Chapter 5

Glucosylated cholesterol in skin: Synthetic role of extracellular glucocerebrosidase

Glucosylated cholesterol in skin: Synthetic role of extracellular glucocerebrosidase

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Abstract

The existence of glucosylated cholesterol (GlcChol) in tissue has recently been recognized. GlcChol is generated from glucosylceramide (GlcCer) and cholesterol through transglucosylation by two retaining β-glucosidases, GBA and GBA2. Given the abundance of GBA, GlcCer and cholesterol in the skin's stratum corneum (SC), we studied the occurrence of GlcChol.

A significant amount of GlcChol was detected in SC (6 pmol/mg weight). The ratio GlcChol/GlcCer is higher in SC than epidermis, 0.083 and 0.011, respectively. Examination of GlcChol in patients with Netherton syndrome revealed comparable levels (11 pmol/mg).

Concluding, GlcChol was identified as a novel component in SC and is likely locally metabolized by GBA. The physiological function of GlcChol in the SC warrants future investigation.

Introduction

The existence of glucosylated cholesterol (GlcChol) has relatively recent been documented [1-3]. GlcChol is present in various tissues in significant amounts. It has become apparent that two cellular retaining β-glucosidases, the lysosomal glucocerebrosidase (GCase; GBA) and cytosol-facing membrane associated glucosylceramidase (GBA2) are able to generate GlcChol from glucosylceramide (GlcCer) and cholesterol (Chol) via a transglucosylation reaction (see Figure 1). Normally, the enzyme GBA2 synthesizes GlcChol and the glycolipid is degraded by the lysosomal GCase [1]. However, when GCase is surrounded by a high concentration of Chol as is the case in Niemann-Pick disease type C, the enzyme also generates GlcChol [1]. In view of occurrence of GlcChol, the skin is of interest, in particular its outer extracellular layer the stratum corneum (SC). Lamellar bodies rich in GlcCer are extruded into the SC

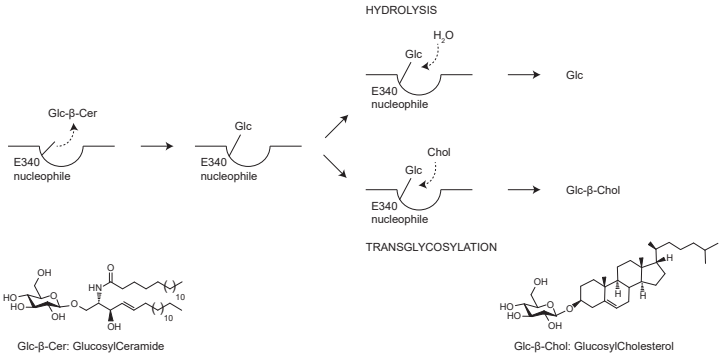

Figure 1. Metabolism of GlcCer by lysosomal GCase. The attack of the glucosidic bond results in cleavage of GlcCer in Cer and the covalent linkage of the glucose (Glc) to the catalytic nucleophile, glutamate 340 [6]. Subsequently, hydrolysis releases Glc. Alternatively, transglucosylation with cholesterol as acceptor results in formation of GlcChol [1].

and the lipid is locally converted by GCase to ceramide (Cer) [4]. This process is essential for the generation of desired barrier properties. The abundant presence of active GCase molecules in the SC has earlier been demonstrated by zymography and labeling with activity-based probes [5, 6]. The importance of GCase in the skin is demonstrated by the dramatic outcome of complete GCase deficiency. GCase-deficient humans and mice do not survive after birth due to major disruption of skin permeability [7]. The collodion baby is the most severe phenotype of Gaucher disease, the inherited lysosomal storage disorder caused by deficiency of GCase [8].

Since the SC contains besides GCase and GlcCer also relative high amounts of Chol, all ingredients for formation of GlcChol appear present. We therefore examined skin regarding the presence of GlcChol. In addition, we studied SC of patients suffering from Netherton syndrome (NTS). Patients with NTS have scaling and superficial peeling of the skin and skin inflammation as a result of uncontrolled serine protease activity [9, 10]. A sensitive LC-MS/MS method for quantitative detection of GlcChol employing an isotope encoded identical standard was used in the investigation [1]. Skin Cer can vary in composition of sphingoid base and fatty acyl moieties. The fatty acyl moiety of the skin Cers is very diverse, ranging from esterified w-hydroxy fatty acids [EO], non-hydroxy fatty acids [N] and a-hydroxy fatty acids [A] [11]. In addition to this, distinct sphingoid base-isoforms of Cers occur like regular sphingosine [S], dihydrosphingosine [DS], phytosphingosine [P] and 6-hydroxysphingosine [H]. The composition of NTS skin regarding [EOS], [NS] and [AS] forms of GlcCer and Cers was earlier studied [12]. In the present investigation we quantified the major GlcCer[S] isoform [13].

Our investigation firstly documents the presence of GlcChol in the SC and the findings are discussed.

Results and discussion

GlcChol levels were measured in full thickness skin, dermatomed skin and SC samples from abdominal skin by LC-MS/MS with $^{13}C_6$-encoded GlcChol as internal standard (see Figure 2). In parallel, samples were deacylated and GlcCer with regular sphingosine (GlcCer[S]) was determined with C17-sphinganine as internal standard.

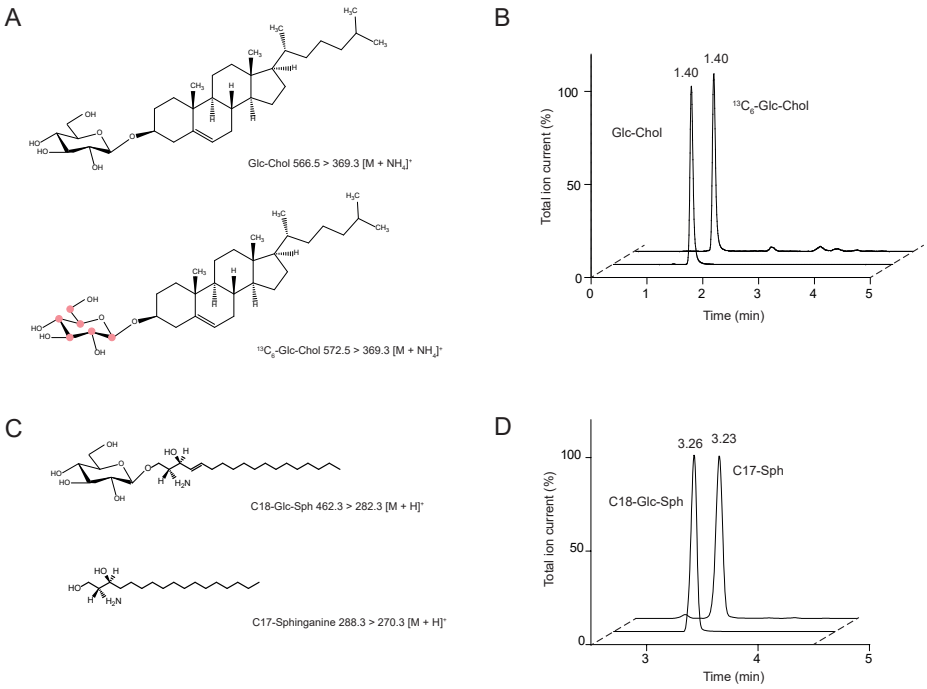

Figure 2. Quantification of GlcChol and C18-GlcSph in skin samples by LC-MS/MS. A. The structure of GlcChol and its isotope, 13C6-labeled GlcChol. B. LC-MS/MS chromatogram of pure GlcChol and its ^{13}C-labeled isotope added to SC lipid extract. The ammonium-adduct is the most abundant m/z for both compounds. The product ion, m/z 369.3, is the common fragment for both compounds. C. The structure of deacylated forms of C18-GlcCer[S] and internal standard Cer[DS] d17.0/16.0. D. LC-MS/MS chromatogram after deacylation: C18-GlcSph in SC lipid extract and Cer[DS] d17.0/16.0 after addition of pure Cer[DS] d17.0/16.0. The H^+-adduct is the most abundant m/z for both compounds.

Table 1 shows the levels of GlcChol and GlcCer[S] and the ratio GlcChol/GlcCer[S] in full thickness and dermatomed human abdominal skin and SC. GlcChol was detected in all samples. The highest levels of GlcChol as well as the highest GlcChol/GlcCer ratio were detected in the SC.

Next, we determined GlcChol and GlcCer[S] in NTS SC samples, being 11.1 +/- 3.7 pmol/mg and 44 +/- 14.6 pmol/mg. Lower levels of GlcCer[S] were detected

Table 1: GlcChol and GlcCer[S] in different fractions of human abdominal skin.

	pmol/mg wet weight		ratio
Sample	**GlcChol**	**GlcCer [S]**	**GlcChol/GlcCer[S]**
Full thickness skin	0.63	63.54	0.010
Epidermis	3.12	272.49	0.011
SC	5.98	72.21	0.083

compared to the levels in the abdominal SC, resulting in a higher ratio GlcChol/GlcCer. Higher levels of GlcCer can be found when the outermost cell layer of the viable epidermis is still present after SC isolation by trypsinization. This method was used to isolate SC from abdominal skin, but was not required for the NTS SC sheets. In Figure 3 lipid data for individual skin samples are shown.

Table 2: Absolute amounts of GlcChol and GlcCer[S] as mean +/- SEM (pmol/mg SC).

	pmol/mg wet weight		ratio
	GlcChol	**GlcCer [S]**	**GlcChol/GlcCer[S]**
NTS	11.1 ± 3.7	44.0 ± 14.6	0.37 ± 0.08

Our investigation reveals the presence of GlcChol in the SC of human skin. Our finding is not entirely surprising given the local abundance of the enzyme GCase, GlcCer and Chol in the SC. The occurrence of GlcChol has earlier been noted for snake skin as well as chicken skin [14, 15], but these investigations received no follow-up. Glucosylated sterols are actually not rare in nature. In plants and algae, glucosylated sterols (sterolins) are abundant metabolites [16].

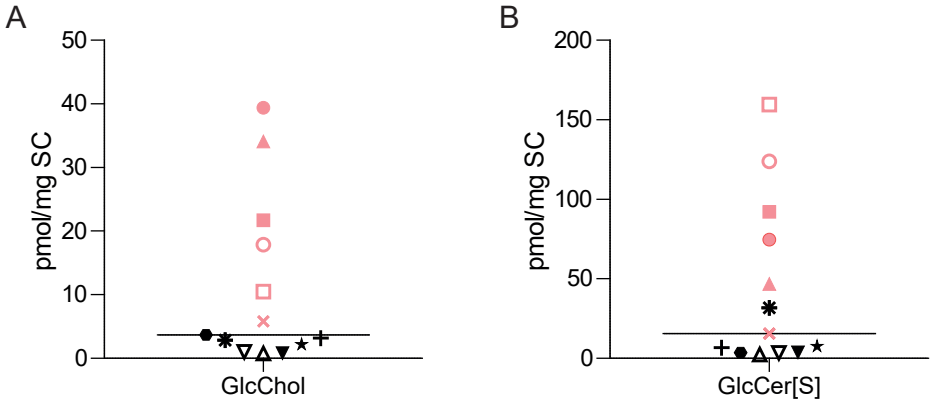

Figure 3. Individual distribution of lipid data. A. Dot plot GlcChol level per individual NTS subject. B. Dot plot GlcCer[S] level per individual NTS subject. Individuals with GlcChol levels above median are depicted in pink.

The likely biosynthetic pathway for GlcChol in the SC involves transglucosylation of cholesterol with GlcCer as glucose donor (see Figure 4). The physiological function of GlcChol in the skin is presently unknown. It might be speculated that it assists, similar to cholesterol sulfate, desquamation [30]. Our investigation of SC samples obtained from NTS and AD patients indicates that GlcChol is still formed in these pathological conditions. Clearly, further research is warranted to establish the function of GlcChol in the SC.

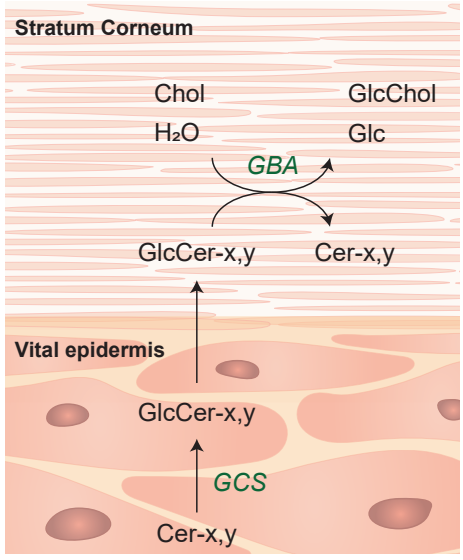

Figure 4. GlcChol formation in the skin.

Materials and Methods

Skin acquisition and preparation. All human skin samples used were obtained with consent and in accordance with the Declaration of Helsinki. Abdominal skin was obtained from a local hospital following cosmetic surgery and used within 24 h after surgery. Subcutaneous fat was removed from full thickness skin using a surgical scalpel. The SC side of the skin was wiped with 70% ethanol in deionized water. After fixing the full skin on a stryofoam support and using a Padgett Electro Dermatome Model B (Kansas City, USA), the skin was dermatomed to a thickness off 300-400 µm as described previously. Subsequently the SC was isolated using a trypsin digestion procedure. SC sheets were harvested from 13 patients suffering from NTS after informed consent.

Lipid extraction. After wet weight determination lipids were extracted with a methanol chloroform extraction (1:1 v/v). ^{13}C-labelled GlcChol and Cer[DS] d17.0/16.0 in methanol (both used as an internal standard) were added followed by a Bligh and Dyer extraction as described previously [1, 13]. Half of the lipid extract was deacylated prior to GlcCer[S] measurement [17].

LC-MS/MS analysis. For all experiments a Waters Xevo-TQS micro instrument was used. The instrument consisted of a UPLC system combined with a tandem quadruple mass spectrometer as mass analyzer. Acquired data were analyzed with Masslynx 4.1 Software (Waters, Milford MA, USA). Tuning conditions and MS settings for GlcChol and GlcCer[S] in ES+ (electrospray positive) mode are as published previously [1, 13].

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