SKIN LIPID ABNORMALITIES IN GAUCHER'S DISEASE

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Summary

In the epidermis of terrestrial vertebrates stratum corneum lipids are known to play an important role as regulators of skin permeability; particularly ceramides, some of which with very long chain, hydroxy fatty acids, are important components of this "barrier" function. Ceramides are derived from the hydrolysis of previous existing glucosylceramide during the terminal stages of epidermal differentiation, due to the presence of a lysosomal-like glucosylceramidase localized in the lamellar bodies in the space between stratum granulosum and the inferior layer of stratum corneum: the deficiency of glucosylceramidase, localized in lysosome, is responsible of Gaucher's disease.

In this study we investigated the glycolipids and ceramide distribution in the epidermis of 4 Gaucher's patients (two of type 1 and two of type 3), compared with the same parameters in 4 healthy donors.

The comparison between the pathological samples and the healthy ones, indicates an impairment of the degradation of glucosylceramide to ceramide in the lamellar bodies of Gaucher's patients stratum corneum, as shown by the high percentage of the former (range: 48-69% of the total glycolipids), and the complete absence of the latter.

We are now evaluating the usefulness of topical therapy (in addition to the replacement therapy with Ceradase) with the use of a lotion containing commercially available 3 hydroxy ceramide, which fatty acids composition is closely related to that of the main ceramide present in human skin.

Riassunto

Nell'epidermide dei vertebi terrestri i lipidi dello strato corneo giocano un ruolo importante nella regolazione della permeabilità della pelle; in particolare le ceramidi, alcune delle quali contengono acidi grassi idrossilati a lunga catena, sono elementi importanti della funzione "barriera". Esse derivano dall'idrolisi di preesistenti glucosilceramidi durante le fasi terminali della differenziazione dell'epidermide, dovuta alla presenza di una glucosilceramidasi localizzata nei corpi lamellari presenti nello spazio tra lo strato granulosum e il margine inferiore dello strato corneo: il deficit di glucosilceramidasi, localizzata nei lisosomi, è responsabile della malattia di Gaucher.

In questo studio abbiamo analizzato la distribuzione dei glicolipidi e delle ceramidi nell'epidermide di 4 pazienti affetti dalla malattia di Gaucher (2 del tipo 1 e 2 del tipo 3), confrontati con gli stessi parametri di 4 donatori sani.
Il confronto tra i campioni prelevati da soggetti malati e soggetti sani, rivela uno squilibrio nella degradazione del glucosilceramide a ceramide nei corpi lamellari dello strato comeo dei pazienti affetti da Gaucher, come dimostrato dall'alta percentuale dei primi (dal 48 al 69% dei glicolipidi totali) e dalla completa assenza delle ceramidi.

Stiamo adesso valutando gli eventuali vantaggi ottenibili con una terapia topica (in aggiunta alla terapia sostitutiva con Ceradase) basata sull'uso di una lozione contenente 3-idrossiceramide la cui composizione in acidi grassi è assai simile a quella della principale specie ceramidica presente nella pelle umana.
INTRODUCTION

Lipids are considered to play an important role in the structure, differentiation and function of the epidermis. During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically (1,2). These changes are consistent with the requirement for cutaneous waterproofing (2-5).

In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function (6-11), and solvent extraction studies have shown that the progressive removal of sphingolipids, rather than non-polar lipids, is associated with proportional abnormalities in barrier function (11).

Stratum corneum lipids major constituent, ceramide, has been shown to be associated to both water retaining capacity of the skin and permeability barrier (10-13); ceramide is thought to derive primarily from glucosylerceramide which is practically absent in the exterior layer of the stratum corneum. This suggests the presence in the stratum corneum of hydrolytic enzymes; in fact, data from the literature show that a number of catabolic enzymes, such as sphingomielinase, triacylglycerol hydrolase (14), phospholipase A (15), steroid sulphatase (16), and b-glycosidase (17-19) are localised in the lower part of the horny layer.

Together all these data lead to the hypothesis that the hydrolysis of glucosylerceramide to ceramide plays an important role in the formation and the maintenance of epidermal permeability barrier (12). This hydrolizing steps however, is only one aspect of the complex process by which the permeability barrier is build-up. Other possible biochemical events associated with stratum corneum barrier formation might be the hydrolysis of sphingomyelin by sphingomyelinase and/or the breakdown of phospholipids by a variety of phospholipase.

For these reasons we analysed the distribution of glycosphingolipids in the stratum corneum of 4 skin biopsies from patients affected by Gaucher's disease, the commonest lysosomal storage disorder, characterised by an accumulation of glucosylerceramide in the reticuloendothelial system and, in the neuronophagic forms, in the central nervous system.

Three phenotypes of Gaucher's disease are recognised: type 1, without neurological involvement; type 2, with acute neurological involvement and lethal within the first 2 years of life; type 3, with chronic neurological involvement. The association of cutaneous abnormalities with Gaucher's disease is still a controversial problem. Skin manifestations are reported to be infrequent and non-specific, such as diffuse brown or yellow-brown pigmentation and easy tanning (20). Hyperpigmentation is probably due to a greater deposit of melanin; infant iron metabolic alterations characteristic of the disease may interact with tyrosinase activity (19,20,21,22,23).

On the other hand cutaneous alterations are particularly evident in Gaucher's disease type 2; this phenotype causes death after few days or months from the birth and shows the characteristic symptoms of "collodion baby" such as eczepod, eclopibion, petechias, purpura, and bright and tight skin.

Purpura, ecchimosys, telangiectasia, pallor and sometimes a yellow skin colour can be caused by hepatic invasion of Gaucher's cells (20,21), but it is not at all clear that a cause - and - effect relationship exists between the glycolipids storage in different organ and the cutaneous disorder.

The histological examination of the cutaneous biopsy in these patients has shown the presence of exfoliative lamellar ichthyosis with normal derma and epidermis, but with a great thickening of stratum corneum and follicular keratosis.

A female type 1 Gaucher's disease patient, dia-
Skin lipid abnormalities in Gaucher’s disease

Diagnosed when 50 years old, had thickened, hardened and xerotic skin; palms of hands and soles had hyperkeratosis and rhagades; on the contrary unguis modifications were not noticed; hair appears dry without dandruff. A biopsy on the right forearm with punch 4.0, showed little thickened skin, without the ichthyotic desquamation such as seen in type II (Celleno L. and Melchiorre M. personal communication).

MATERIALS AND METHODS

PATIENTS AND CONTROLS

The skin biopsies were taken from patients with type I and type 3 Gaucher’s disease under enzyme replacement therapy with Ceradase and were kindly provided by Dr. B. Bembi (C.H. “Burlo Garofalo” and Institute of Pediatrics, University of Trieste); the skin from healthy controls were surgically removed from a 52, a 54 and a 67 years old women abdomen and a 52 years old woman arm.

Gaucher patients’ features in terms of age, type, severity of the disease and period of therapy are reported in Table I.

REAGENTS

Commercial chemicals were of the highest available grade; Silicic acid (200-400 mesh) for column chromatography, standard neutral glycolipids and ceramides for thin-layer-chromatography were from Sigma Chemical Co. (St. Louis, USA); Silica gel plates (Kieselgel 60, HPTLC 10x10) were from Merck GmbH (Darmstadt).

LIPID EXTRACTION AND PURIFICATION

The lipids were extracted according to the method of Brunngraber E.G. et al. (24). Briefly, each sample was handily accurately homogenised in a mixture of phosphate buffer (10mM, pH 6.8)/ tetrahydrofuran (THF) 1:8. After centrifugation, at 10000 rpm for 10 min., the pellet was extracted three more times with the same solvent mixture in a different ratio (phosphate buffer/THF 1:4).

The 4 supernatants collected after each centrifugation were pooled and partitioned by addition of diethylether (1/3 of the total volume). The mixture was gently shaken and centrifuged at 2000 rpm for 10 min.; the lower phase was removed; to the remaining upper phase, 1/10 of the total volume of water was added and the mixture was centrifuged again in the same conditions. The two lower phases were collected to-

<table>
<thead>
<tr>
<th>Name</th>
<th>Age (y)</th>
<th>Gaucher Type</th>
<th>Time of Therapy (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.L.</td>
<td>20</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>F.A.</td>
<td>28</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P.M.</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>S.M.</td>
<td>33</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*: years
_/_: not yet in therapy with Ceradase.
The developing solvent system was: chloroform gel plate previously activated at 37°C for 1 hr.

The qualitative and quantitative percentage distribution of each class of neutral glycolipids was achieved by high performance thin layer chromatography (HPTLC) on 10x10 cm silica gel plate previously activated at 120°C for 1 hr.

The developing solvent system was: chloroform / methanol / water (110:40:6, by vol., running time: 40 min) and the visualising reagent was the diphenylamine spray (0.25g of diphenylamine in aniline / acetone / orthophosphoric acid, 0.25:25:2.5, by vol.). After spraying, the plate was heated at 120°C for 20 min.

The densitometric analysis was performed with a Camag II TLC Scanner (I = 660nm), connected to a Shimadzu C-R 3A Chromatopak system; the concentration of each compound was determined using a standard calibration curve, obtained spotting pure reference standards in different amounts.

The qualitative evaluation of ceramides was also achieved by HPTLC. Plate was activated as above described. Developing solvent system: chloroform / methanol / water (95:5:0.5, by vol.). The compounds were then visualised by spraying the plate with a mixture of anisaldehyde / acetic acid / sulphuric acid (0.25:25:0.5, by vol.). After spraying, the plate was heated at 120°C for 10 min.

The densitometric analysis was performed at 610nm; the concentration of each compound was determined as above described.

RESULTS

Tables II and III, respectively, show our results concerning the content of each neutral glycolipid, as µg/mg of fresh tissue and their percentage of the total. Total glycolipids as well as glucosylceramide amount are higher in patients as compared to control simples (Table II).

Glucosylceramide was the most remarkable neutral glycolipid in all of our samples, both as indicated by its absolute amount or by its relative percentage (Table III); its range was 35-46% of the total glycolipids in three out of controls, and 48-69% in the pathologic samples. In one control its percentage reached the value of 70%; this subject showed also the highest amount on the µg/mg tissue basis. The other glycolipid content and distribution do not seem to have any apparent correlation with the disease. Lactosylceramide was missing in two out of the four samples while pentahexosylceramide was ab-
Table II Glycolipids content in the skin biopsies analyzed.

<table>
<thead>
<tr>
<th>NAME</th>
<th>TISSUE WEIGHT (mg)</th>
<th>TOTAL GLYCOSYL CERAMIDE</th>
<th>LACTOSYL CERAMIDE</th>
<th>SULPHATIDE CERAMIDE</th>
<th>TRIHEXYOSYL CERAMIDE</th>
<th>TETRAHEXYOSYL CERAMIDE</th>
<th>PENTAHEXYOSYL CERAMIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.L.</td>
<td>40,390</td>
<td>0.180</td>
<td>0.087</td>
<td>0.030</td>
<td>0.022</td>
<td>0.032</td>
<td>0.009</td>
</tr>
<tr>
<td>F.A.</td>
<td>15,490</td>
<td>0.170</td>
<td>0.114</td>
<td>n.d.a</td>
<td>n.d.a</td>
<td>n.d.a</td>
<td>0.014</td>
</tr>
<tr>
<td>P.M.</td>
<td>9,400</td>
<td>0.744</td>
<td>0.415</td>
<td>n.d.a</td>
<td>0.019</td>
<td>0.022</td>
<td>0.288</td>
</tr>
<tr>
<td>S.M.</td>
<td>8,600</td>
<td>0.648</td>
<td>0.449</td>
<td>0.023</td>
<td>0.011</td>
<td>0.027</td>
<td>0.139</td>
</tr>
<tr>
<td>F.M.</td>
<td>334,200</td>
<td>0.124</td>
<td>0.088</td>
<td>0.011</td>
<td>0.015</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>P.A.</td>
<td>393,060</td>
<td>0.086</td>
<td>0.030</td>
<td>0.031</td>
<td>0.008</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td>C.J.</td>
<td>65,450</td>
<td>0.096</td>
<td>0.042</td>
<td>0.015</td>
<td>0.004</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>B.J.</td>
<td>826,000</td>
<td>0.026</td>
<td>0.012</td>
<td>0.003</td>
<td>0.003</td>
<td>0.004</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The results are given as µg/mg of fresh tissue; the samples referred to as R.L., F.A., P.M. and S.M. are the pathologic samples; F.M., P.A., C.J. and B.J. are the healthy controls.

* not detectable.

sent in three out of the four Gaucher's samples. One of the patient in therapy with Ceradase (R.L.) showed a decrease in glucosylceramide absolute content if compared with the other patient (Table II); percent distribution of the stored compound in the two patients under enzyme replacement therapy (R.L. and P.M.) are more similar to the normal ones (Table III).

According to the method we used, total ceramides were only detectable in control samples (Table IV, panel a), where we analysed also the distribution of different molecular species; the presence of higher amounts of OH-ceramide and 3OH-ceramide was noticed in all the four samples. In C.J. subject the hydroxy species are the only one present.

**DISCUSSION**

In this study we have investigated the amount of ceramide and glucosylceramide in the skin of Gaucher's disease patients, compared with healthy control samples.

Gaucher's disease is due to the deficiency of glucosylceramide β-glycosidase. (E.C.: 3.2.1.45, β-D-glucosyl-N-acylsphingosine glycohodrolase) a lysosomal enzyme: on the contrary the hydrolysis of skin glucosylceramide takes place in the lamellar bodies at the interface between the upper part of stratum granulosum and the lower part of stratum corneum. However it was suggested that the lysosomal and the lamellar bodies enzymes should have similar characteristics, even though their topology is different; this suggestion is further supported by the ascertained presence in the lamellar bodies of a proton pump (26) to secure the acidic conditions for the optimum of activity of the enzyme. The similarities between the two enzymes is further supported by the demonstration that in an animal model for Gaucher's disease, one of the most impressive clinical sign was the collapse of water barrier. Holleran W. M. and coworkers (23) showed the correlation between the deficit of glucocerebrosidase and the cutaneous barrier permeability, the ultrastructural
Table III
Glycolipid percentage and distribution in the pathologic samples 
(a) and in the healthy ones (b).

<table>
<thead>
<tr>
<th></th>
<th>R.L.</th>
<th>F.A.</th>
<th>P.M.</th>
<th>S.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% GLUCOSYLCERAMIDE</td>
<td>48.25</td>
<td>66.84</td>
<td>55.77</td>
<td>69.28</td>
</tr>
<tr>
<td>% LACTOSYLCERAMIDE</td>
<td>16.41</td>
<td>n.d.a</td>
<td>n.d.a</td>
<td>3.53</td>
</tr>
<tr>
<td>% SULPHATIDE</td>
<td>12.43</td>
<td>3.88</td>
<td>2.51</td>
<td>1.58</td>
</tr>
<tr>
<td>% TRIHEXOSYLCERAMIDE</td>
<td>17.71</td>
<td>5.28</td>
<td>2.99</td>
<td>4.10</td>
</tr>
<tr>
<td>% TETRAHEXOSYLCERAMIDE</td>
<td>5.19</td>
<td>15.53</td>
<td>38.72</td>
<td>21.51</td>
</tr>
<tr>
<td>% PENTAHEXOSYLCERAMIDE</td>
<td>n.d.a</td>
<td>8.47</td>
<td>n.d.a</td>
<td>n.d.a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F.M.</th>
<th>P.A.</th>
<th>C.J.</th>
<th>B.J.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% GLUCOSYLCERAMIDE</td>
<td>70.83</td>
<td>35.70</td>
<td>44.04</td>
<td>46.10</td>
</tr>
<tr>
<td>% LACTOSYLCERAMIDE</td>
<td>8.83</td>
<td>36.07</td>
<td>15.23</td>
<td>9.70</td>
</tr>
<tr>
<td>% SULPHATIDE</td>
<td>12.12</td>
<td>9.84</td>
<td>3.78</td>
<td>9.90</td>
</tr>
<tr>
<td>% TRIHEXOSYLCERAMIDE</td>
<td>5.14</td>
<td>3.53</td>
<td>6.23</td>
<td>16.80</td>
</tr>
<tr>
<td>% TETRAHEXOSYLCERAMIDE</td>
<td>1.00</td>
<td>6.71</td>
<td>4.03</td>
<td>6.00</td>
</tr>
<tr>
<td>% PENTAHEXOSYLCERAMIDE</td>
<td>2.06</td>
<td>8.15</td>
<td>26.70</td>
<td>11.50</td>
</tr>
</tbody>
</table>

a: not detectable

aspect of stratum corneum and the epidermis lipid content. This study was performed using healthy mice and homozygous mice for the Gaucher's disease gene. In these ones Odland bodies are modified and incomplete, there is an increase of trans epidermal water loss, a decrease of epidermal ceramide and of glucocerebrosidase activity, which was reported to be no more
than 1% as compared to normal animals. This experimental model could justify the alteration found in newborn skin with Gaucher's disease type II. In fact these modifications should derive from incomplete intercellular lamellae formation caused by a reduced transformation of glucosylceramide into ceramide. However experimental data indicate also that the "storage" of glucosylceramide in the skin was more responsible of the barrier disruption rather than the absence of ceramide (12). Also in other lysosomal pathology the epidermal barrier function is compromised by tissue accumulation of ceramide: clinical expression of the classic form of Fabry disease, due to the deficiency of α-galactosidase A, are cutaneous vascular lesions (angiectases) that progressive increase in the number and size with patient's age (27). On the other hand Farber disease, another lysosomal pathology, associated with deficiency of acid ceramidase leading to tissue accumulation of ceramides, shows granulomatous lesions in the skin (28). Possibly more than one process are involved in

### Table IV

Ceramide content (a; mg/mg fresh tissue) and distribution (b; %) in healthy controls.

<table>
<thead>
<tr>
<th>NAME</th>
<th>OH-CERAMIDE</th>
<th>CERAMIDE</th>
<th>3OH-CERAMIDE</th>
<th>TOTAL CERAMIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.M.</td>
<td>0,109</td>
<td>0,033</td>
<td>0,118</td>
<td>0,260</td>
</tr>
<tr>
<td>P.A.</td>
<td>0,064</td>
<td>0,007</td>
<td>0,079</td>
<td>0,150</td>
</tr>
<tr>
<td>C.J.</td>
<td>0,171</td>
<td>n.d.a</td>
<td>0,179</td>
<td>0,351</td>
</tr>
<tr>
<td>B.J.</td>
<td>0,055</td>
<td>0,021</td>
<td>0,041</td>
<td>0,118</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NAME</th>
<th>% OH-CERAMIDE</th>
<th>% CERAMIDE</th>
<th>% 3OH-CERAMIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.M.</td>
<td>41,842</td>
<td>12,668</td>
<td>45,530</td>
</tr>
<tr>
<td>P.A.</td>
<td>42,508</td>
<td>4,606</td>
<td>52,886</td>
</tr>
<tr>
<td>C.J.</td>
<td>48,834</td>
<td>n.d.a</td>
<td>51,166</td>
</tr>
<tr>
<td>B.J.</td>
<td>46,963</td>
<td>17,990</td>
<td>35,040</td>
</tr>
</tbody>
</table>

a: not detectable
the skin barrier formation. Small differences in these catabolic processes seem to exist among the different regions of the keratinizing epithelium, resulting in subtle variations in lipid composition of the stratum corneum, so explaining the different physico-chemical organization and fluidity of intracellular lipids (29). However the conversion of glucosyleramides to ceramides remains one of the more important step in the formation of the barrier, also due to the topology of these complex lipids (30). Holleran et al. found that the induced barrier abnormalities were not reversed by coapplication of ceramide (8). No indications on the chemical structure of the used ceramide were presented. It is noteworthy that the most abundant species of ceramide present in the skin has a peculiar fatty acid composition as shown by Uchida et al. (31). Due to the small amount of extracted lipids we were unable to perform reliable analysis on the fatty acid composition of our samples.

In conclusion our results seem to indicate that the glycosidase, normally present in the lamellar bodies, failed in our patients to hydrolyse the glucosyleramide to ceramide, as shown by the complete absence of the latter in the pathological samples but its presence in a relative high amount in the healthy subjects. Due to the relevant role of ceramide in the cutaneous permeability barrier function, these findings might justify the presence of a mild to a severe dry skin in our patients. To our knowledge this is the first report on glucosyleramide and ceramide content in the skin of Gaucher disease patients. In fact despite many studies of glycolipid content in various tissues of these patients it appears that skin has been never analyzed, probably because, mainly in type I, the alterations in epithelia are not of sufficient clinical significance compared to the other more severe problems associated to the disease.

In order to solve the question whether or not the coapplication of ceramide could improve the skin abnormalities in Gaucher's disease patients, we prepared a special emulsion containing 0.5 - 1% 3OH-ceramide, which fatty acid composition is more closely related to what reported by Uchida et al.(32)

The treatment of the patients is now started and the preliminary results seem very encouraging.

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References


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