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Potential application of oat-derived ceramides in improving skin barrier function: Part 1. Isolation and structural characterization

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ABSTRACT

The impaired epidermal barrier and skin dryness in chronic skin conditions such as atopic dermatitis, psoriasis and aged skin are associated with the depletion of ceramides (CERs) in the *stratum corneum*. Previously, the beneficial effects of phyto-CERs, mainly from wheat and rice, in replenishing the depleted epidermal CERs and restoring the skin barrier have been shown. However, very few efforts have been made to exploit CERs from other plants for dermal applications. In an attempt to explore alternative plant source of CERs, glucosylceramides (GlcCERs) were isolated from the lipid extract of Ethiopian oat grain (*Avena abyssinica*). The GlcCER species were separated on a reversed phase HPLC and the structure of individual GlcCERs were identified by tandem MS with atmospheric pressure chemical ionization interface. The glycosidic linkage of the GlcCERs was cleaved by acid treatment and the predominant CERs species were isolated using column chromatography and preparative LC–MS. Further structural characterization of the CERs was made by HR/ESI–MS and NMR analyses. All the detected oat-derived GlcCER species consisted of C18 dihydroxy sphingoid bases amide-linked with α-hydroxylated saturated fatty acids (C16-C24). The two predominant GlcCER species consisted of sphingenine (d18:1) amide-linked to hydroxypalmitic acid (h16:0) and hydroxyarachidic acid (h20:0). The molecular formulae of the two major CERs assigned by HR/ESI–MS were identical to the ones identified by LC/APCI-MS/MS. The structural information was also supported by ^{1}H, ^{13}C, ^{1}H COSY NMR and HMBC spectral analyses. The amount of GlcCERs in oat grain, quantified by HPTLC, was found to be 193.5 mg/kg. The results indicated the similarity of oat CERs with commercial plant CERs (with comparable GlcCER content) suggesting its potential as source of CERs for oral (as dietary supplements) as well as topical applications.

1. Introduction

Ceramides (CERs) are the backbone of the intercellular lipid membranes of the *stratum corneum* (SC) and play important roles in maintaining the skin barrier function. They are the simplest types of sphingolipids consisting of sphingoid bases (SBs; which can be dihydrosphingosine, sphingosine, phytosphingosine or 6-hydroxysphingosine) amide-linked to fatty acids (FAs; either non-hydroxylated, α-hydroxylated or ester-linked ω-hydroxylated) [1,2]. The levels of CERs in chronic skin conditions such as atopic dermatitis, psoriasis and aged skin were shown to be reduced resulting in an impaired skin barrier and dryness [3,4]. Plant-derived CERs are potential alternative to the animal-based or synthetic CERs to replenish the natural skin CERs lost due to ageing and different skin conditions and restore the skin barrier function.

Plant sphingolipids are diverse group of lipids composed of polar head groups attached to CERs and include glycosylceramides, glycosyl inositol phosphor-ceramides and free CERs. They play important roles in membrane stability and permeability, signaling and cell regulation as well as cell-to-cell interactions [5–7]. Glycosylceramides are the major

Abbreviations: AMD, automated multiple development; APCI, atmospheric pressure chemical ionization; CER, ceramide; d18:0, sphinganine (dihydrosphingosine); d18:1, sphingenine; d18:2, 4,8-sphingadienine; ELSD, evaporative light scattering detector; ESI, electrospray ionization; FA, fatty acid; GlcCER, glucosylceramide; ^{1}H COSY, correlation spectroscopy; HMBC, heteronuclear multiple bond correlation; LC, liquid chromatography; MS/MS, tandem mass spectrometry; SB, sphingoid base; SC, *stratum corneum*

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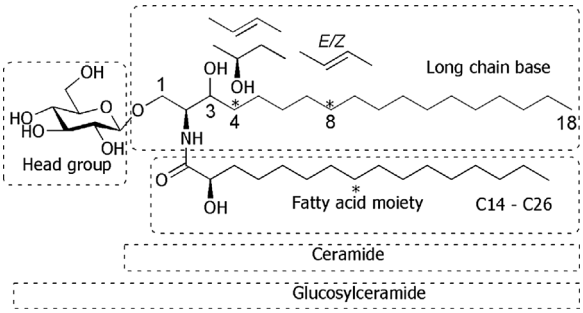

Fig. 1. Chemical structure of plant GlcCERs showing the variation of CER backbones. The FAs are predominantly α-hydroxylated and they vary in chain length and ω-9-desaturation*. The long chain bases are amide linked with the FA moieties and they vary with the degree of desaturation and hydroxylation on C-4 and/or C-8 desaturation* [19,20].

and most extensively characterized plant sphingolipids, the most abundant being mono-glucosylceramides (GlcCERs). Fig. 1 shows the chemical structure of representative plant GlcCERs. The polar head groups are attached to C-1 of the CER moieties with glycosidic linkage [6]. Plant GlcCERs consist of dihydroxy and trihydroxy SBs with C-4 and/or C-8 desaturation (Fig. 1**S,** Supplementary Material; the C-8 double bond being characteristic for plant CERs) and α-hydroxy FAs (ranging from C14 – C26) [8]. GlcCERs are naturally found in many cereals, tubers and legumes dietary sources such as wheat [8–10], rice [11–13], corn [13,14], potato [15], sweet potato [15], soybean [8,16] and konjac [17,18].

Liquid chromatography tandem mass spectrometry (LC–MS/MS) is a powerful analytical tool for qualitative analysis of GlcCERs [13]. The LC allows the separation of the complex mixtures of GlcCERs in plants. The structural identification is based on the fragmentation patterns of the GlcCERs through the transition of precursor ions to characteristic product ions of the SBs (Table 1 and **Fig. S1**, Supplementary Material) [21–24]. Ionization techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have been used for the analysis of sphingolipids [8,15,22,25–27]. Ion trap MS/MS with ESI have been used for the structural identification of GlcCERs obtained from different plant sources [13,23]. Despite the fact that APCI has greater applicability for the analysis of hydrophobic molecules [26], there are limited published data on the analysis of plant GlcCERs using LC/APCI-MS/MS.

Attempts have been made to replenish the depleted CERs of diseased and aged skin with synthetic and animal-based CERs. However, due to the fact that the synthesis of CER is an expensive and laborious procedure and unestablished safety profile of animal-based CERs [28,29], there is a need for alternative sources of CERs. Recent studies involving animal models [30–33] as well as healthy human subjects [30,34–36] have shown that oral intake of phyto-derived CERs improves the skin hydration and skin barrier function. There are also plenty of marketed plant CER-based dietary supplements meant for skin barrier reinforcement mainly from two plant sources, rice and wheat. Nevertheless, very few efforts have been made to exploit CERs from other plants for dermal applications.

The dermatological benefit of colloidal oatmeal has been documented for decades [37–39]. It contains high lipid content including polar lipids and FAs (3–18%) compared to other cereal grains [40]. Although earlier studies have investigated the FA and SB compositions of GlcCERs derived from oat leaf [41,42] and oat root [43], to our knowledge, the GlcCER composition of oat grain is not yet reported. Previous studies have shown that the GlcCER species obtained from seed, leaf and root tissues display different SB and FA profiles. While dihydroxy bases and C16–C20 saturated hydroxy FAs (the predominant being α-hydroxypalmitic acid (h16:0)) are enriched in seed tissues, trihydroxy bases and very long-chain (C20–C26) saturated and ω-9 monounsaturated hydroxy FAs occur abundantly in leaf tissues [6,44]. In these earlier studies, the GlcCERs were first hydrolyzed and then the resulting components and derivatives were analyzed making the pairing of specific SB with a FA in the intact molecular species more difficult. However, LC–MS/MS analysis can easily provide this information.

In this study, therefore, we report an LC–MS/MS-based structural identification and HPTLC-based quantification of GlcCERs isolated from Ethiopian oat grain (*Avena abyssinica*). Furthermore, the preparation of CERs from oat GlcCERs (acid-induced deglucosylation) and further structural characterization of the predominant oat CERs by 1D/2D NMR and HR-MS are also presented. Oat belongs to Poaceae family which has been shown to have plants which are rich sources of GlcCERs such as wheat and rice. Investigation of oat provides alternative plant source of CERs that could have potential benefits in improving the barrier function of diseased and/or aged skin.

Table 1
Characteristic product ions generated during tandem MS analysis of plant GlcCERs containing various C18 SBs [13,22,23].

C18 Sphingoid Moiety	Target Product Ion (*m/z*)		
	$[M + H\text{-}H_2O]^+$	$[M + H\text{-}2H_2O]^+$	$[M + H\text{-}3H_2O]^+$
Sphinganine/dihydrosphingosine (d18:0)	284.3	266.3	
4-Sphingenine/sphingosine (d18:1)		264.3	
4-Hydroxysphinganine/ phytosphingosine (t18:0)		282.3	264.3
8-Sphingenine (d18:1)		264.3	
4, 8-Sphingadienine (d18:2)		262.3	
4-Hydroxy-8-sphingenine (t18:1)		280.3	262.3
2-Amino-9-4,8,10-Octadecatriene-1,3-diol (d18:3)		260.3	

2. Materials and methods

2.1. Materials

Soybean GlcCER containing mainly d18:2/h16:0 (> 99%) (Avanti Polar Lipids, Alabaster, AL, USA), CER [AP] and CER [AS] (Evonik-Industries, Essen, Germany) and N-(R,S)-α-hydroxy-hexadecanoyl-D-*erythro*-dihydrosphingosine (BIOTREND Chemikalien GmbH, Köln, Germany) were used as reference standards. 1, 4-dioxane anhydrous (99.8%) and 4.0 M HCl in dioxane were obtained from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. The extraction solvents, isopropanol and *n*-hexane, were purchased from Grüssing GmbH, Filsum, Germany. The following solvents were used for the purification and analysis of GlcCERs: chloroform, methanol (VWR International GmbH, Darmstadt, Germany) and formic acid (Merck KGaA, Darmstadt, Germany). The Ethiopian oat grain (*Avena abyssinica,* Poaceae, local name: Ajja) was purchased from a local market in Holleta, Ethiopia in July 2015.

2.2. Extraction and purification of GlcCERs

The extraction and purification of oat GlcCERs were carried out following the methods described earlier [45,46]. Briefly, the dehulled oat grain was ground and the powdered grain (1 kg) was extracted with isopropanol/*n*-hexane/H_2O (55:20:25, v/v/v, 3 L) (ultrasonic assisted, 30 min). The dried lipid extract (59.3 g) was fractionated in a mixture of $CHCl_3$, MeOH and H_2O (1:1:1, v/v/v, 0.5 L each). The aqueous and $CHCl_3$ phases were separated and washed with $CHCl_3$/MeOH (1:1, 0.2 L each) and H_2O/MeOH (1:1, 0.2 L each) mixtures, respectively. The $CHCl_3$ fractions were combined and evaporated to dryness. The dried non-polar fraction (19.2 g) was further purified with column chromatography (400 g of Silica gel 60 (0.063–0.200 mm), Merck KGaA, Darmstadt, Germany) with $CHCl_3$/MeOH stepwise gradient elution (first elution: $CHCl_3$, 1.5 L; second elution: $CHCl_3$/MeOH (9:1, v/v),