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Distribution of Gangliosides in Human Epidermis, Dermis and Whole Skin

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Abstract

As very little information is available regarding human skin ganglioside, an investigation was made on the different patterns of gangliosides in samples of whole skin and on epidermis and dermis separated from such samples. The profile of monosialogangliosides of epidermis showed GM3 and GD3 as major species, with GM1 and an unknown species as minor ones. Di- and trisialogangliosides were also present in small amounts in the total human epidermis although they were mostly absent from the gangliosides of basal keratinocytes. The dermis had a more complex pattern, with GM3, GD3 and significant amounts of b-series gangliosides. Samples from donors of different ages were analyzed and a slight increase of gangliosides was noticeable along with age in the skin, with dermis accounting for this increase.

Keywords Epidermis; Dermis; Skin; Gangliosides; Sphingolipids

Introduction

Nowadays, it is well known that sphingolipids are present in all eukaryotic cell membranes and they are involved in many biological activities, such as growth regulation [1], differentiation, apoptosis [2], autophagy, cell detoxification pathways and cell to cell signalling. It was shown that in keratinocytes, the main cell type of the epidermis, aging induces a continuing change in the sphingolipid profile, from the proliferating cells to the differentiated ones [3]. Many studies report that the modulation of functions by sphingolipids depends mainly on the constitutive sphingoid bases [4].

In epidermis, due to the singular phenomena of differentiation, from the basal layer to the stratum corneum, the lipid profile changes as the keratinocytes phenotype changes. In live epidermis from the basal layer of keratinocytes containing mainly phospholipids, cholesterol and fatty acids to the stratum granulosum containing lipids bodies made of phospholipids and glucosyceramides. The upper layer of the epidermis, the stratum corneum, contains protein-bound lipids and the intercellular matrix of the corneocytes layer is made mainly of different types of ceramides [5].

During proliferation, differentiation and aging of keratinocytes, sphingolipid metabolism is continuously changing [3]. Whereas the most undifferentiated layers of the epidermis contain mostly membrane lipids such as phospholipids, the differentiated keratinocytes contain ceramides, cholesterol and fatty acids predominantly.

The lamellar bodies contains mostly glucosylceramides (named also epidermosides [5] consisting of N-(O-linoleoyl)- omega-hydroxy fatty acyl sphingosyl glucose and N-(O-linoleoyl)-omega- hydroxy fatty acyl phytosphingosyl glucose, which are then hydrolyzed by glucocerebrosidases into (acyl)ceramides, major components of the intercellular matrix of corneocyte layers.

Besides epidermosides, one specific class of sphingolipids which has been less studied in normal skin are the sialoglycosphingolipids, or gangliosides, reported for the first time by Gray and Yardley in 1975.

These glycosphingolipids located on the outer leaflet of the plasma membrane are ligands for cytokines, hormones and growth factors and involved in the control of cellular proliferation and intercellular communication [6].

It was shown that in epidermis, GM3 is the predominant ganglioside (from 51.5% in breast tissue to 73.8% in foreskin tissue), followed by GM2 (from 12.7% in abdomen tissue to 19.9% in face tissue), followed by GM1 and GD3 and some traces of GD1a [7]. On the other hand, Hersey et al. [8], detected GD2 in normal skin. Concerning the presence of the 9-O-acetyl-GD3 it was found as a marker of basal cell carcinoma and melanoma of skin [9,10] but not in normal epidermis [7].

Concerning the presence of the gangliosides in dermis it was shown that GM3 is the predominant ganglioside (from 58.2 % in face tissue to 73.3 % breast tissue), followed by GD3 (from 12.2% in breast tissue to 19.4% in face tissue), followed by GT1b, GD1a, GM1 and GM2 [7].

Several studies were done on the inhibition of sphingolipid metabolism [11] but only few ones showed products capable of stimulating this metabolism [12,13] in keratinocytes. In this respect, we showed that the de novo biosynthesis of gangliosides in cultured keratinocytes of aged donors could be strongly increased by a peptidic hydrolysate [14].

We showed that in elder people, the lipid biosynthesis turnover drops significantly as compared to young donors in keratinocytes [14] and others studies suggested that this variation is also subjected to the season cycle [15].

In this paper, we aimed at the comparison of the ganglioside profiles of the whole skin versus epidermis and dermis of donors of different ages. We described the changes in monosialogangliosides and

polysialo-gangliosides as markers of the skin layers, which could lead to novel therapeutic targets.

Materials and Methods

Materials

All solvents were of analytical grade and were purchased from Carlo-Erba (Peypin, France). Standard gangliosides were from Matreya (Pleasant Gap, PA, USA) and from Sigma (L'Isle d'Abeau, France).

Human skin biopsies of 2 cm^2 obtained by plastic surgery (mostly from abdomen) were taken from 5 young donors (26, 28, 31, 32 and 35 years old) and 5 old donors (51, 53, 56, 58 and 62 years old). Each skin was sliced in two equal parts. On one slice it was extract the lipids. The other slice from whole skin was subjected to an enzymatic treatment with trypsin in order to separate the epidermis and dermis. Then each tissue (whole skin, epidermis or dermis) was placed in a mixture of chloroform-methanol 2:1 (v/v) under slow stirring overnight at 4°C. After filtration, the delipidized tissue was dried and a protein assay was done with the Coomassie blue method.

Basal keratinocytes isolated from human skin biopsies of about 75 cm^2 were cultivated in 4 flasks at 10^6 cells density per flask. In two flasks, the KSFM was enriched with 10% of fetal serum and potato extract at 0.5 % final concentration [14].

Lipid extraction and analysis

Gangliosides were recovered from aqueous phase as previously described [16], and chemically assayed by the periodate-resorcinol method [17]. The gangliosides were then migrated on silica gel HPTLC plates [18].

The distribution of components was determined by scanning densitometry with a CS-930 Chromatoscan (Shimadzu, Kyoto, Japan). Quantitative results obtained before and after treatment were compared by Student's T-Test, $P \leq 0.05$ being accepted as significant.

Results and Discussion

Few research studies were developed in this respect about the profile of the ganglioside with depth and the age in skin of humans or in general mammalian skin. A significant variability was shown in the ganglioside pattern between epidermis and dermis [7].

Here we show some comparative profile of the gangliosides in epidermis, dermis and whole skin of a 58 years old donor (Figure 1). As shown before, GM3 and GD3 were the predominant gangliosides of epidermis and dermis, accounting together for about 65% of the total amount of gangliosides, followed by small amounts of GM1. The disialogangliosides GD1a and GD1b and the trisialoganglioside GT1b seen in the whole skin were mostly found in the dermis. It can be seen in Figure 1 an unknown skin ganglioside species that seems to be specifically enriched in epidermis and that migrates on HPTLC between GM1 and the GD3 doublet. This ganglioside may be associated with the differentiation process of keratinocytes since it is barely visible in the gangliosides purified from basal keratinocytes (Figure 2). Its chemical structure is still under investigation and the data will be soon published.

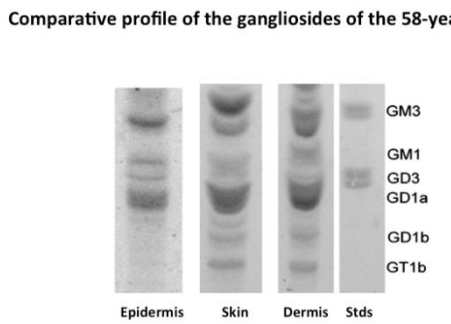

Figure 1: HPTLC of comparative profiles of the gangliosides of skin, epidermis and dermis from a 58 years old donor. Standard (Std): mixed gangliosides GM3, GD3 from human melanoma tumors.

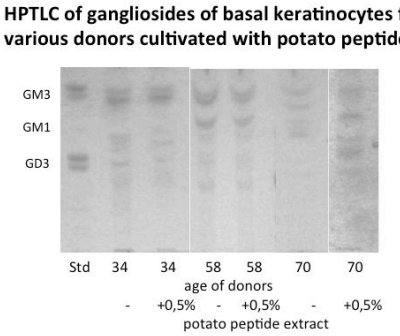

Figure 2: HPTLC of the gangliosides of basal keratinocytes taken from skin explants from three donors 28, 58 and 70 years old. The gangliosides taken out of the keratinocytes incubated 48 h with 0.5 % potato hydrolysate were spotted against the control ones. Standard (Std): mixed gangliosides GM3, GD3 from human melanoma tumors.

After the comparative ganglioside profiles of epidermis and dermis compared to the whole skin, we looked at the possibility of a variation with age for the ganglioside profile of the different layers of skin, as it was observed a slight variation with age of the ceramide profile in stratum corneum [18]. Previously, we reported [14] that basal keratinocytes taken from different donors have a different profile of gangliosides as a function of the donor's age, showing GM3 and GM1 as the major compounds and traces of the other gangliosides. Gangliosides decrease along with age in basal keratinocytes, suggesting deficiencies in the chain of biosynthetic enzymes. However, as shown in Figure 2, treatment with a potato hydrolysate at 0.5% final

concentration led to a strong increase in all gangliosides species, mostly due to a de novo biosynthesis, as evidenced by radiolabelling with [^{14}C]-serine. The observed increase normalized the ganglioside content of these keratinocytes up to the level seen in young donors, but did not increase the gangliosides beyond the amount seen in young donors. Those results suggest that the activity of the enzymes involved in the ganglioside biosynthesis is hampered along with age by factors that remain to be elucidated, and the treatment with potato peptides brought the level of activity of these enzymes back to a normal level. This pattern is possible with in vitro cultured keratinocytes where proliferation is ensured in adequate experimental conditions since the maximum GM3 is synthetized.

Figure 3 shows the ganglioside profile of the whole skin for several donors from 28 to 58 years old. Some increase of GD3 is noticeable along with a slight accumulation of the di- and trisialosyl gangliosides GD1a, GD1b and GT1b.

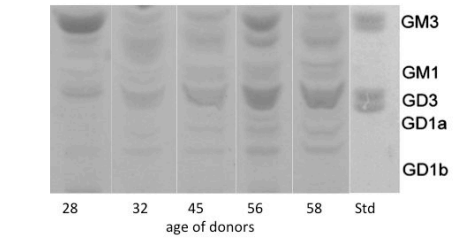

Figure 3: HPTLC of the gangliosides of whole skin (25 mg dry weight) from various donors 28 to 58 years old. Standard (Std): mixed gangliosides GM3, GD3 from human melanoma tumors.

Gangliosides are separated into several series (a,b,c) depending on their structure [19]. Each series is associated with its own pathway of biosynthesis, each one involving several specific glycosyl and sialyltransferases. From the profile of gangliosides in several epidermal samples (Figure 4), one can see mostly gangliosides of the a-series already shown in basal keratinocytes (Figure 2) with a fairly constant amount in GM3 and GD3, a slight decrease in GM1 and a heterogeneous variation in GD1a and GD1b. The dermis (Figure 5) has higher ganglioside content than the epidermis with a more complex pattern that combines a- and b-series of gangliosides, accounting for about two thirds of the skin gangliosides. This is consistent with the well- known ganglioside composition, with GM3, GM1, GD3 and GD1a, of human fibroblasts that are a major cell type of the dermis [20]. A similar profile is observed in the GM3 and GD3 amounts along with age and an important increase is noticeable for GM1, GD1a, GD1b and GT1b. The only previous study showing gangliosides in human dermis could only work on three samples [7], so the present findings on the relative amounts of gangliosides in epidermis and dermis to account respectively for one third and two thirds of the whole skin gangliosides remain to be confirmed. The significant proportion of b-series gangliosides in the dermis.

In normal skin, it was shown that gangliosides such as GM3, GD3 and GD1b inhibit the proliferation of keratinocytes whereas highly sialylated gangliosides (GT1b and GQ1b) promote the differentiation of keratinocytes [21]. As noticed, the live dermis and epidermis keep a certain level of the whole gangliosides to promote differentiation and epidermisation, as it was shown by the co-localization of GT1b with the expression of keratin 1 and desmosome formation [22,23].

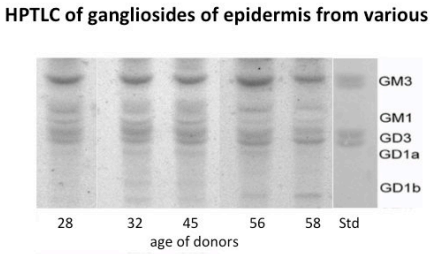

Figure 4: HPTLC of the gangliosides of epidermis (25 mg dry weight) from various donors 28 to 58 years old. Standard (Std): mixed gangliosides GM3, GD3 from human melanoma tumors.

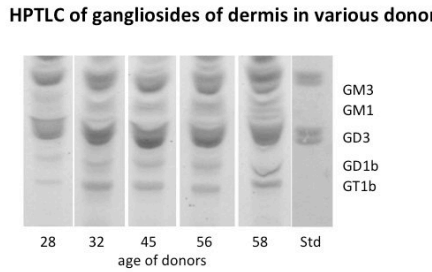

Figure 5: HPTLC of the gangliosides of dermis (25 mg dry weight) from various donors 28 to 58 years old. Standard (Std): Mixed gangliosides GM3, GD3 from human melanoma tumors.

An accumulation in glycolipids is observed in the case of patients with psoriasis vulgaris where the glucosyceramide-beta-glucosidase level is physiologically altered, leading to the impairment of the water permeability barrier of epidermis [24]. This could be reversed in culture with keratinocytes provided from biopsy of patients with psoriasis and ichtyosis and supplemented in medium with GM3.

It could be hypothetized that an accumulation of monosialylated gangliosides occurs in dermis or epidermis through aging to ensure extracellular matrix homeostasis.

Otherwise, concerning epidermis, it is known that caveolins 1, 2 and 3 in skin are partially co-localised with glucocerebrosidase, but only caveolin 1 expression is induced by the calcium levels in skin.

On the other hand, caveolin 1 is co-localized with putative lamellar-granules lipids such as cholesterol-enriched domains and GM1 [25], suggesting that they are involved in the lamellar granules biogenesis, since the caveolin 3 is co-localized at the interface of the stratum granulosum- stratum corneum of the stratum corneum playing an important role in barrier function formation [26].

This could stand for another proof of the accumulation of precursors lipids and especially of some complex gangliosides in aged skin due to down regulation of their trafficking to the surface.

Conclusions

It was noticed for aged donors skin versus the young donors a slight increase in complex gangliosides, linked to the deficiency in enzymatic turnover mechanism as suggested by the previous study with potato hydrolsate which increased the global amount of lipids and of gangliosides such as GM3 and GD3 in basal keratinocytes.

In another aspect, in patients with cutaneous pigmentation deficiency, it was reported a GM3 synthase deficiency suggesting that the functions of these sphingolipids in normal skin physiology reveal new features.

In a general way, the skin improves along with aging its protection to counterbalance the reduced de novo sphingolipid metabolism, proliferation and slower desquamation [27], along with larger sized-corneocytes and an increased location variability [28].

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