PS is also considered to be part of the skin's natural defence system (Wolf et al., 1997; Lambers and Streekstra, 1998; Park et al., 2002). Because of these properties, PS is an attractive candidate for topical use.

This investigation was focused on two poorly soluble ceramides, ceramide 3 (C3), the most abundant ceramide in healthy human skin, and the non-skin identical ceramide 3B (C3B). By topical application, both ceramides were able to improve skin barrier recovery indicated by increasing of skin hydration (Lambers and Roehl, 1999; De Paepe et al., 2002).

The aim of this work was to study the effect of a positively charged nanoemulsion cream containing phytosphingosine, incorporating ceramides and SC lipids (PNSC cream), on skin properties such as skin hydration, elasticity and skin erythema in healthy female volunteers. In this respect, PNSC cream was compared to (1) a positively charged nanoemulsion cream without SC lipids in order to evaluate the importance of the SC lipids on skin hydration and elasticity, (2) a negatively charged nanoemulsion cream in order to investigate the influence of the phytosphingosine and surface charge on the penetration enhancement, and (3) Physiogel® cream, a SC lipid containing formulation already on the market, in order to classify the effect of PNSC cream. Since ceramides may increase the water content of the skin (Lambers and Roehl, 1999), the skin hydration and elasticity were chosen as parameters to estimate the extent of penetration of ceramides into the skin. Erythema measurements were used as an indication of the skin tolerance to the formulations. Additionally, an ex vivo spreadability study was carried out in order to gain insight regarding the enhanced topical penetration effect of the o/w nanoemulsion as a function of surface charge.

2. Experimental methods

2.1. Materials

Ceramide 3 (C3), ceramide 3B (C3B) and phytosphingosine (PS) were kindly provided by Degussa, Essen, Germany. Lipoid E-80® (LE80; a mixture of phospholipids ex ovo with at least 80% phosphatidylcholine) was obtained from Lipoid KG, Ludwigshafen, Germany. The cosmetic oil, Eutanol G (octyldodecanol), and the preservative potassium sorbate were purchased from Caelo, Caesar & Loretz GmbH, Hilden, Germany and conformed with European Pharmacopoeia specifications. The antioxidant D,L-α-tocopherol and myristic acid were supplied from Synopharm, Barsbüttel, Germany and from Carl Roth GmbH & Co. KG, Karlsruhe, Germany. Tween 80® (T80) was supplied from Uniqema, Everberg, Belgium. Physiogel was kindly provided by Stiefel Laboratorium GmbH, Offenbach. All used ingredients were of pharmaceutical grade.

Table 1
Composition of the nanoemulsions used in the studies

Compounds	Nanoemulsion composition (%, w/w)		
	Positively charged		Negatively charged
	PNSC	PN	NNSC
Oil phase			
Eutanol G	20	20.8	20
Lipoid E-80®	2	2	2
Myristic acid	–	–	0.6
Phytosphingosine	0.6	0.6	–
Ceramide 3B	0.2	–	0.2
Ceramide 3	0.2	–	0.2
Palmitic acid	0.2	–	0.2
Cholesterol	0.2	–	0.2
Vitamin E	0.03	0.03	0.03
Ethanol	–	–	2
Water phase			
Tween 80	2	2	2
Glycerol	2.5	2.5	2.5
Potassium sorbate	0.1	0.1	0.1
Water to	100	100	100

PNSC, positively charged nanoemulsion with stratum corneum lipids; PN, positively charged nanoemulsion without stratum corneum lipids; NNSC, negatively charged nanoemulsion with stratum corneum lipids.

2.2. Production of nanoemulsions and creams

Aqueous and oil phases were prepared separately (Table 1). The aqueous phase, containing T80, glycerol, potassium sorbate, and bidistilled water, was heated to 50 °C under slight mixing. PS, for positively charged nanoemulsion, C3 and C3B were dissolved in Eutanol G above 100 °C and then cooled down to 75 °C. Then, LE80, cholesterol, palmitic acid, α-tocopherol and myristic acid, for negatively charged nanoemulsion, were dissolved in the oil phase and cooled down to 50 °C. Now, ethanol was added in the oil phase of negatively charged nanoemulsions to keep the lipids dissolved. The two phases were merged and prehomogenized with an Ultra-Turrax (Janke and Kunkel GmbH, Staufen, Germany) at 8000 rpm for three minutes and further homogenized with a high pressure homogenizer (Micron Lab 40, APV Systems, Germany) at 50 °C, 500 bar and eight homogenization cycles (Yilmaz and Borchert, 2005). After immediate cooling to room temperature and filtering through a membrane filter (polytetra-fluorethylene filter, Sartorius AG Germany, pore size 1.2 μm), 0.3–0.4% Carbopol 940 was added to the nanoemulsions by stirring with a Unguator® E 100 (GAKO Konietzko GmbH, Bamberg, Germany) for 30–60 min to obtain creams which were stored at a temperature of 2–8 °C.