J. Soc. Cosmet. Scientists Korea
Vol. 34, No. 1, March 2008, 43-50

신규 유사세라마이드의 합성과 그 특성

김 진 국 · 김 경 태 · 박 선 희 · 이 방 용 · 김 기 호 · 김 영 희†

(주)바이오랜드 생명공학연구소
(2008년 2월 22일 접수, 2008년 2월 25일)

Synthesis of Novel Pseudo-ceramide and Its Properties

Jin Guk Kim, Kyoung Tae Kim, Sun Hee Park, Bang Yong Lee, Ki Ho Kim, and Young Heui Kim†

R&D Center, Bioland Ltd., Songjeong-ri, Byongchon-myun, Cheonan-si, Chungnam 330-863, Korea
(Received February 22, 2008; Accepted February 25, 2008)

요 약: 세라마이드는 콜레스테롤, 지방산과 함께 각질층을 구성하는 주요성분으로 각질층의 피부장벽 기능에 중요한 역할을 하며, 아토피 피부염, 건선, 건성습진, 주부습진 등과 같은 염증성 피부질환 방지에 도움이 되는 것으로 알려져 있다. 본 연구에서 2-(2-amino-ethylamino)-ethanol (AEEA)을 출발물질로 하여 신규 유사세라마이드 BPC-16을 합성하고, 그 물리화학적 성질과 화장품의 신규 소재로 활용하기 위하여 세포독성, 보습, 각질제거효능 등을 평가하였다. 합성된 BPC-16을 이용하여 콜레스테롤, 스테아린산을 포함하는 에멀젼을 제조했고, 편광현미경에서 'Maltese Cross'를 확인함으로써 BPC-1이 라멜라 에멀젼을 형성함을 확인하였다. 단층배양세포와 삼차원배양세포에서의 세포독성 실험에서 BPC-16은 보습과 피부손상회복 효과를 보이는 10 mM 이하의 농도에서 독성이 없음을 확인하였다. BPC-16의 보습효능을 확인하기 위하여 Vapormeter와 Corneometer를 이용하여 임상실험을 실시하였으며, 우수한 효과를 확인할 수 있었다. 또한, 피부손상에 대한 회복효과와 Visioscan을 이용한 각질량의 변화 실험에서도 우수한 효과를 확인하였다.

Abstract: Ceramides, a constituent of stratum corneum lipids, play a crucial role in the formation and maintenance of the epidermal permeability barrier. As in many other skin disorders, atopic dermatitis and psoriasis show decrease and transformation of the ceramides. The application of ceramide has been demonstrated to be efficient in the repair of these skin disorders. Nevertheless, natural ceramides are still too expensive and small in quantity to be used as a cosmetic ingredient. Although a lot of pseudo-ceramides have been developed and on the market until now, those pseudo-ceramides did not fully meet the consumer's needs, therefore, there is still a demand for a novel pseudo-ceramides. We synthesized a novel pseudo-ceramide BPC-16 from 2-(2-amino-ethylamino)-ethanol (AEEA), which was characterized by structures having both amide bonds and hydroxyl groups as hydrophilic units, as well as two long alkyl chains. We formulated emulsion with BPC-16, cholesterol, stearic acid, and other components to make an emulsion. These emulsion showed a typical optical anisotropy on cross-polarized microscopy. This 'Maltese cross' appearance is a characteristic figure observed in concentric lamellar emulsion under cross-polarized microscopy. In cytotoxicity assay using MTT in monolayer and three dimension (3D) cell culture, a BPC-16 showed only negligible cytotoxicity up to the effective concentration for barrier repair and moisturization (less than 10 mM). In the measurement of TEWL, this BPC-16 showed significant recovery of water-retaining properties when it was topically applied to either SDS-induced dry skin or normal skin compared to that of base cream. This novel pseudo-ceramide BPC-16 showed as effective in skin barrier repair and moisturization as natural ceramides.

Keywords: *skin barrier, ceramide, moisturization, transepidermal water loss (TEWL), desquamation*

† 주 저자 (e-mail: biolandrnd@biolandkorea.com)

1. Introduction

Skin is a surrounding barrier of body, which protects the organs from the external environment. Skin is composed of the epidermis, dermis, and hypodermics. Epidermis is the first visible region of skins surface, it is highly connected with cosmetics[1]. Epidermis is composed of stratum corneum, stratum lucidum, stratum granulosum, stratum spinusum, and stratum basale. The stratum corneum, a major part in the barrier function of skin, is composed of protein-rich corneocytes and intercellular lipid. Intercellular lipids were composed of ceramides (50 %), cholesterols (25 %), free fatty acids and something else[2-4]. About 20 layers of thin corneous cells are overlapped to form stratum corneum, which is mainly composed of keratin and protein. The moisture of stratum corneum is protected by the amino acids, called by natural moisturizing factor (NMF), their metabolites (pyrrolidone carboxylic acid: high hygroscopic metabolite of glutamine), and skin lipid. Skin lipid is composed of both sebum secreted from sebaceous glands and lipid from derived epidermis, and 0.4 ~ 0.05 mg/cm^2 of lipids are always maintained in human skin[5,6].

Ceramides, a constituent of stratum corneum lipids, play a crucial role in the formation and maintenance of the epidermal permeability barrier. As in many skin disorders, atopic dermatitis and psoriasis show decrease and transformation of ceramides[7,8]. These changes are proposed to be important also for the pathogenesis of atopic dermatitis[9]. The improvement of the defective barrier may relieve the symptoms, prevent aggravation of the disease and possibly, decrease the amount of the corticoid needed. Supplementation of the deficient ceramides or complex lipid mixtures is a logical therapeutic approach with minimum side effects [10,11]. Nevertheless, natural ceramides are such expensive and small in quantity as to be a burden to use as a cosmetic ingredient, and a lot of pseudo-ceramides have been developed and on the market until now [12,13]. But those pseudo-ceramides did not fully satisfy consumers needs, therefore, there is still a demand on novel pseudo-ceramides.

In this study, we designed a novel pseudo-ceramide BPC-16 from 2-(2-amino-ethylamino)-ethanol (AEEA) to mimic natural ceramide's properties. A new pseudo-ceramide BPC-16 was synthesized through an efficient synthetic procedure and its physico-chemical properties, cell viability and barrier-repairing ability were evaluated.

2. Materials and Methods

2.1. Materials and Instruments

Chemicals and organic solvents were purchased from Sigma-Aldrich (MI, USA) and reagent grade unless otherwise indicated. Solvents were purified in common methods before use. The reactions were routinely carried out under inert atmosphere. Melting point was measured using an MEL-TEMP (Laboratory Devices inc. USA). ^{1}H and ^{13}C-NMR spectra were recorded on a Varian-Mercury plus 400 spectrometer (USA). The ^{13}C-NMR spectra were then calibrated and reported, using tetramethylsilane (TMS) as an internal standard. The tablet of BPC-16, were obtained by KBr pellet. The infrared absorption (IR) spectra were recorded on a FT-IR spectrometer (MIDAC Corperation, M series, USA). In a cytotoxicity assay, absorbance at 570 nm was read by ELISA reader (Tecan A-5082, Austria). Optical anisotropy was observed under the cross-polarized light microscope (Nikon 200 POL, Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM), keratinocyte-serum free media (K-SFM), fetal bovine serum (FBS), and antibiotics were purchased from Life Technologies (Grand Island, NY, USA). Insert used for skin equivalent was purchased from Milipore. TEWL value was measured by using an Vapometer (Delfin SWL3 type, Finland).

2.2. Synthesis of Hexadecanoic Acid (2-Hexadecanoylamino ethyl)-(2-Hydroxy ethyl) Amide (Pseudo-ceramide BPC-16)

To a solution of MgO (1.6 g, 40 mM) in 100 mL of water at room temperature was added AEEA (2.08 g, 20 mM) in 100 mL of 1,4-dioxane. Palmitoyl chloride (10.99 g, 40 mM) in 10 mL of 1,4-dioxane was drop-

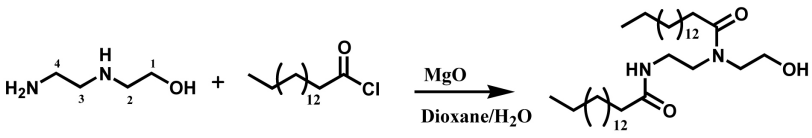

Figure 1. Synthesis of pseudo-ceramide BPC-16.

wise added to this solution for 1 h with vigorous stirring. After completion of the addition, the combined solution was stirred for another 4 h. The disappearance of palmitoyl chloride was checked by TLC. The reaction mixture was filtered and washed with 50 mL of H_2O. The filtered cake was dissolved in 300 mL of $CHCl_3$ with stirring at 50 ℃, and then filtered. Recystallization from $CHCl_3$ at room temperature gave 6.74 g (11.6 mM yield : 58 %) of BPC-16 as a white powder. Mp : 85 ~ 89 ℃. IR 1570 (NH), 1619 (C = O), 1640 (C = O), 2847 (CH), 2916 (CH), 3100 (NH) cm^{-1}, ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 0.879 (t, 6H), 1.251 (s, 48H), 1.594 (s, 4H), 1.815 (s, 1H), 2.132 (q, 2H), 2.366 (q, 2H), 3.462 ~ 3.556 (m, 6H), 3.766 (d, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 14.27, 22.81, 25.75, 29.47, 29.61, 29.77, 32.02, 33.53, 36.90, 39.66, 46.24, 52.06, 60.74, 174.35, 175.33.

2.3. Cross-polarized Microscope Observation

Test formulation and control formulation were observed under the cross-polarized microscope (magnification × 400). These formulations were oil-in-water (O/W) formulation that contains BPC-16, cholesterol, stearic acid, and other components.

2.4. Cell Culture

Human normal fibroblast cells were purchased from American Type Culture Collection (ATCC). Cells were cultured in DMEM supplemented with 10 % FBS and 10 % antibiotics at 37 ℃ in a humidified atmosphere of 95 % air and 5 % CO_2. The cells were changed with fresh medium every two or three days and subcultured with 0.05 % typsin in 0.53 mM ethylenediaminetetraacetic acid (EDTA). Normal keratinocytes were isolated from the foreskin tissue and cultured in K-SFM.

Table 1. Composition of Basic O/W Emulsion

Components	Emulsion 1 (%)	Emulsion 2 (%)
Pseudo-ceramide BPC-16	0.5	-
Cholesterol	1	1
Palmitic acid	1.5	1.5
Lecithin	4	4
Polysorbitan 60	0.5	0.5
Miglyol 8810	8	8
1,3-Buthylene glycol	4	4
Water	80.5	81

2.4.1. Cell Viability Assay in Monolayer Culture

Human fibroblasts were seeded at a density of 1 × 10^5 cells into 24-well plates and cultured at 37 ℃ in 5 % CO_2. After 1 day, cells were added fresh medium containing 2 % serum and treated with samples for 24 h. 100 uL of 2.5 mg/mL MTT was treated into well plate and incubated at 37 ℃ for 4 h. Then, the medium containing MTT were discarded and MTT formazan product were extracted with 1 mL of dimethyl sulfoxide (DMSO) and measured at 570 nm by ELISA reader.

2.4.2. Cell Viability Assay in Three Dimension Culture

Dermal equivalents were made using type I collagen, 5 × DMEM, raft buffer (2.2 % Na_2HCO_3, 200 mM HEPES, 0.05 N NaOH) and 1 × 10^4 human fibroblasts per mL. The ration of type I collagen, 5 × DMEM and raft buffer was 7 : 2 : 1. The cells and collagen mixtures were poured into 12-well plates and gelated at 37 ℃ for 1 h. Dermis was made in insert and cultured for 3 days in ascorbic acid-containing DMEM with 10 % FBS. The normal keratinocytes were seeded on dermis at the density of 2 × 10^5 cells/mL and incubated for 7 days with supplemented ascorbic acid-containing DMEM with 10 % FBS and K-SFM (1 : 1). Then, the me-

dium of insert was discarded for induction of keratinization and cultured for 5 days. Samples were treated on epidermis for 24 h and 1 mL of 0.25 mg/mL MTT was treated into insert. After incubating at 37 ℃ for 4 h, MTT solution was discarded and MTT formazan product was extracted with 2 mL DMSO and measured at 570 nm by ELISA reader.

2.5. Moisturizing Effect of BPC-16

2.5.1. Moisture Content Measurement by Using Corneometer

The 5 subjects visited the research agency after showering without any soap in the morning. It was washed away with the water flowing on the both elbows. A quadrangle with 1.5 × 1.5 cm was drawn on both elbows with the position fixed. In a relaxed state, the subjects rested for at least 30 min in the controlled-room with both forearms uncovered. Each test area was measured 5 times as a baseline prior to the first application with Corneometer CM825 (Courage & Khazaka, Germany), and we took the mean values except the maximum and minimum of total 5 values.

2.5.2. Transepidermal Water Loss (TEWL) Measurement by Using Vapometer

A group of fermale volunteers, ranging in age from 23 to 30 years, were recruited. Transepidermal water loss (TEWL) was measured at 22 ± 2 ℃ and 55 ± 5 % relative humidity with a Vapometer.

Moisturization test was carried out without the skin damage. 8 mg of each sample (0.5 % of BPC-16 emulsion, control emulsion, water) was spread over a 4 cm^2 area of the ventral forearm (2 cm × 2 cm; 2 mg/cm^2), followed by TEWL measurements being taken at regular intervals, for a total of 6 h.

2.6. Desquamation Reduction Effect of BPC-16

The image picture was taken from both elbows with Visioscan VC98 (Courage & Khazaka, Germany) and analyzed to record the measurement value. In the application protocol, 2 mg/cm^2 of the cream (0.5 % BPC-16) was uniformly spread on the right site of the elbow. The other side of right elbow remained untreated.

After standing over 1, 2, and 3 days in the same condition as mentioned above on the day 0, the test was carried out with Visioscan, and the sample was treated on it. After stopping the treatment of the sample, we measured desquamation on the tested area as same method as first day's measurement by Visioscan.

2.7. Barrier-repairing Effect

We damaged the skin by the treatment of 1 % sodium dodecyl sulfate (SDS) solution for 1 h. After washing the damaged skin with water tissue, 8 mg of each sample (0.5 % of BPC-16 emulsion, control emulsion, water) was spread over a 4 cm^2 area of the ventral forearm (2 cm × 2 cm; 2 mg/cm^2), followed by TEWL measurements being taken at regular intervals, for a total of 6 h.

3. Results and Discussion

3.1. Synthesis of Pseudo-ceramide BPC-16

Pseudo-ceramide BPC-16 was obtained through the reaction of AEEA and palmitoyl chloride with the aid of MgO. It was characterized by structures having amide bonds and hydroxyl groups as hydrophilic units, as well as two long alkyl chains. AEEA, the starting material, have main two peaks at 2.76 ppm for $H_{2,3,4}$ and 3.62 ppm for H_1 in the ^{1}H-NMR spectrum (scheme 1). The structure of the product was supported by the down field chemical shift of the $H_{2,3,4}$ of AEEA shifted from 2.76 ppm to 3.46 ppm due to the adjacent carbonyl group coming from palmitoyl chloride. BPC-16 exhibited the characteristic absorption at 1619 cm^{-1}, 1640 cm^{-1}, which are carbonyl stretching vibration in IR spectrum. The new characteristic amide band appeared at 1570 cm^{-1}. This result suggested that new amide bonds were synthesized.

3.2. Cross-polarized Microscope

The multi-lamellar emulsion was generated by repeat heating and cooling of intercellular lipid components, which is extracted with acetone/ether from skin substance of human, with water. The multi-lamellar

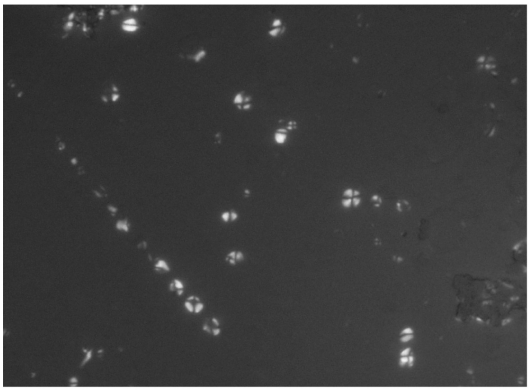

Figure 2. Cross-polarized microscopic observations of pseudo-ceramide BPC-16 (Nikon 200 POL, Tokyo, Japan).

emulsion showed the specific 'Maltese cross' in lamellar emulsion by cross-polarized microscope[14,16]. 'Maltese cross', appeared in intercellular lipid components of human, was observed in emulsion 1 (0.5 % BPC-16) by cross-polarized microscope (Figure 1). In Figure 1, it was confirmed that BPC-16 formed lamellar emulsion.

3.3. Cell Viability Assay

We evaluated cell cytotoxicity of pseudo-ceramide BPC-16 by MTT assay using monolayer cell culture *in vitro*, samples were prepared at various concentrations. Figure 2 showed the result that did not show any cytotoxicity. Moreover, we evaluated cell cytotoxicity by using three dimention (3D) cell culture, pseudo-ceramide BPC-16 did not show any cytotoxicity up to the effective concentration for barrier repair and moisturization (less than 10 mM) (Figure 3).

3.4. Moisturizing Effect of BPC-16

The stratum corneum layer of epidermis existing in the outermost of the skin prevents skin-dryness by suppressing the evaporation of the water in the skin. Ceramides are the main components of the stratum corneum layer, and the lamellar layer formed by ceramides in the stratum corneum has the main effect on the moisturizing on the skin[15].

3.4.1. Moisture Content Measurement by Using Corneometer

Subjects were evaluated at baseline and at 1, 2, 3 and 6 day using Corneometer. The results of moisture content measured with Corneometer are as in Table 2.

Table 2. Moisturizing Effect of Pseudo-ceramide BPC-16 by Using Corneometer CM 825

Time (day)	Treated site		Untreated site	
	Average	S.D.	Average	S.D.
0	16.07	5.37	18.40	8.15
1	18.27	6.12	18.73	7.76
2	19.27	6.82	19.20	9.22
3	20.80	7.09	18.87	7.89
6	22.33	6.65	18.73	6.91

The data were represented as mean values (± standard deviations) of five experiments.

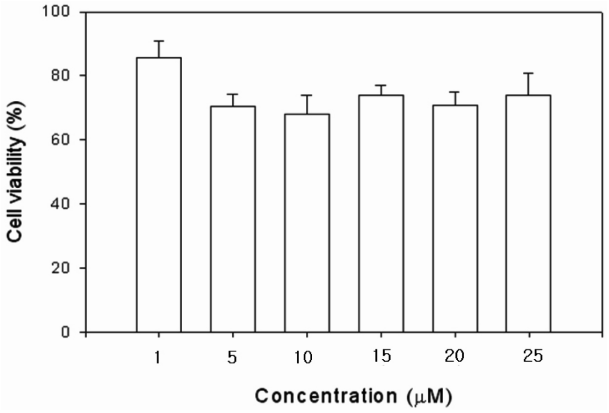

Figure 3. Cytotoxicity of pseudo-ceramide BPC-16 in monolayer cell culture. The data were represented as mean values (± standard deviations) of five experiments.

To analyze and verify the results, the statistical processing program included in the Microsoft Excel 2000 version was employed. As a results of the statistical processing, treated site showed the good moisturizing effect for the period (days 2, 3, and 6) with the statistically significant level compared with the before treatment ($p < 0.05$). Compared with the untreated site, treated site showed the statistically significant ($p < 0.05$) moisturization effect at day 3 when the product was used and until 3 days (6 day) after the use was discontinued as well. From these facts, it was determined that the BPC-16 provided the good moisturization effect.

Table 3. Change of The Desquamation Score Before and After Using Visioscan

Time (day)	Treated site		Untreated site	
	Average	S.D.	Average	S.D.
0	11.74	1.81	13.68	0.98
1	11.78	0.87	11.56	1.30
2	12.22	0.16	11.74	1.11
3	11.51	2.52	11.45	1.62
6	12.03	1.90	11.34	1.87

The data were represented as mean values (± standard deviations) of five experiments.

3.4.2. Transepidermal Water Loss (TEWL) Measurement by Using Vapometer

Moisturizing effect of BPC-16 (emulsion 1) applied on the skin was measured by the comparison of the TEWL value with control emulsion (emulsion 2, without BPC-16) and water (Figure 4). The TEWL value of emulsion 1 containing BPC-16 showed the lowest value of the tested samples, supporting that pseudo-ceramide BPC-16 formed so efficient lamellar structure as to prevent the skin from losing the water contained in the skin.

3.5. Desquamation Reduction Effect of BPC-16

With Visioscan, the variation of desquamation score was observed and it was again analyzed with Visioscan software 2000 after 0, 1, 2, 3 and 6 days. The results are as in Table 3. After running through Microsoft Excel 2000 version for analysis and verification, the result of desquamation score was significant decreased ($p < 0.05$) for the period (days 1, 2, 3, and 6) after application of the BPC-16. Compared with the untreated site, the statistically significant effect of desquamation reduction was observed at days 1, 2, and 3 when the product was used and 3 days after the BPC-16 wasn't used further. From these results, it was determined that the BPC-16 provided the good desquamation reduction effect.

3.6. Barrier-repairing Effect

In order to evaluate the recovery effect on damaged skin, TEWL measurement was carried out by Vapormeter. After the intentional injury by SDS treatment,

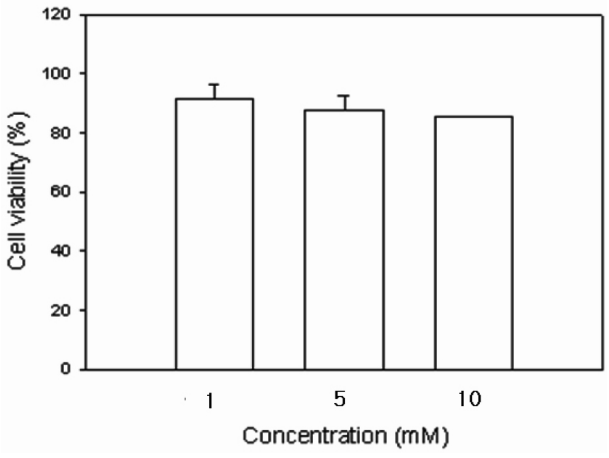

Figure 4. Cytotoxicity of pseudo-ceramide BPC-16 in 3D cell culture (skin equivalents were prepared from type I collagen, 5X DMEM, raft buffer and human fibroblasts. Then, the normal keratinocytes were seeded on it). The data were represented as mean values (± standard deviations) of five experiments.

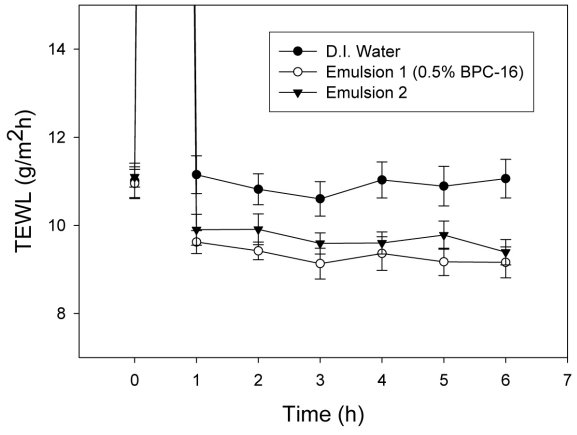

Figure 5. Transepidermal water loss profile of pseudo-ceramide BPC-16 by using Vapormeter. The data were represented as mean values (± standard deviations) of five experiments.

we measured the TEWL values before and after the treatment of pseudo-ceramide BPC-16 emulsion (0.5 % O/W emulsion) comparing to control emulsion (not containing pseudo-ceramide BPC-16) and water (Figure 5). Pseudo-ceramide BPC-16 emulsion (0.5 % O/W emulsion) had beneficial effect on recovery from SDS-induced barrier disruption more than that of control emulsion.

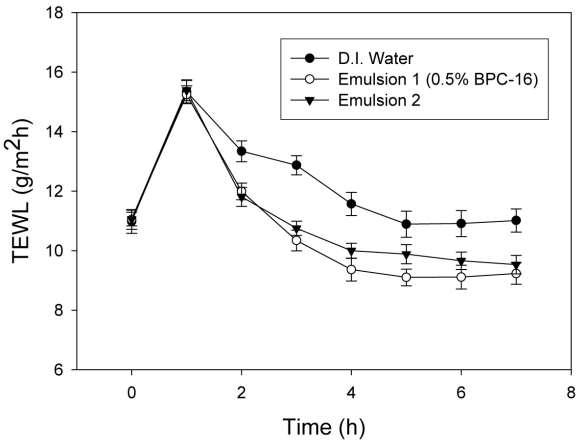

Figure 6. Barrier repair of SDS-damaged skin by pseudo-ceramide BPC-16 by using Vapormeter. The data were represented as mean values (± standard deviations) of five experiments.

The results showed that BPC-16 made a rapid recovery of the skin itself by replacing a natural ceramides, because BPC-16 has the similar characteristic structure with natural ceramides on the human skin.

4. Conclusion

In this study, we synthesized novel pseudo-ceramide BPC-16 (hexadecanoic acid (2-hexadecanoylamino-ethyl)-(2-hydroxy-ethyl)-amide) from AEEA and fatty acid having both amide bonds and hydroxyl groups as hydrophilic units, as well as two long alkyl chains. BPC-16 showed only negligible cytotoxicity up to the effective concentration for barrier repair and moisturization by MTT assay using monolayer and three dimensional (3D) cell culture. Emulsion, formulated with this BPC-16 was found the 'Maltese cross' pattern which clearly showed the existence of multi-lamellar structure of lipids. In the measurement of TEWL on the SDS-induced dry skin, the BPC-16 showed a significant recovery of water-retaining properties could be shown when these BPC-16 (0.5 % in O/W emulsion) applied topically from the damage compared to that of base cream. Also, BPC-16 (0.5 % in O/W emulsion) was applied to normal skin. It showed an increase of moisturization. *In vivo* efficacy test using Corneometer and Visioscan VC98, a cream containing BPC-16 was treated. As a result, we found that BPC-16 showed statistically significant ($p < 0.05$) improvement effects in moisturizing and desquamation reduction compared with the untreated area. We obtained a novel BPC-16 through the practical and economical pathway, and this BPC-16 showed as much effective in skin barrier repair and moisturization as natural ceramides.

References

1. A. V. Rawlings and C. R. Harding, Moisturization and skin barrier function, *Dermatol. Ther.*, **17**, 43 (2004).
2. P. M. Elias, Epidermal lipids, barrier function, and desquamation, *J. Invest. Dermatol.*, **80**, 44 (1983).
3. G. M. Gray, R. J. White, and H. J. Yardley, Lipid composition of the superficial stratum corneum cells of the epidermis, *Br. J. Dermatol.*, **106**, 59 (1982).
4. P. M. Elias and G. Menon, Structural and lipid biochemical correlates of the epidermal permeability barrier, *Adv. Lipid. Res.*, **24**, 1 (1991).
5. I. R. Scott, C. R. Harding, and J. G. Barratt, Histidine-rich protein of the keratohyalin granules : source of the free amino acids, urocanic acid, and pyrrolidone carboxylic acid in mammalian stratum corneum, *Biochem. Biophys. Acta.*, **719**, 110 (1982).
6. J. A. Bouwstra, H. W. Groenink, J. A. Kempenaar, S. G. Romeijn, and M. Ponec, Water distribution and natural moisturizer factor content in human skin equivalents are regulated by environmental relative humidity, *J. Invest. Dermatol.*, **128**(2), 378 (2008).
7. Y. Werner, M. C. Myers, and D. A. Taylor, Electron probe analysis of human skin : determination of water concentration profile, *J. Invest. Dermatol.*, **90**, 218 (1988).
8. K. Akimoto, N. Yoshikawa, Y. Higaki, M. Kawashima, and G. Imokawa, Quantitative analysis of stratum corneum lipids in xerosis and asteatotic eczema, *J. dermatol.*, **20**, 1 (1993).
9. F. S. Larsen and J. M. Hanifin, Secular change in the occurrence of atopic dermatitis, *Acta. Derm.*

Venereol., **176**, 7 (1992).
10. G. Imokawa, A. Abe, K. Jin, Y. Higaki, M. Kawashima, and A. Hidano, Decreased level of ceramides in stratum corneum of atopic dermatitis. An etiologic factor on atopic dry skin, *J. Invest. Dermatol.*, **96**, 523 (1991).
11. C. Geilen, T. Wieder, and C. E. Orfanos, regulatory role in cell proliferation, differentiation and apoptosis in human epidermis, *Arch. Dermatol. Res.*, **289**, 559 (1997).
12. A. Yamamoto, S. Serizawa, M. Ito, and Y. Sato, Stratum corneum lipid abnormalities in atopic dermatitis, *Arch. Dernatol. Res.*, **283**(4), 219 (1991).
13. M. Man, K. R. Feingold, and P. M. Elias, Optimization of physiological lipid mixtures for barrier repair, *J. Invest. Dermatol.*, **106**(5), 1096 (1996).
14. F. Caboi and M. Monduzzi, Didodecyldimethylammonium bromide vesicles and lamellar liquid crystals, *A Multinuclear NMR and Optical Microscopy Study. Langmuir.*, **12**, 3548 (1996).
15. K. R. Feingold, Permeability barrier homeostasis: its biochemical basis and regulation, *Cosmet. Toilet.*, **112**, 49 (1997).
16. A. Frey-Wyssling, Submicroscopic morphology of protoplasm and its derivatives, 219, Elsevier, New York (1948).