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Permeability and lipid organization of a novel psoriasis stratum corneum substitute

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ABSTRACT

Lipids in the uppermost layer of the skin, the stratum corneum (SC), play an important role in the skin barrier properties. The main lipid classes are ceramides, cholesterol and free fatty acids. In previous studies a stratum corneum substitute (SCS) was developed, solely prepared from the SC lipids. The SCS mimics the lipid barrier properties of SC very closely. The present study aimed to design a psoriasis SCS (PS-SCS) mimicking several aspects of the lipid composition in SC from psoriasis patients. This PS-SCS showed a different lipid organization than SCS. The main differences were a reduced presence of an orthorhombic packing and an increased level of crystalline cholesterol. These changes resulted in lower flux of hydrocortisone across PS-SCS than across SCS and SC, which was most likely attributed to the higher level of phase separated crystalline cholesterol in PS-SCS. As propylene glycol (PG) is often used in dermatological formulations, in subsequent studies the interaction of PG with SC and SCS membranes was also investigated. These studies revealed that PG increased the permeability of hydrocortisone, mainly by selectively extracting cholesterol from SCS membranes and SC. This may play an important role in the penetration enhancing effect of PG.

1. Introduction

The natural function of the skin is to protect the body from losing water and to prevent invasion of foreign matter from the environment. This barrier function is located in the outermost layer of the skin, the stratum corneum (SC). Human SC consists of 15–20 layers of dead cells, referred to as the corneocytes. The corneocytes are surrounded by a cornified envelope and embedded in a lipid matrix (Madison et al., 1987; Swartzendruber et al., 1989). The densely crosslinked cornified envelope reduces the partitioning of drugs into the corneocytes. For this reason the tortuous intercellular route is considered the main pathway for drug diffusion across SC (Johnson et al., 1997; Meuwissen et al., 1998). Consequently the intercellular lipid matrix is considered to play an important role in the skin barrier function. Therefore, the lipid matrix has been under active investigation for many years.

The main lipid classes in the SC are ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA). In human SC 12 CER subclasses are identified (Ponec et al., 2003; Masukawa et al., 2008; van Smeden et al., 2011). The CERs differ from each other by the head group architecture and the acyl chain length. The CER head groups are either a sphingosine (S), phytosphingosine (P), dihydroxysphingosine (dS) or a 6-hydroxysphingosine (H) base. The acyl chain is a non-hydroxy (N), α-hydroxy (A) or an ester linked to an ω-hydroxy (EO) chain. The chain length of the ω-hydroxy acyl chains varies mainly between C30 and C34 (Motta et al., 1993). In human SC the intercellular lipids are arranged in two crystalline lamellar phases with repeat distances of approximately 13 nm and 6 nm, referred to as the long periodicity phase (LPP) and the short periodicity phase (SPP), respectively (Bouwstra et al., 1991). Several studies have reported that CER EOS is crucial for the formation of the LPP (McIntosh et al., 1996; Bouwstra et al., 1998a; Schreiner et al., 2000). Furthermore, in human SC the lipids mainly form a dense orthorhombic lateral packing, while a hexagonal lateral packing is also present (Gay et al., 1994; Pilgram et al., 1999; Damien and Boncheva, 2010). When drugs are administered topically, it is a major challenge to overcome the skin barrier function. Excised human or animal skin can serve as predictive models for skin diffusion characteristics of new chemical entities. However,

Abbreviations: SCS, stratum corneum substitute; PS-SCS, psoriasis stratum corneum substitute; PG, propylene glycol; CER, ceramides; CHOL, cholesterol; FFA, free fatty acids; S, sphingosine; P, phytosphingosine; dS, dihydroxysphingosine; H, 6-hydroxysphingosine; N, non-hydroxy; A, α-hydroxy; EO, ω-hydroxy; LPP, long periodicity phase; SPP, short periodicity phase; FTIR, Fourier transform infrared spectroscopy; SAXD, small angle X-ray diffraction.

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the availability of human skin is low and the interindividual variability of human and animal skin is high. In addition human skin equivalents have been used to predict skin diffusion characteristics. However, the permeability of these models is higher than the permeability of excised skin (Schafer-Korting et al., 2008). Consequently, in order to obtain favorable permeability properties of topical drug candidates, there is an urgent need for reproducible alternative systems mimicking the barrier properties of human skin very closely. A recently reported artificial lipid membrane is the stratum corneum substitute (SCS) (de Jager et al., 2006a). The SCS is a lipid membrane consisting of synthetic CER, CHOL and FFA supported by a porous membrane. The lipids in the SCS form both lamellar phases, the LPP and the SPP with a predominantly orthorhombic packing (de Jager et al., 2006b; Groen et al., 2008). Therefore, the lipid organization of the SCS resembles the lipid organization in human SC closely. In addition the steady state flux of moderately hydrophobic to moderately lipophilic model compounds through SCS and human SC has been shown to be very similar (de Jager et al., 2006a; Groen et al., 2008).

Diseased skin is often characterized by an impaired skin barrier function. The impaired barrier function of psoriasis skin has been demonstrated by elevated trans epidermal water loss (TEWL) compared to that of normal skin (Motta et al., 1994; Ghadially et al., 1996). Psoriasis is a benign skin disease characterized by hyperproliferation of keratinocytes, abnormal epidermal differentiation, activation of angiogenesis, vasodilation, and the presence of inflammatory cell infiltrate in the dermis and epidermis (Schon and Boehncke, 2005). Besides these changes in the viable skin psoriasis scales have been demonstrated to contain an increased level of CHOL and a decreased level of FFA in comparison to SC of normal skin (Motta et al., 1995). In addition the CER composition is also different, the most prevalent being 40% reduction of CER(EOS) as compared to normal SC (Motta et al., 1993). No information is available on the CER and FFA chain length distribution in psoriasis SC. To determine whether the reported changes in lipid composition contribute to the impaired barrier function of psoriasis skin, it is of interest to design a SCS mimicking lipid composition of psoriasis skin as closely as possible. In a very recent study a psoriasis SCS including the increased level of CHOL and a decreased level of FFA has been presented (Groen et al., 2011a). The psoriasis SCS model designed in the present study is a further development of this model as it also contains the reported CER composition of psoriatic scales (Motta et al., 1993).

Propylene glycol (PG) is readily used as a solvent for lipophilic drugs in dermatological formulations. Besides its solubilizing properties, PG also acts as a penetration enhancer in topical formulations (Williams and Barry, 2004). Therefore additional information on its influence on SC lipids of normal and of diseased skin is of great interest.

The aim of the present study was to design a novel psoriasis stratum corneum substitute (PS-SCS) mimicking the lipid composition in psoriasis skin as closely as possible. Using the existing SCS and the PS-SCS the effect of lipid composition on the permeability properties and lipid organization were examined. In addition the influence of PG on the permeability and lipid organization in SCS models and SC was evaluated.

2. Materials and methods

2.1. Materials

Synthetic CER(EOS)C30, CER(NS)C24, CER(NP)C24, CER(AS)C24 CER(NP)C16, and CER(AP)C24 were generously provided by Cosmoferm B.V. (Delft, The Netherlands). C24 e.g. indicates 24 carbon atoms in the lipid acyl chain. Palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), tricosanoic acid (C23:0), lignoceric acid (C24:0), cerotic acid (C26:0) and cholesterol were purchased from Sigma–Aldrich Chemie GmbH (Schnelldorf, Germany). Trypsin (type III, from bovine pancreas) and trypsin inhibitor (type II-S from soybean) were obtained from Sigma–Aldrich (Zwijndrecht, The Netherlands). Albumin (Fraction V from bovine serum) was obtained from Merck (Darmstadt, Germany). Hydrocortisone was provided by LEO Pharma (Ballerup, Denmark). Dialysis membrane disks (cutoff value of 5000 Da) were obtained from Diachema (Munich, Germany). Nuclepore polycarbonate filter disks (pore size of 50 nm) were purchased from Whatman (Kent, UK). All other chemicals were of analytical grade. All aqueous solutions were prepared with Millipore water (Billerica, MA, USA).

2.2. Isolation of SC from human skin

SC was isolated from human abdominal or mammary skin within 24 h after cosmetic surgery. Briefly, following removal of the subcutaneous tissue, the skin was dermatomed resulting in a thickness of approximately 250 μm using a Padgett Electro Dermatome Model B (Kansas City, KS, USA). The SC was separated from the viable epidermis after overnight incubation with trypsin (0.1% (w/v) in phosphate buffered saline pH 7.4) at 4 °C and afterwards at 37 °C for 1 h. After rinsing the SC with trypsin inhibitor (0.1% (w/v) in phosphate buffered saline pH 7.4), the SC was rinsed twice with Millipore water. Finally the SC was left to dry at room temperature and subsequently stored on silica, protected from light and under gaseous argon until use.

2.3. Preparation of lipid membranes

To prepare SCS membranes, lipid mixtures of equimolar CER, CHOL and FFA were prepared by dissolving appropriate amounts of the individual lipids in chloroform: methanol (2:1). The composition of the synthetic CER closely resembles the composition in pig SC (Bouwstra et al., 1996a) and is provided in Table 1. The FFA composition was C16:0, C18:0, C20:0, C22:0, C23:0, C24:0, and C26:0 in molar ratios of 1.8, 4.0, 7.7, 42.6, 5.2, 34.7, and 4.1, respectively (Wertz and Downing, 1991). After evaporation of organic solvent under nitrogen, the lipid mixtures were re-dissolved in hexane: ethanol (2:1) resulting in a total lipid concentration of 4.5 mg/ml. The preparation of the lipid mixtures for the PS-SCS membrane was performed in the same manner. However, the molar ratio of CER, CHOL and FFA resembles more closely the lipid composition in psoriatic scales (Motta et al., 1993, 1995). The composition is provided in Table 1. To spray the lipids on the polycarbonate filter disks a Linomat IV (Camag, Muttenz, Switzerland) connected to gaseous nitrogen and extended with a *y*-axis was used. With a 100 μl Hamilton syringe the lipid solution was sprayed in an area of 8 mm × 8 mm at a rate of 5.0 μl/min, a scan speed of 1.0 cm/s and a distance of approximately 1 mm from the filter. The lipid loaded filters were equilibrated just above the melting temperature of the membrane for 10 min. Hence, the equilibration was at 80 °C (SCS membranes) or at 65 °C (PS-SCS membranes). Subsequently the membranes were cooled to room temperature during at least 30 min. This resulted in a thin layer of approximately 300 μg of total lipids in the diffusion area of the membrane.

2.4. Diffusion experiments

In vitro diffusion studies were performed using in-line diffusion cells (Permegear, Bethlehem, PA, USA) with a diffusion area of 0.28 cm^2. SC and dialysis membrane discs were left for hydration in 10 mM saline phosphate buffer pH 7.4 (PBS) 1 h prior to the experiment. For the various studies different SC donors were used and