32nd IFSCC Congress
London 19–22 September 2022

Epidermal hydrating and anti-melanogenic effects of rice-derived glucosylceramides and elasticamide on cell basis evaluation

Poster ID
111

Hiroshi Shimoda[1], Shogo Takeda[1], Kenchi Miyasaka[1], Akari Yoneda[1], Yoshiaki Manse[2], Toshio Morikawa[2*]

[1]Oryza Oil & Fat Chemical Co., Ltd.,[2] Pharmaceutical Research and Technology Institute, Kindai University.

Introduction:

Skin ceramides (Cer) are lipids mainly existing in stratum corneum and play pivotal rolls on epidermal barrier function with other lipids and moisturizing proteins such as filaggrin. Especially 12 major Cer form intracellular lipids in stratum corneum. In terms of plant-derived major Cer, glucosylceramides (GlcCer) dominantly exist in variety of botanical resources and have well studied about clinical effects on skin barrier function.[1)] Besides, as other biological effects of GlcCer on skin, maize GlcCer mixture has been reported to suppress melanogenesis in B16 melanoma cells.[2)] Rice GlcCer consist of multiple molecules, which are composed of different types of sphingoid bases and multiple length of free fatty acids. In addition there are free Cer as minor constituents including elasticamide (ceramide[AP]) near GlcCer fraction. However, no study results have been reported regarding epidermal hydration and anti-melanogenic effects of these single molecules. Therefore, in this study, we evaluated the effects of these compounds on epidermal moisturizing and anti-melanogenic effects .

Materials & Methods:

1. Isolation of GlcCer and elasticamide: GlcCer and elasticamide (Figure 1) were purified from GlcCer-rich fraction of the by-product rice bran oil by a normal phase middle pressure column chromatography and reversed-phase HPLC. [1)]

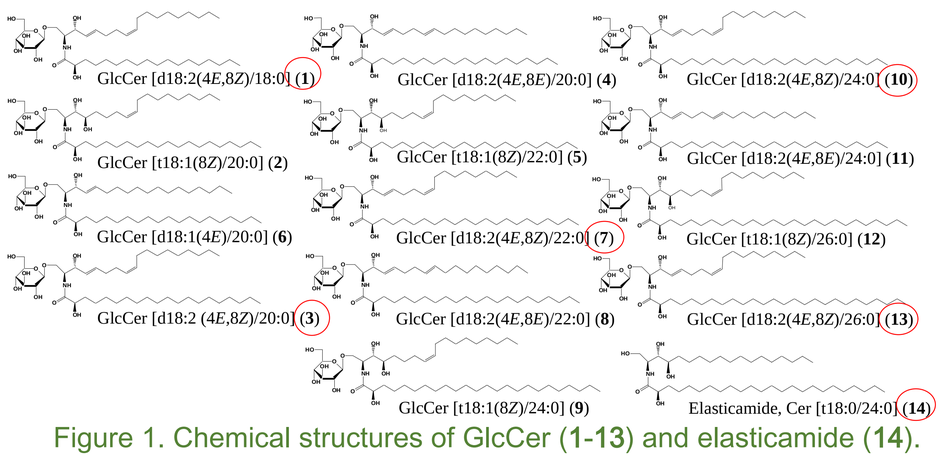

Figure 1. Chemical structures of GlcCer (**1-13**) and elasticamide (**14**).

2. Epidermal hydrating effects: Reconstructed human epidermal keratinization (RHEK) model (J-TEC) was used to measure transepidermal water loss (TEWL) chronologically after adding samples (10 μM) to the membrane side of RHEK. For evaluation of the mechanism of elasticamide (**14**) and GlcCer [d18:2 (4*E*,8*Z*)/26:0] (**13**) in RHEK, we determined Cer contents by TLC and measured firagglin protein expressions of by western blotting analysis.

3. Anti-melanogenic effect: B16 melanoma cells were used to induce melanogenesis by theophylline. For GlcCer [d18:2 (4*E*,8*Z*)/20:0] (**3**) and elasticamide (**14**), we also evaluated anti-melanogenic effects in a human 3D cultured epidermal melanocytes (MEL-300-A, Kurabo Ind. Ltd.).

Results & Discussion:

1. Moisturizing effects of GlcCer (1-5, 7, 9-13) and elasticamide (14) in RHEK: As a result of TEWL from the surface of RHEK, 10 mM of GlcCer[d18:2(4*E*,8*Z*)] including GlcCer[d18:2(4*E*,8*Z*)/18:0] (**1**),GlcCer[d18:2(4*E*/8*Z*)/ 20:0] (**3**), GlcCer[d18:2(4*E*/8*Z*)/22:0] (**7**), GlcCer[d18:2(4*E*/8*Z*)/24:0] (**10**), GlcCer[d18:2 (4*E*/8*Z*)/26:0] (**13**) and elasticamide (**14**) decreased TEWL by 7-day treatment (Figure 2). Among GlcCer, TEWL was improved depending on the length of fatty acids and **13** exhibited most potent hydrating effect. The hydrating effect of **14** was as strong as **13**.

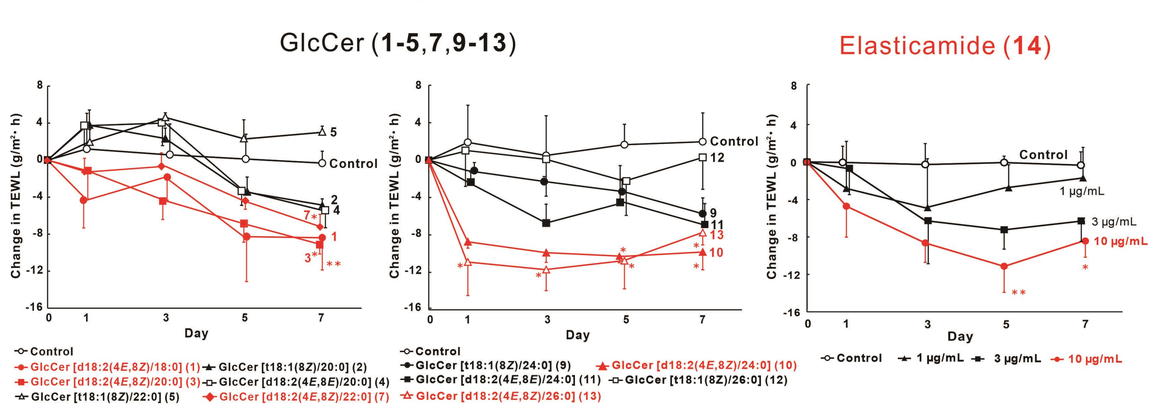

Figure 2. Effect of GlcCer (**1-5**, **7**, **9-13**) and elasticamide (**14**) on TEWL in RHEK Each value represents the means ± S.E. (N=3-4). Asterisks denote significant differences from the control group, *p<0.05, ** p<0.01.

2. Effect of elasticamide (14) and GlcCer (13) on Cer and filaggrin in RHEK: To find the epidermal hydration mechanism, we determined Cer contents in RHEK. However all GlcCer (**1-5**, **7**, **9-13**) had no effect on total Cer contents (See full papaer). On the other hand, **14** (10 μg/mL, 14.3 μM) significantly increased total Cer and Cer[NS/NDS] (Figure 3A). 1 μM GlcCer (**13**) enhanced filaggrin production.

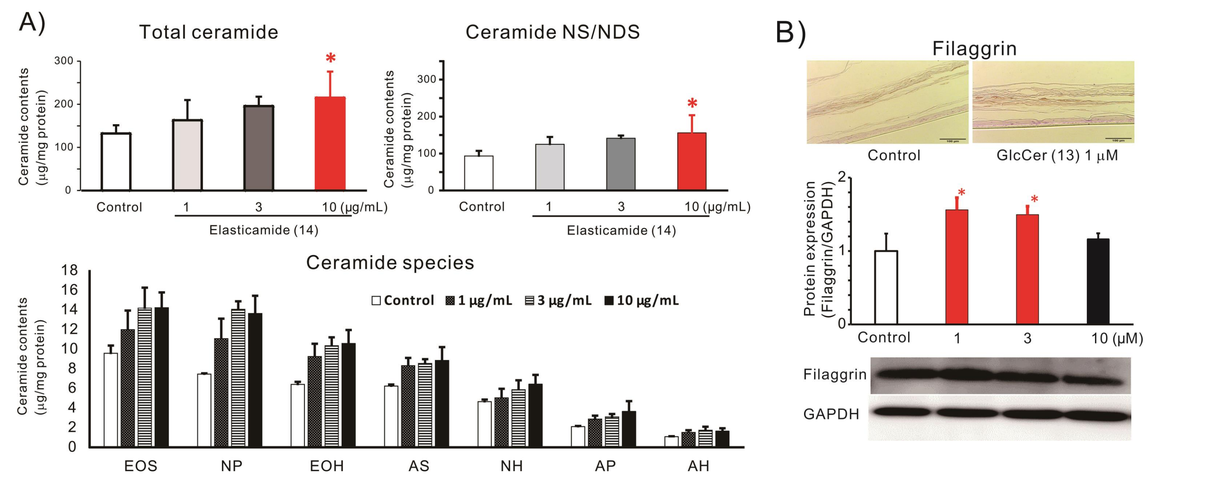

Figure 3. Effects of elasticamide (**14**) on Cer contents and of GlcCer (13) on filaggrin in RHEK: A) Changes in Cer species treated with **14**. B) Filaggrin production by **13**. Each value represents the means ± S.E. (N=4). An asterisk denotes significant difference from the control group, *p<0.05.

3. Effects of GlcCer (1-11) and elasticamide (14) on melanogenesis in B16 melanoma cells:

As shown in Table 1, GlcCer [d18:2(4*E*,8*Z*)/18:0] (**1**) significantly suppressed melano-genesis without cytotoxicity. GlcCer [d18:2(4*E*,8*Z*)/20:0] (**3**) also inhibited melanogenesis.
However, **3** exhibited cytotoxicity at a concentration >3 μg/mL. The IC50 values of **1** and **3** were less than 10 μg/mL. GlcCer[d18:2 (4*E*,8*E*)/20:0] (**4**) and GlcCer [d18:1(4*E*)/20:0] (**6**) significantly suppressed melanogenesis. GlcCer (**9-11**) with very long fatty acid lengths affected cell survival, but not melanogenesis.
Elasticamide (**14**) strongly suppressed melanogenesis at concentrations with an IC50 value of 3.9 μM.

Table 1. Inhibitory activities of GlcCer (1-11) and elasticamide (14) against theophylline-stimulated melanogenesis and cell viability in B16 melanoma cells

Sample Conc. (μg/mL)	1	3	10	IC_{50} (μM)
GlcCer[d18:2(4*E*, 8*Z*)/16:0]	8.2±3.7 (96.4±1.0)	4.3±5.3 (97.5±0.8)	4.3±3.0 (95.9±0.9)	
GlcCer[d18:2(4*E*, 8*E*)/16:0]	8.1±3.3 (96.8±0.6)	10.8±3.4 (96.7±0.9)	5.8±3.3 (95.2±0.4)	
GlcCer[d18:2(4*E*,8*Z*)/18:0] (1)	29.7±1.3** (90.6±5.8)	45.3±3.4** (84.1±1.1)	69.8±2.9** (95.8±2.8)	6.6
GlcCer[t18:1(8*Z*)/20:0] (2)	5.3±6.7 (85.8±3.6)	15.9±4.7 (79.7±10.4)	-12.9±8.2 (78.1±4.9)	
GlcCer[d18:2(4*E*,8*Z*)/20:0] (3)	22.9±2.4** (91.6±5.4)	45.8±2.1** (70.3±3.2**)	63.8±1.4** (63.2±2.9**)	5.9
GlcCer[d18:2(4*E*,8*E*)/20:0] (4)	36.1±1.1** (88.6±3.3)	44.0±2.5** (88.2±5.6)	45.6±1.3** (91.0±2.1)	>10
GlcCer[t18:1(8*Z*)/22:0] (5)	-5.9±4.2 (115.1±8.3)	1.6±5.1 (86.4±5.1)	11.2±1.5 (81.9±6.3)	
GlcCer[d18:1(4*E*)/20:0] (6)	17.3±1.5 (97.7±1.5)	4.8±2.5 (97.4±0.8)	32.7±4.8** (95.8±0.2*)	>10
GlcCer[d18:2(4*E*,8*Z*)/22:0] (7)	7.8±8.1 (81.5±4.1)	-22.7±5.2 (97.1±7.9)	0.0±7.8 (100.6±7.0)	
GlcCer[d18:2(4*E*,8*E*)/22:0] (8)	13.7±5.4 (94.6±2.6*)	22.5±3.8* (97.3±1.4)	0.0±2.6 (95.7±0.9)	
GlcCer[t18:1(8*Z*)/24:0] (9)	18.8±2.1* (49.6±2.7**)	26.6±4.5** (51.3±1.9**)	21.0±2.8* (63.1±2.9**)	
GlcCer[d18:2(4*E*,8*Z*)/24:0] (10)	8.2±3.7 (62.4±5.5**)	4.3±5.3 (73.6±5.5**)	4.3±3.0 (101.3±4.3)	
GlcCer[d18:2(4*E*,8*E*)/24:0] (11)	5.7±1.5 (48.5±0.5**)	2.5±0.9 (68.8±1.1**)	8.5±0.9 (84.1±1.3**)	
Elasticamide, Cer[t18:0/24:0](14)	21.7±5.2** (98.1±6.0)	46.4±2.7** (100.8±7.2)	63.4±2.7** (86.3±6.7)	3.9

Cytotoxicity was indicated in parenthesis. Each value represents the means ± S.E. (N=4). Asterisks denote significant differences from the control group, *:p<0.05, **:p<.0.01.

4. Effects of GlcCer (3) and elasticamide (14) on melanogenesis in normal melanocytes:

Elasticamide (**14**) significantly decreased the content of melanin, whereas **3** did not in a human 3D cultured epidermal melanocytes. Observations of the upper side of the culture melanocytes and a microscopic analysis of Fontana-Masson staining showed that **14** suppressed melanin production as well in the basal layer (see full paper).

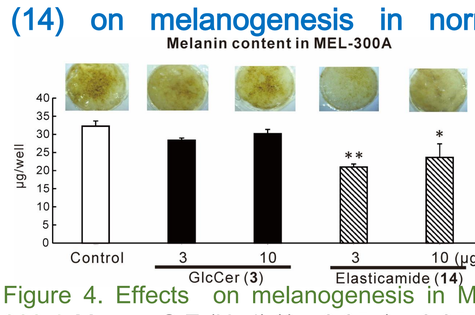

Figure 4. Effects on melanogenesis in MEL-300-A Means±S.E.(N=4).**p<0.05, *p<0.05.

Conclusions:

GlcCer [d18:2(4*E*,8*Z*)] exhibited fatty acid length-dependent moisturizing effects in a epidermis model. The mechanism seems to involve enhancement of filaggrin expressions. Whereas, elasticamide showed the strong moisturizing effect by accelerating SC Cer [NS/NDS] production. In terms of anti-melanogenic effects, GlcCer [d18:2(4*E*,8*Z*)] with C18 or C20 fatty acids and elasticamide suppressed melanin production in melanoma cells. The inhibitory mechanism of GlcCer was not clarified, however; the effect of elasticamide involved suppression of TYRP1 and ATP expression. This study is the first report which examined moisturizing and anti-melanogenic effects of Cer species in single molecule level.

Acknowledgements:

This work was supported by the New Aichi Creative Research and Development Subsidy [grant numbers: 118-20, 2020] and Oryza Oil & Fat Chemical Co., Ltd.

References:

1) Takara T, et al. Functional Foods Health Disease 11:385 (2021),
2) Kinoshita M, et al. J Oleo Sci 56:645 (2007).