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Research paper

Delivery of oat-derived phytoceramides into the *stratum corneum* of the skin using nanocarriers: Formulation, characterization and *in vitro* and *ex-vivo* penetration studies

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ABSTRACT

Deficiency or altered composition of *stratum corneum* (SC) lipids such as ceramides (CERs), causing skin barrier dysfunction and skin dryness, have been associated with skin diseases such as atopic dermatitis and psoriasis, and ageing. Replenishing the depleted native CERs with exogenous CERs has also been shown to have beneficial effects in restoring the skin barrier. Phyto-derived CERs such as oat CERs were shown to be potential for skin barrier reinforcement. To effect this, however, the oat CERs should overcome the SC barrier and delivered deep into the lipid matrix using the various novel formulations. In an attempt to demonstrate the potential use of oat CERs, lecithin-based microemulsions (MEs) and starch-based nanoparticles (NPs) were formulated and characterized. Besides, ME gel and NP gel were also prepared using Carbopol®980 as a gelling agent. The *in vitro* release and penetration (using artificial four-layer membrane system) and *ex vivo* permeation (using excised human skin) of oat CERs from the various formulations were investigated. The results revealed ME enhanced the *in vitro* release and penetration oat CERs compared to the other formulations. On the other hand, the NPs retarded the release of oat CERs and small quantities of oat CERs incorporated into NP gel penetrated into the deeper layers of the multilayer membranes. The penetration-enhancing effect of ME was also observed in the *ex vivo* permeation studies where significant quantities of oat CERs were found in the acceptor compartment. Compared to the ME, the ME gel exhibited reduced depth and extent of oat CERs permeation. As compared to NP gel, ME gel enhanced the degree of permeation of oat CERs into the deeper layer of the skin. Generally the gel formulations were effective in concentrating oat CERs in the SC where they are needed to be.

1. Introduction

Skin is the largest organ of the body forming an effective barrier protecting the body from various types of stimulation and damage and preventing water loss from the body [1]. *Stratum corneum* (SC), the outermost layer of the skin, consists of several layers of keratinized corneocytes embedded in a lipid matrix of ordered lamellar structure [2]. The SC lipid matrix is composed of approximately equimolar ratios of ceramides (CERs), cholesterol, and free fatty acids (FAs). Epidermal CERs (Fig. S1, Supplementary material) play important structural roles in maintaining the skin barrier function and water-retaining properties [3]. Even though the unique lamellar arrangement of SC lipid matrix has not yet been fully elucidated, it has been shown that the deficiency or disturbance of SC lipids may lead to disruption of lipid organization which, in turn, affects the skin barrier function [4]. Several chronic skin diseases such as atopic dermatitis [5,6] and psoriasis [7,8] and aged skin [5,9] are associated with depletion or disturbance of SC lipids mainly CERs. One of the approaches used to treat skin dryness and skin barrier dysfunction associated with depletion and/or disturbance of SC lipids such as CERs is direct replacement of the depleted lipids [4].

Abbreviations: APCI, Atmospheric Pressure Chemical Ionization; CER, Ceramide; DS, Degree of Substitution; EE, Encapsulation Efficiency; FA, Fatty Acid; GlcCER, Glucosylceramide; LBME, Lecithin Based Microemulsion; LC, Loading Capacity; LC–MS, Liquid Chromatography Mass Spectrometry; ME, Microemulsion; NP, Nanoparticle; PhytoCER, Phytoceramide; RSD, Relative Standard Deviation; SA, Starch Acetate; SANP, Starch Acetate Nanoparticle; SB, Sphingoid Base; SC, *Stratum Corneum*

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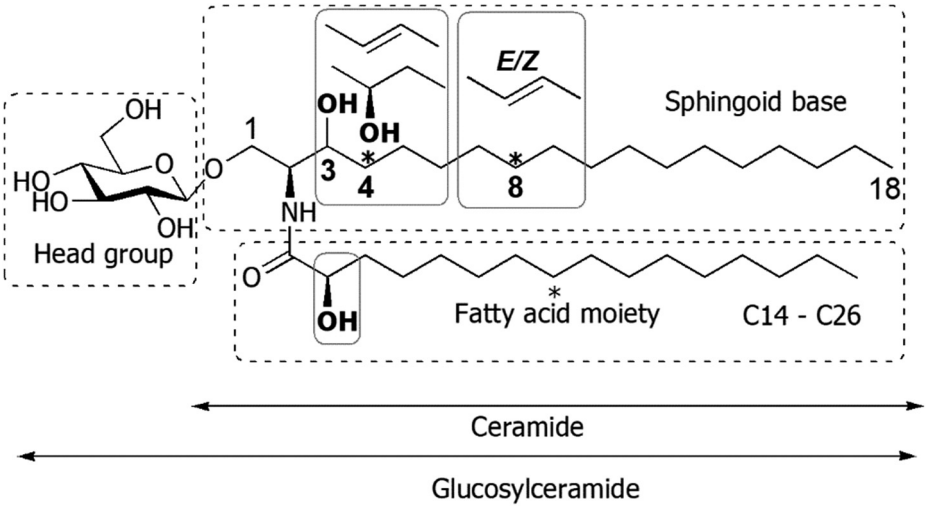

Fig. 1. Chemical structure of plant GlcCERs. The FAs are predominantly α-hydroxylated and they vary in chain length (C14 - C26) and ω-9-desaturation*. The SBs vary with the degree of desaturation or hydroxylation on C-4 and/or C-8 desaturation*.

The CERs are mostly obtained from animal such as bovine brain and synthetic or semi-synthetic sources. Nowadays, CERs are also produced by biotechnological approach [10]. Due to unestablished safety profile of animal-based CERs and the laborious and expensive synthetic procedure, safe and low cost alternative source of CERs are needed. The depleted native skin CERs can potentially be replaced with CERs isolated from edible plants. In plants, CERs are mainly found as constituents of glucosylceramides (GlcCERs) and glycosyl-phosphoryl inositol CERs; the most abundant being GlcCERs [11] (Fig. 1). Even though plant and mammalian CERs are structurally related, there are variations in terms of chain length and the degree of hydroxylation and unsaturation in their FA and sphingoid base (SB) moieties [12]. Plant GlcCERs consist of dihydroxy or trihydroxy SBs, which are mostly characterized by a double bond at position 8, amide-linked with α-hydroxy FAs [13,14]. The dominant SBs in plants include 4,8-sphingadienine (d18:$2^{\Delta4,8}$), 4-hydroxy-8-sphingenine (t18:$1^{\Delta8}$) and 8-sphingenine (d18:$1^{\Delta8}$) (Fig. S2, Supplementary material) [15]. The FAs are mostly α-hydroxylated with a chain length of C14-C26; the principal being C16, C20, C22 and C24 saturated α-hydroxy FAs [16]. On the other hand, sphingosine (d18:$1^{\Delta4}$), sphinganine (d18:0) and phytosphingosine (t18:1) are the dominant SBs in human skin [13,15]. Skin CERs contain non-hydroxy, α-hydroxy or ω-hydroxy FAs, the later having a chain length up to C32 and mostly ester-linked with unsaturated FA [3,13].

The beneficial effects of oral intake of plant CERs (PhytoCERs) for skin hydration and skin barrier reinforcement have been indicated in several studies [17–19]. So far, however, little effort has been made to investigate the possibility of delivering PhytoCERs topically for the stabilization of SC lipid lamellae in diseased, aged and affected skin. *In vitro* as well as *in vivo* studies are, therefore, needed to investigate the skin permeation profile of PhytoCERs and for better understanding of the influences of the structural variations between plant and human skin CERs on the stabilization of SC lipid bilayer.

Most of the studies supporting the beneficial effects of CER-based topical formulations were unable to show the depth and extent of permeation of the CERs into various layers of the skin. The CERs intended to replenish the depleted CERs in the SC have to be delivered deep into the SG-SC interface where the SC lipid organisation into lipid bilayers takes place [4,20,21]. Since the penetration of CERs across the skin from conventional formulations such as ointments and creams is poor [22,23], other formulation strategies have been investigated to improve the poor solubility, facilitate the permeation and target the delivery of CERs into the SC [22,24–26].

Nano-sized carriers such as microemulsions (MEs) and nanoparticles (NPs) have been shown to be promising vehicles for dermal and transdermal delivery of drugs [27]. MEs are optically isotropic, transparent one phase systems which are formed spontaneously by mixing appropriate amounts of lipophilic and hydrophilic components with surfactant/co-surfactant [24]. They have the advantages such as high drug-loading capacity, drug-permeation enhancing effects, long-term stability and ease of preparation [28,29]. The high drug solubilization capacity is attributed to the enormous interfacial area and existence of microenvironments of different polarity within the same single-phase system [29]. A wide range of hydrophilic and lipophilic actives can be solubilized in MEs as there are plenty of combinations of ME constituents which principally can form MEs [30]. Previously lecithin-based MEs (LBMEs) have been formulated and characterized for controlled delivery of CER [AP] into the SC [26]. On the other hand, polymer-based NPs are another interesting nanocarriers for effective delivery of drugs to a target site. In dermal and transdermal delivery, they present enormous surface area allowing homogeneous drug release [31,32]. They also act as reservoirs for controlled delivery of drugs into the SC, controlling the drug permeation into the deeper layers of the skin [33,34]. The NPs are closely in contact with the SC, which increases the partition coefficient of the drug into the SC thereby facilitating the penetration of the drug into the SC [34,35].

Oat PhytoCERs can potentially be used in topical formulations intended for skin barrier reinforcement. The structural characterization as well as the method of hydrolysis of oat GlcCERs (Fig. S3, Supplementary material) have been reported by Tessema et al. [36]. Elsewhere, an LC-MS method for the quantification of oat CERs in skin permeation studies has also been reported [37]. In an attempt to deliver oat CERs into the SC, in this study, LBMEs and starch-based NPs containing oat CERs were formulated and characterized. Besides, the *in vitro* release and penetration (using an artificial multilayer membrane system) and *ex vivo* skin permeation (using excised human skin) of oat CERs were also investigated.

2. Materials and methods

2.1. Materials

Soybean GlcCER (> 99% by TLC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). CER [AS] were obtained from Evonik-Industries (Essen, Germany). Hydrogen chloride solution (4.0 M in dioxane), 1, 4-dioxane anhydrous (99.8%), Pluronic® F-127 and 1,2-pentanediol were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Euxyl® PE 9010 was supplied by Schülke & Mayr