Altered Levels of Sphingosine and Sphinganine in Psoriatic Epidermis

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Background: Ceramides are the main lipid component of the stratum corneum and are a structurally heterogeneous and complex group of sphingolipids of which sphingoid bases are the basic structural constituents. Altered levels of sphingoid bases have been reported in skin conditions that involve dryness and barrier disruption, including atopic dermatitis. Objective: The purpose of this study was to investigate the altered levels of sphingoid bases in psoriatic epidermis and their relationship with the clinical severity of the psoriasis. Methods: Samples from the lesional and non-lesional epidermis were obtained from eight psoriasis patients. Levels of sphingosine and sphinganine were analyzed by high-performance liquid chromatography. The expression of ceramide synthase and ceramidase proteins, which are related to sphingosine and sphinganine metabolism, were measured using Western blot analysis. Results: Levels of sphingosine and sphinganine in the lesional epidermis were significantly higher than those in the non-lesional epidermis. Although there was no altered ceramide synthase and ceramidase, there was a highly significant positive correlation between the % change of ceramidase, the degradative enzyme of ceramide into sphingosine, and the Psoriasis Area Severity Index (PASI) score. Conclusion: The levels of sphingosine and sphinganine were significantly increased in psoriatic epidermis and the % change of ceramidase was positively correlated with the clinical severity of psoriasis. (Ann Dermatol 25(3) 321–326, 2013)

Keywords- Ceramidases, Dihydroceramide desaturase, Psoriasis, Sphinganine, Sphingosine

INTRODUCTION

Ceramides are the primary lipids in the stratum corneum and their depletion is thought to be one of the etiological factors of barrier disruption in various skin conditions. Marked depletion of ceramides in the stratum corneum has been reported in such conditions, particularly in patients with psoriasis and atopic dermatitis (AD). In our previous study, we found that the decreased levels of ceramide in psoriatic skin lesions caused epidermal hyperplasia via downregulation of apoptotic signal cascades such as protein kinase C-alpha (PKC-α) and c-jun N-terminal kinase (JNK). In the de novo synthesis of ceramide, sphinganine (Sa) is generated by enzymatic condensation of serine and palmitoyl-Co A by serine palmitoyltransferase, and Sa is further acylated into ceramides. The newly synthesized ceramide is promptly metabolized to glucosylceramide or sphingomyelin and is ultimately degraded into sphingosine (So) and fatty acids by ceramidase. Ceramides are a structurally heterogeneous and complex group of sphingolipids, of which sphingoid bases are basic structural constituents. So and Sa exhibit anti-microbial activity and prevent the adherence of bacteria to cells, but their functions remain otherwise largely unknown. Changes in the levels of sphingoid bases have been described in skin conditions involving dryness and barrier disruption,
including AD. However, only limited information is available on alterations in sphingoid bases in psoriasis. Therefore, the aim of this study was to examine the levels of sphingoid bases in the epidermis of psoriasis patients and to assess their relationship with psoriasis severity.

MATERIALS AND METHODS

Patients and skin biopsies

Eight Korean patients with psoriasis (two women, six men) ranging in age from 21 to 58 years gave informed consent and participated in this study. All subjects had psoriasis vulgaris as identified through clinical and histologic assessment and had not been treated either systemically or topically for at least one month before punch biopsies were obtained. Using a 4-mm punch, biopsies were taken from lesional and non-lesional skin on the lower extremities, back, or arms. The epidermis was separated as described previously. Specifically, the epidermis was separated from whole-skin biopsies by overnight incubation at 4°C in a 1:1 (v/v) mixture of Dispase solution (Roche Molecular Biochemicals, Mannheim, Germany) and Hank's balanced salt solution (HBSS; Gibco BRL, Life Technologies, Rockville, MD, USA).

Assessment of the clinical severity of psoriasis

The clinical severity was assessed using the Psoriasis Area Severity Index (PASI) score, which is calculated as follows: PASI = 0.1 (Et + Ih + Dh) Ah + 0.2 (Eu + Lu + Du) Au + 0.3 (Et + It + Dt) At + 0.4 (El + Ii + Dl) Al, where E=erythema, I=infiltration, D=desquamation, A=area, h=hand, u=upper extremities, t/trunk, and l=lower extremities. A numerical value is given to the extent of the lesions in each area as follows: 1=10%, 2=10–30%, 3=30–50%, 4=50–70%, 5=70–90%, and 6=90–100%. E, I, and D are scored on a five-point scale (0=no symptoms, 1=slight, 2=moderate, 3=marked, and 4=very marked) to obtain a final PASI score between 0 and 72. The PASI scores of the patients who took part in this study ranged between 1.8 and 23.7; this range corresponds to mild and moderate psoriasis. Only patients with PASI scores <25 were enrolled in this study in order to determine whether alterations in the levels of ceramides and ceramide-related apoptotic signaling molecules are closely correlated to the clinical severity of mild to moderate psoriasis.

Lipid extraction

Epidermal tissue isolated from each 4 mm punch biopsy was homogenized with a Polytron homogenizer in 500 μl of ice-cold phosphate-buffered saline. After centrifugation at 240×g for 5 min, aliquots (50 μl of supernatant) of the epidermal homogenates were collected for protein determination by a modified Lowry method using bovine serum albumin as the standard. Sphingoid bases were extracted by adding 350 μl of MeOH, 150 μl of 1 M NaCl and 300 μl of CHCl3 to the prepared homogenates. A 35 μg aliquot of 3N NaOH was also added to adjust the pH to 10–11 in order to facilitate the separation of sphingoid bases in the upper phase. As an internal standard, 100 pmol of the non-naturally occurring species C17 So and C17 S1P was added followed by vigorous vortexing for 1 h. After centrifugation at 240×g for 3 min, the lower phase was evaporated under N2 gas and then dissolved in 500 μl of 0.15 M methanolic KOH with vortexing for 20 min at 37°C. Then, 500 μl of CHCl3, 100 μl of 2 N-NH4OH and 400 μl of alkaline water were added and the mixture was vortexed for an additional hour. After centrifugation at 240×g for 3 min, the lower phase was washed twice with 800 μl of alkaline water and the CHCl3 was evaporated using a SpeedVac concentrator.

O-phthalaldehyde derivatization and high-performance liquid chromatography (HPLC) analysis of sphingoid bases

The extracted lipid fraction was resolved in 120 μl of MeOH. A 20 μl volume of o-phthalaldehyde (OPA) derivatization reagent (Sigma, St Louis, MO, USA; 50 mg ortho-phthalaldehyde, 1 ml ethanol, 100 μl 2-mercaptoethanol, and 50 ml 3% (w/v) boric acid solution) was added and the mixture was allowed to stand for 30 min at room temperature. OPA reacts with the primary amine group of the sphingoid bases and becomes highly fluorescent at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. The derivatives were analyzed using a Jasco (Tokyo, Japan) PU-980 pump, an AS-1559 autosampler, and a Jasco FP-920 fluorescence detector. The isotropic eluent comprised of methanol: deionized distilled water (92:8 v/v) with 0.1% triethylamine was flowed at the rate of 1 ml/min. A 70 μl aliquot of the derivatives was injected then separated on an C18 column (Waters, Sunfire 4.6×1.5 mm internal diameter) kept at room temperature, and the fluorescence was measured at an emission wavelength of 455 nm and an excitation wavelength of 340 nm. C17 So (8.5 min), C18 So (10.5 min), C18 Sa (14.2 min) were detected.

Western blot analysis

Isolated epidermis was added to 200 μl of Folch solution (CHCl3 : MeOH, 2 : 1, v/v mixture) and homogenized using a Polytron homogenizer, and 200 μl of 0.1 M KCl was subsequently added. The mixture was centrifuged at...
Table 1. Ceramidase expression in non-lesional and lesional epidermis tissue, PASI scores and disease duration in patients with psoriasis

| Patient No. | Ceramidase expression (% signal intensity) | PASI score | Psoriasis duration |
|-------------|-------------------------------------------|------------|-------------------|
|             | Lesion | Non-lesion | Change*                     |                  |
| 1           | 15.16  | 29.25     | 51.83                     | 1.80             | 3.00            |
| 2           | 13.75  | 10.82     | 127.06                    | 2.40             | 1.00            |
| 3           | ND     | 53.02     | 0.00                      | 3.20             | 2.00            |
| 4           | 40.07  | 28.15     | 142.36                    | 3.40             | 20.00           |
| 5           | 50.46  | 38.91     | 129.69                    | 4.30             | 3.00            |
| 6           | 146.75 | 91.82     | 159.82                    | 4.60             | 4.00            |
| 7           | 66.17  | 11.73     | 564.11                    | 9.30             | 8.00            |
| 8           | 43.53  | 9.31      | 467.52                    | 23.70            | 10.00           |
| Mean±standard error of measurement | 46.99±16.21 | 34.13±9.84 |                  |                  |

PASI: Psoriasis Area Severity Index, ND: not detected. *% Change in ceramidase expression was calculated as (lesion/non-lesion level)×100.
Expression of ceramide synthase in non-lesional and lesional epidermis, PASI scores and disease duration in patients with psoriasis

| Patient No. | Ceramide synthase expression (% signal intensity) | PASI score | Psoriasis duration |
|-------------|-----------------------------------------------|------------|-------------------|
|             | Lesion | Non-lesion | % Change* |                     |                     |
| 1           | 28.46  | 38.79      | 73.35     | 1.80                | 3.00                |
| 2           | ND     | 77.91      | 0.00      | 2.40                | 1.00                |
| 3           | 54.93  | 77.17      | 71.18     | 3.20                | 2.00                |
| 4           | ND     | ND         | 0.00      | 3.40                | 20.00               |
| 5           | 85.89  | 96.70      | 88.82     | 4.30                | 3.00                |
| 6           | 100.20 | 18.88      | 530.65    | 4.60                | 4.00                |
| 7           | 18.75  | ND         | 0.00      | 9.30                | 8.00                |
| 8           | 42.00  | 49.40      | 82.02     | 23.70               | 10.00               |

Mean±standard error of measurement 41.28±13.18 44.86±13.08

PASI: Psoriasis Area Severity Index, ND: not detected. *% Change in ceramide synthase expression was calculated as (lesion/non-lesion level)×100.

DISCUSSION

The fundamental function of the epidermis is to serve as an efficient barrier against water loss through the skin. The epidermal barrier is comprised of the extracellular lipid-enriched membranes of the stratum corneum and is organized into membranous multi-layers with a repetitive lamellar structure. The intercellular lipid lamellae are composed predominantly of ceramides, cholesterol, and fatty acids that originate from polar lipid precursors provided by cells in the basal layer of the epidermis. Ceramides are the primary lipids in the stratum corneum. During keratinization, almost all of the epidermal phospholipids found abundantly in the basal layer disappear and ceramides, which are synthesized de novo from phospholipid intermediates, remain within the stratum corneum. Ceramide-rich intercellular lipid lamellae are thought to be of particular importance in maintaining the structural integrity of the epidermal barrier. Depletion of ceramides in the stratum corneum has been suggested as an etiological factor of dryness and barrier disruption in skin conditions such as AD and essential fatty acid
deficiency.24. Ceramides are a structurally heterogeneous and complex group of sphingolipids containing derivatives of So bases joined by an amide linkage to a variety of fatty acids. Differences in chain length, type and the extent of hydroxylation and saturation are responsible for the heterogeneity of epidermal sphingolipids24. Sphingoid (long-chain) bases are the basic structural constituents of sphingolipids, which are thought to be synthesized by the acylation of sphingoid bases to form a ceramide, with subsequent glycosylation and other modifications leading to the formation of complex sphingolipids such as neutral glycosylsphingolipids, and phosphosphingolipids (e.g., sphingomyelin)25. So and Sa, which are free sphingolipids of the stratum corneum, have been shown to strongly inhibit both bacteria and fungi in vitro. Furthermore, So was found to be significantly downregulated in the skin of patients with AD compared with healthy controls. Alterations in the ratio of So to Sa due to a ceramide metabolic abnormality in the stratum corneum can affect membrane integrity in AD.13. Additionally, as lipid second messengers, they mediate antiproliferative and apoptotic effects via activation of several signal transduction molecules such as PKC-α and JNK5-7. However, there are only a few studies that have examined the relationship between sphingoid bases and psoriasis. In this study, we measured the levels of the precursor and degradation products of two ceramides, Sa and So, in both psoriatic and non-lesional skin. The levels of both proteins were significantly higher in psoriatic skin compared to in the non-lesional epidermis, indicating that in psoriatic skin, ceramide synthesis is decreased and the degradation of these proteins is increased. These results suggest that ceramide levels, which are regulated by a balance in the activity of ceramide generating enzymes such as CerS in the de novo synthesis pathway and degradative enzymes such as CDase, are not well-controlled in the epidermis of psoriasis patients.

We measured the expression of CerS and CDase in both the psoriatic epidermis and non-lesional epidermis. CerS is one of the enzymes related to the synthesis of ceramide, and CDase is a catalytic enzyme that is related to a decrease in ceramide levels. The results of this experiment showed that the levels of CerS were decreased in psoriatic skin, whereas the levels of CDase were increased. However, there was no significant difference in the levels of CerS and CDase between psoriatic epidermis and non-lesional epidermis. We also evaluated the relationship between the PASI score and increased levels of CDase to determine the relationship between CDase and psoriasis severity. The percent change in the ratio of lesional/non-lesional epidermis and the PASI score showed a significant and direct correlation. These findings confirm those of our previous study, namely that decreased levels of ceramide in psoriatic skin are responsible for increased levels of CDase in psoriatic skin lesions and that, furthermore, increased levels of this protein are highly correlated with the clinical severity of psoriasis. These results are also consistent with those found in a previous study of CDase activity in AD.

However, there are some limitations to our study. First, we could not show the relation between psoriasis patients and healthy volunteers. Of course, it is well known that the ceramide level of healthy skin is decreased compared with that of psoriasis and AD patients, based on the results of previous study. However, earlier studies have not directly compared between healthy volunteers and psoriasis patients in terms of the levels of Sa and So. In addition, our study also had a relatively small sample size. Therefore, further studies should include more patients, as well as comparison with healthy volunteers. In conclusion, we demonstrated that So and Sa levels are significantly increased in psoriatic epidermis and that increased expression of CDase is positively correlated with the clinical severity of psoriasis. Given that psoriasis is an inflammatory skin disease, several cytokines or growth factors secreted by keratinocytes, Langerhans cells, or T-cells in psoriatic skin lesions may affect the regulation of ceramide synthesis and expression of CDase. However, in light of the study limitations as given above, further study is required to confirm this hypothesis.

ACKNOWLEDGMENTS

This work was supported by a 2011 grant from Kyung Hee University (KHU-20110254).

REFERENCES

1. Elias PM, Menon G. Structural and lipid biochemical correlates of the epidermal permeability barrier. In: Elias PM, editor. Skin lipid. Advances in lipid research. San Diego: Academic Press, 1999:24-26.
2. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? J Invest Dermatol 1991;96:523-526.
3. Matsumoto M, Umemoto N, Sugihara H, Uehara M. Difference in ceramide composition between “dry” and “normal” skin in patients with atopic dermatitis. Acta Derm Venereol 1999;79:246-247.
4. Chung S, Kong S, Seong K, Cho Y. Gamma-linolenic acid in borage oil reverses epidermal hyperproliferation in guinea pigs. J Nutr 2002;132:3090-3097.
5. Cho Y, Lew BL, Seong K, Kim NI. An inverse relationship between ceramide synthesis and clinical severity in patients with psoriasis. J Korean Med Sci 2004;19:859-863.

6. Lew BL, Cho Y, Kim J, Sim WY, Kim NI. Ceramides and cell signaling molecules in psoriatic epidermis: reduced levels of ceramides, PKC-alpha, and JNK. J Korean Med Sci 2006;21:95-99.

7. Aschrafi A, Franzen R, Shabahang S, Fabbro D, Pfeilschifter J, Huwiler A. Ceramide induces translocation of protein kinase C-alpha to the Golgi compartment of human embryonic kidney cells by interacting with the C2 domain. Biochim Biophys Acta 2003;1634:30-39.

8. Huwiler A, Fabbro D, Pfeilschifter J. Selective ceramide binding to protein kinase C-alpha and -delta isoenzymes in renal mesangial cells. Biochemistry 1998;37:14556-14562.

9. Ruvolo PP. Ceramide regulates cellular homeostasis via diverse stress signaling pathways. Leukemia 2001;15:1153-1160.

10. Bibel DJ, Aly R, Shinefield HR. Topical sphingolipids in antisepsis and antifungal therapy. Clin Exp Dermatol 1995;20:395-400.

11. Ohnishi Y, Okino N, Ito M, Imayama S. Ceramidase activity in bacterial skin flora as a possible cause of ceramide deficiency in atopic dermatitis. Clin Diag Lab Immunol 1999;6:101-104.

12. Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, Imokawa G. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by Staphylococcus aureus. J Invest Dermatol 2002;119:433-439.

13. Aburai K, Yoshino S, Sakai K, Sakai H, Abe M, Loiseau N, et al. Physicochemical analysis of liposome membranes consisting of model lipids in the stratum corneum. J Oleo Sci 2011;60:197-202.

14. Macheleidt O, Kaiser HW, Sandhoff K. Deficiency of epidermal protein-bound omega-hydroxyceramides in atopic dermatitis. J Invest Dermatol 2002;119:166-173.

15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-275.

16. Ruwisch L, Schafner-Koring M, Kleuser B. An improved high-performance liquid chromatographic method for the determination of sphingosine-1-phosphate in complex biological materials. Naunyn Schmiedebergs Arch Pharmacol 2001;363:358-363.

17. Min JK, Yoo HS, Lee EY, Lee WJ, Lee YM. Simultaneous quantitative analysis of sphingoid base 1-phosphates in biological samples by o-phthalaldehyde precolumn derivatization after dephosphorylation with alkaline phosphatase. Anal Biochem 2002;303:167-175.

18. Yoon HT, Yoo HS, Shin BK, Lee WJ, Kim HM, Hong SP, et al. Improved fluorescent determination method of cellular sphingoid bases in high-performance liquid chromatography. Arch Pharm Res 1999;22:294-299.

19. Rogers J, Harding C, Mayo A, Banks J, Rawlings A. Stratum corneum lipids: the effect of ageing and the seasons. Arch Dermatol Res 1996;288:765-770.

20. Gray GM, White RJ, Williams RH, Yardley HJ. Lipid composition of the superficial stratum corneum cells of pig epidermis. Br J Dermatol 1982;106:59-63.

21. Hedberg CL, Wertz PW, Downing DT. The time course of lipid biosynthesis in pig epidermis. J Invest Dermatol 1988;91:169-174.

22. Grubauer G, Feingold KR, Harris RM, Elias PM. Lipid content and lipid type as determinants of the epidermal permeability barrier. J Lipid Res 1989;30:89-96.

23. Wertz PW, Cho ES, Downing DT. Effect of essential fatty acid deficiency on the epidermal sphingolipids of the rat. Biochim Biophys Acta 1983;753:350-355.

24. Coderch L, Lopez O, de la Maza A, Parra JL. Ceramides and skin function. J Clin Dermatol 2003;4:107-129.

25. Kobayashi T, Shinnoh N, Goto I. Metabolism of free sphingoid bases in murine tissues and in cultured human fibroblasts. Eur J Biochem 1989;186:493-499.

26. Hong KK, Cho HR, Ju WC, Cho Y, Kim NI. A study on altered expression of serine palmitoyltransferase and ceramidase in psoriatic skin lesion. J Korean Med Sci 2007;22:862-867.