

The efficacy of synthetic pseudo-ceramide for dry and rough lips

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Abstract

OBJECTIVE: Lips can easily become dry and rough, one reason being the characteristics of their ceramide (CER) profile. Lips have lower levels of total ceramides, higher percentages of CER[NS] and CER[AS], and lower percentages of CER[NP] and CER[NH] than skin in other regions of the body. The purpose of this study was to clarify the effects of synthetic pseudo-ceramide (pCer; Cetyl-PG hydroxyethyl palmitamide) to improve the dryness and roughness of the lips of healthy subjects in a formulation that exclude an occlusive effect.

METHODS: Thirty-one Japanese female subjects with normal skin (age range 21–37 years; mean 28.6) were enrolled in this study. A four-week continuous use test was conducted using samples with or without 0.5% or 2.0% pCer. The degree of lip roughness was scored, and values of capacitance, transepidermal water loss (TEWL) and lip surface elasticity were measured. Endogenous CER profiles and absorption levels of pCer in the stratum corneum (SC) were analysed in tape-stripped skin specimens.

RESULTS: Treatment with the pCer-2.0% sample significantly improved the visual roughness score after 2 and 4 weeks compared to 0 weeks and compared to the Blank. Moreover, an improvement in TEWL was observed after 4 weeks of treatment with the pCer-2.0% sample. CER[NP] showed a significant increase in pCer-2.0% treated lips after 4 weeks compared to the Blank. Both pCer-0.5% and pCer-2.0% were significantly absorbed after 2 weeks compared with the Blank.

CONCLUSION: The effect of the synthetic pseudo-ceramide pCer to improve the roughness of lips was shown excluding the effect of occlusiveness derived from the formulation for the first time. Since the improvement of TEWL and absorption of pCer was observed, we concluded that pCer was first absorbed in rough lip areas, improved the ceramide profile and consequently restored the barrier function.

Résumé

OBJECTIF: Les lèvres peuvent facilement devenir sèches et rugueuses, l'une des raisons étant les caractéristiques de leur profil de céramide (CER). Les lèvres présentent des taux plus faibles de céramides totaux, des pourcentages plus élevés de CER[NS] et de CER[AS] et des pourcentages plus faibles de CER[NP] et CER[NH] que la peau des autres régions de l'organisme. L'objectif de cette étude visait à clarifier les effets du pseudo-céramide synthétique (pCer;

cétyl-PG hydroxyéthyl palmitamide) pour améliorer la sécheresse et la rugosité des lèvres de sujets en bonne santé avec une formulation excluant un effet occlusif.

MÉTHODES: Trente et un sujets de sexe féminin japonais ayant une peau normale (tranche d'âge: 21 à 37 ans; moyenne: 28,6 ans) ont été inclus dans cette étude. Une analyse d'utilisation continue de quatre semaines a été effectuée en employant des échantillons avec ou sans pCer à 0,5 % ou 2,0 %. Le degré de rugosité des lèvres a été noté et les valeurs de capacitance, de perte d'eau transépidermique (transepidermal water loss, TEWL) et d'élasticité de la surface des lèvres ont été mesurées. Les profils CER endogènes et les niveaux d'absorption de pCer de la couche cornée (stratum corneum, SC) ont été analysés dans des échantillons cutanés fixés par bande adhésive.

RÉSULTATS: Le traitement avec l'échantillon pCer-2,0% a amélioré de façon significative le score de rugosité visuelle après 2 et 4 semaines par rapport à 0 semaine et par rapport au groupe témoin. En outre, une amélioration de la TEWL a été observée après 4 semaines de traitement avec l'échantillon pCer-2,0%. Après 4 semaines, le CER[NP] a augmenté de manière significative dans les lèvres traitées avec pCer-2,0% par rapport au groupe témoin. Le pCer-0,5 % et le pCer-2,0% ont tous deux été absorbés de manière significative après 2 semaines par rapport au groupe témoin.

CONCLUSION: L'effet du pseudo-céramide synthétique pCer pour améliorer la rugosité des lèvres a été démontré, excluant l'effet de l'occlusion dérivé de la formulation pour la première fois. Puisque l'amélioration de la TEWL et l'absorption du pCer ont été observées, nous avons conclu que le pCer était d'abord absorbé dans les zones rugueuses des lèvres, améliorait le profil du céramide et, par conséquent, rétablissait la fonction barrière.

Introduction

The most common problem of the lips is dryness and chapping [1,2], followed by hardening of the skin due to abnormal desquamation that is peculiar to the lips [3]. These problems are due to the dermatologic characteristics of lips. Specifically, the lips are transition areas from mucous membranes to skin, having a mucosal element but not sebaceous glands [4], and are characterized by a ceramide profile that plays an important role in SC function [5,6]. So far, Kobayashi and Tagami have reported that lips and cheeks have different moisturizing and barrier functions, the lips having a lower SC water content and a higher TEWL than the cheeks [7]. As for ceramides (CERs) of the lips, the following characteristics have been clarified; lips have lower levels of total CER, a higher percentage of CER[NS] and CER[AS], a lower percentage of

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CER[NP] and CER[NH], a lower percentage of CER[NS] with long chains having large total carbon numbers and a higher percentage of CER[NS] with short chains having small total carbon numbers compared with other skin regions of the body [5,6]. The characteristics of the CER profile in the lips compared with the upper arm of healthy males is similar to that of atopic skin compared with normal skin [5]. It is considered that these characteristics lead to dryness and roughness of the lips. Furthermore, very recently, it has been reported that the ceramide [NP]/[NS] ratio in the stratum corneum is a potential marker for skin properties, and the importance of the ceramide profile has attracted attention [8].

Regarding the roughness of the lips, Hikima et al. reported that the water content of the SC is related to lip chapping, and the remaining desmosomes cause hardening of the skin due to abnormal desquamation [3]. As a result of investigating the relationship between rough lip conditions and ceramides, we found that not only the total amount of CER but also the specific ceramide species and their chain lengths greatly influence the maintenance of the SC function of the lips [9].

Generally, lip balm is used to prevent dryness of the lips. This is because it is considered that a lip cream balm approach to increase the occlusivity of the lip film is effective for moisturizing the lips with high TEWL values. However, it has not been clarified to what extent the water occlusive property of a coating film has an effect to improve the roughness of the lips. Therefore, in a previous study we clarified that a highly occlusive formulation consisting of common oils and waxes without a specific care agent, improves lip roughness [10].

Furthermore, since the effect of the continuous use of synthetic pseudo-ceramide (pCer) is observed in atopic dermatitis [11,12], the pCer effect can be expected even on lips having similar profiles to the barrier-disrupted skin of patients with atopic dermatitis. In fact, it has been confirmed that lip balm containing pCer alleviates the symptoms of sensitive lips [13]. The purpose of this study was to clarify the effect of pCer to improve dryness and roughness of the lips in healthy subjects in a formulation that excludes an occlusive effect.

Materials and methods

Ethics

This study was conducted according to the Declaration of Helsinki Principles, and the protocol was approved by the Ethical Committee of the Kao Corporation. Consent to participate in this study was obtained from each subject after the procedures had been explained.

Subjects

Thirty-one Japanese female subjects with normal skin (age range 21–37 years; mean 28.6, standard error 0.67) who were concerned about their dry lips were enrolled in the study. The examination was conducted from February to March in 2016.

Materials

Cetyl-PG hydroxyethyl palmitamide, developed by the Kao Corporation (Tokyo, Japan), was used as the synthetic pseudo-ceramide (pCer) [14].

A sample having low occlusivity that had a low effect to improve lip roughness in a previous study [10], was defined as a blank formulation (Blank), and samples containing 0.5% or 2.0%

Table 1 Characteristics of the samples used for a continuous application test

INCI	Blank	pCer-0.5%	pCer-2.0%
N-(Hexadecyloxyhydroxypropyl)-N-hydroxyethylhexadecanamide	–	0.50	2.00
Paraffin wax	7.00	7.00	7.00
Synthetic hydrocarbon wax	3.00	3.00	3.00
Ceresin	2.00	2.00	2.00
Diisostearyl malate	20.00	19.90	19.50
Isotridecyl isononanoate	20.00	19.90	19.50
Mineral Oil	5.00	5.00	4.90
2, 6-Di-tert-butyl-4-methylphenol	0.03	0.03	0.03
Decanoic acid, 2, 2-dimethyl-1,3-propanediyl ester	Balance	Balance	Balance
	100.00	100.00	100.00
Occlusivity (%)	64.1	65.5	63.1

pCer were defined as test samples (pCer-0.5%, pCer-2.0%). The method of measuring occlusivity is described below. Table 1 shows the formulations and the occlusivity values of samples used for this study. The raw materials shown in Table 1 were adjusted by stirring and melting at 105°C.

All subjects used the Blank one week before the start of the test in order to remove the influence of any agents they had used previously. The thirty-one subjects were divided into three groups and were prohibited from using other lip cosmetics throughout the test period. Each sample was applied four times a day (morning, noon, evening, before going to bed).

Measurement of occlusivity

Ten ml water were placed in a glass reagent bottle (opening ϕ 1.5 cm), and 10 mg of a test sample was applied to one side of a filter paper (4A, Toyo Roshi Kaisha Ltd., Japan) to cover the opening. The weight loss (water loss) of the reagent bottle when it was left to stand in an environment at 30°C and 30% relative humidity (RH) for 12 hours was measured. The water occlusivity was determined by the following equation in comparison with the weight loss when the sample was covered with an uncoated filter paper.

Occlusivity (%) = $(1 - [\text{amount of water loss when applying sample} / \text{amount of water loss when no sample is applied}]) \times 100$.

Degree of lip roughness

The degree of lip roughness in each subject was classified according to the criteria described in Fig. 1 (lip roughness criteria, level s0–3). This roughness criteria were created by authors for this study. Based on this score, the two authors made a blind evaluation.

Biophysical measurements of the SC and measurement of surface elasticity

All measurements were conducted in an air-conditioned room (temperature $20 \pm 3^\circ\text{C}$; RH $40 \pm 10\%$). Each subject washed her lips with a make-up remover (Sofina beaute' make-up cleansing gel) and a face wash (Sofina cleanse mild washing foam), and

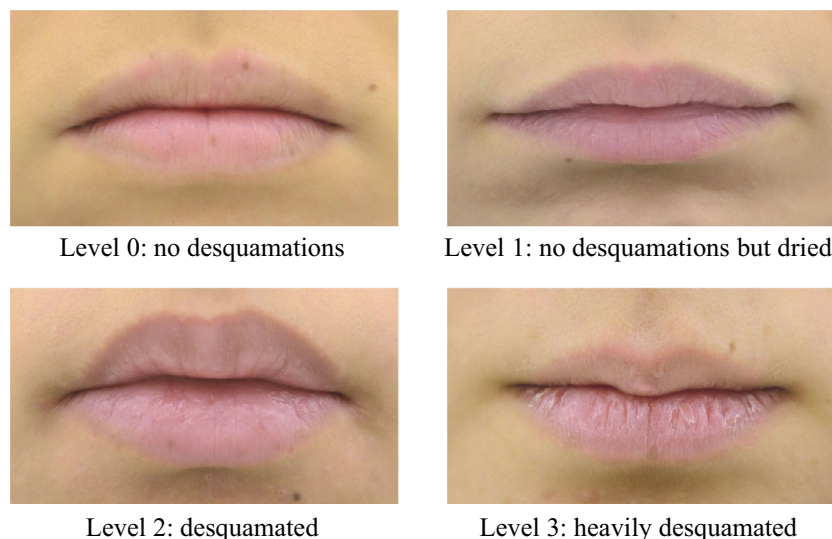


Figure 1 Lip roughness scores.

acclimated for 15 min. The measurement locations were at the centre of the lower lip.

Transepidermal water loss was measured 4 times with a closed-chamber VAPO SCAN AS-VT100RS (Asahi Techno Lab, Yokohama, Japan) and the mean value was determined. Capacitance was measured six times with a Corneometer CM 825 (Courage + Khazaka electronic GmbH, Köln, Germany) and the mean value was determined.

Elasticity was measured four times with a Cutometer SEM 575 (Courage + Khazaka electronic GmbH, Köln, Germany) and the mean value was determined. A measuring probe with a diameter of 2 mm that applied a constant suction of 300 mbar was used. Both the absorption time and the liberation time was 5 s. *Uf* represents the skin suction distance and indicates the softness of the skin surface.

The subjects were divided into three groups so that their degree of lip roughness and their capacitance were almost equal.

Tape stripping and lipid analysis by LC/MS

Three continuous SC tape strips (2.5 × 3.5 cm) were obtained from each lip site by making them evenly adhere with a finger for several seconds and then stripping an adhesive acrylic film (465#40; Teraoka Seisakusho, Tokyo, Japan). The samples were subjected to LC/MS analysis to assess the levels of 11 major ceramide species [5,15]. The tape strips were cut into two equal half-strips: one half was used for ceramide measurement and the other half was used for quantifying protein.

Ceramide measurement

Three half-strips were immersed in 0.9 ml chloroform/methanol/2-propanol (10:45:45; v/v/v) and were then sonicated for 10 min to extract ceramides. Next, an NS-C35 (d18: 1/17:0) ceramide solution as an internal standard reagent was added to part of the extract to prepare an LC/MS measurement sample solution. Its final concentration was 50 nmol/L. The lipid solution was subjected to

reverse phase LC/MS. The equipment used was an Agilent 1100 series LC/MSD SL system equipped with a multi-ion source, and the column was an L-column ODS (2.1 mm i.d. × 150 mm, manufactured by Chemicals Evaluation and Research Institute). Chromatographic separation of lipids was achieved by gradient analysis of two liquids at a column temperature of 40°C and a flow rate of 0.2 mL/min. CER[NDS], [NS], [NH], [NP], [ADS], [AH], [AP], [EOS], [EOH], [EOP] and pCer were detected by selected ion monitoring.

Quantification of SC exfoliation protein

Three other half-strips were dipped in 0.9 mL of a 0.1 mol/L NaOH aqueous solution containing 1% SDS, then were alkali-hydrolysed for 150 min in an incubator set at 60°. The solution was water-cooled and returned to room temperature, then 0.1 mL of 2 mol/L HCl aqueous solution was added to neutralize the solution, and the mixture was sufficiently stirred and used as a sample solution for SC protein measurement.

After adding 20 µL of each sample solution for protein measurement and 200 µL of colouring solution (BCA protein assay kit (Thermo)) to 96-well micro test plates for cell culture, the mixtures were reacted for 30 min in an incubator set at 37°C. Next, the plates were incubated at room temperature for 15 min, after which the absorbance was measured using a microplate reader ($\lambda = 570$ nm). For quantification, an absolute calibration curve method using an albumin standard solution (Albumin Standard Fraction V, Ampoules (Thermo)) was used.

Calculation of ceramide quantitative value

Ceramide quantitative values were standardized by the SC protein weight and were adjusted to the final quantitative value.

Statistical analysis

The efficacy by continuous use application examination was determined using the Bonferroni test, giving a *P*-value < 0.05 as the

level of statistical significance. This test was also used to assess the relationship between two samples.

Results

Changes in appearance score

Figure 2 shows the changes in the roughness score of lips before and after treatment. A significant improvement effect was observed in the pCer-2.0% sample after 2 and 4 weeks compared to 0 weeks. Even in the pCer-0.5% sample, an improvement tendency was observed after 2 weeks and a significant improvement effect was observed after 4 weeks compared to 0 weeks. In addition, in the comparison between groups, the improvement effect was observed after 2 and 4 weeks for the pCer-2.0% sample compared to the Blank. Remarkable examples of improvement in pCer-2.0% group are shown in Fig. 3.

Changes in SC functions and elasticity

Figure 4 shows the changes in capacitance, TEWL and maximum elongation value U_f of lips before and after treatment. First, with regard to the capacitance, there was a general tendency for

improvement after 2 and 4 weeks compared to 0 weeks. For pCer-0.5%, a significant improvement was observed after 2 and 4 weeks. For pCer-2.0%, there was a significant improvement after 4 weeks. However, the Blank also showed improvement after 2 weeks compared to 0 weeks, but decreased after 4 weeks compared to 2 weeks. There was no significant difference in the capacitance between the groups.

Regarding TEWL, the Blank did not show much change, but the pCer-blended samples showed an improvement tendency after 4 weeks compared to 0 weeks, and a significant improvement was observed for pCer-2.0%. Moreover, when compared between the groups, pCer-0.5% and pCer-2.0% showed significant improvement effects compared to the Blank after 4 weeks, indicating a concentration-dependent effect.

The maximum elongation value U_f of the viscoelasticity tended to increase in all groups after 2 weeks compared with 0 weeks, and among them, it was significantly increased in pCer-0.5%. After 4 weeks, it decreased in contrast to the Blank, but significantly increased in pCer-0.5% and tended to increase in pCer-2.0%. Comparing between the groups, pCer-0.5% and pCer-2.0% significantly increased compared to the Blank after 4 weeks.

Changes in ceramide profile

Figure 5 shows the results of changes in the composition of the ceramide profile. For the Blank sample, there was a significant increase in CER[ADS] after 2 and 4 weeks and a significant decrease in CER[EOH] after 2 weeks. For pCer-0.5%, there was a significant increase in CER[NH] and a significant decrease in CER[NS] and CER[AH] after 2 weeks. For pCer-2.0%, there were significant increases in CER[ADS] and CER[EOS] after 2 weeks and in CER[EOH] after 4 weeks, and significant decreases in CER[NS] and CER[AH] after 2 weeks and in CER[AS] after 4 weeks.

When comparing between the groups, for CER[NS], although there was a significant difference between pCer-2.0% after 2 weeks and pCer-0.5% after 4 weeks compared with the Blank, the opposite change was obtained for pCer-2.0% after 2 weeks was lower than the Blank and pCer-0.5% after 4 weeks was higher than the Blank. For CER[NH], a significant increase was observed after 2 weeks for pCer-0.5% and pCer-2.0% compared with the Blank. CER[NP] showed a significant increase in pCer-2.0% after 4 weeks compared to the Blank. CER[ADS] decreased significantly after 4 weeks of pCer-0.5% and pCer-2.0% compared with the Blank. Furthermore, pCer-0.5% showed a significant decrease in CER[EOS] and CER[EOP] after 4 weeks compared to the Blank.

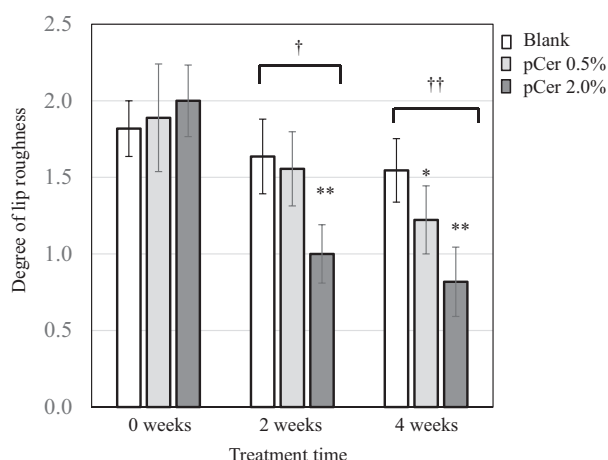


Figure 2 Improvement effect on lip roughness by continuous use of each test sample (** $P < 0.01$, * $P < 0.05$, vs. 0 Weeks, †† $P < 0.01$, † $P < 0.05$, vs. the Blank, Bonferroni's test).



Figure 3 Remarkable examples of improvement in pCer-2.0% group.

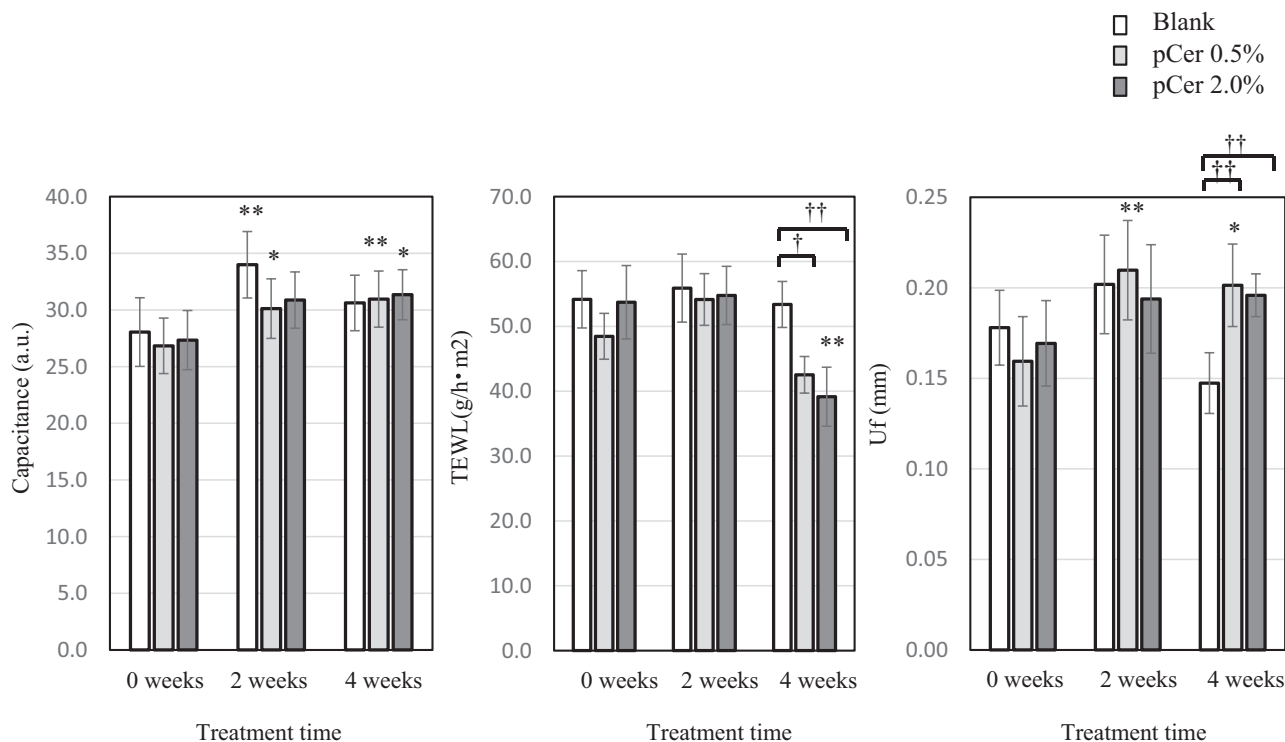


Figure 4 Improvement effect on SC function and elasticity by continuous use of each test sample. Left panel; capacitance, Centre panel; TEWL. Right panel; maximum elongation value U_f (** $P < 0.01$, * $P < 0.05$, vs. 0 Weeks, †† $P < 0.01$, † $P < 0.05$, vs. the Blank, Bonferroni's test).

Changes in the amount of pCer absorption into the SC

The absorption amount of pCer was also analysed. The difference from the state at week 0 before continuous use was evaluated, and the results are shown in Figure 6. pCer was significantly absorbed after 2 and 4 weeks of pCer-2.0% compared with 0 weeks, and the amount was higher after 2 weeks than after 4 weeks. Comparing between groups, both pCer-0.5% and pCer-2.0% were significantly absorbed after 2 weeks and pCer-2.0% was absorbed after 4 weeks compared with the Blank.

Discussion

So far, it has been reported that highly occlusive formulations affect the improvement of lip roughness [10]. Since the occlusivity of the formulations carried out in this study was around 65%, which is lower than the occlusivity of a formulation that did not show any effect of improving the roughness of the lips [10], it is considered that the effects of the synthetic pseudo-ceramide pCer could be verified. The results demonstrate for the first time that pCer has an effect to improve the roughness of the lips, which is remarkable from the visual observations. Since the effect of pCer concentration was observed, it is considered that the blending amount of pCer also affects the improvement of roughness. According to a study by Isoda et al. [13], a lip balm containing vaseline and pCer was continuously applied to sensitive lips, but those results did not reveal how the occlusive property of the formulation of pCer acts. This time, the effect of pCer could be directly verified

because the effect of pCer was tested without using vaseline after reducing the effect of the occlusive property.

From the above results, the parameter that has the highest correlation with lip roughness is the SC water content. Although there was an improvement tendency in all groups compared with 0 weeks, it was hard to consider that the improvement tendency of the SC was due to pCer alone because the Blank also improved after 2 weeks. The TEWL was significantly improved after 4 weeks, and a positive dose-response of pCer was also observed. Although TEWL has not been previously correlated with lip roughness, pCer is considered to have an effect to improve the barrier ability by improving the TEWL.

Regarding the viscoelasticity of the lip surface, the maximum elongation value U_f in the pCer-blended samples increased after 2 and 4 weeks compared to the initial value at 0 weeks. That is, the surface is more easily distended and softened. These results confirmed that the initial value of U_f of the subjects had a high positive correlation with the capacitance ($R = 0.521$, $P < 0.01$, Pearson's test), and U_f is the effect of water on the corneal surface. Although the capacitance itself was not significantly improved compared to the Blank, the surface conditions seemed to be improved because the U_f after 4 weeks was significantly increased compared to the Blank.

For ceramide profiles, when the ceramide amount, ceramide compositions, and ceramide carbon number were all analysed, the composition changes were the most pronounced. Previously, we reported that there was a correlation between the roughness of lips and the level of specific ceramide species [9]. Since changes in the ceramide profile due to continuous use like this study are short-term, it was

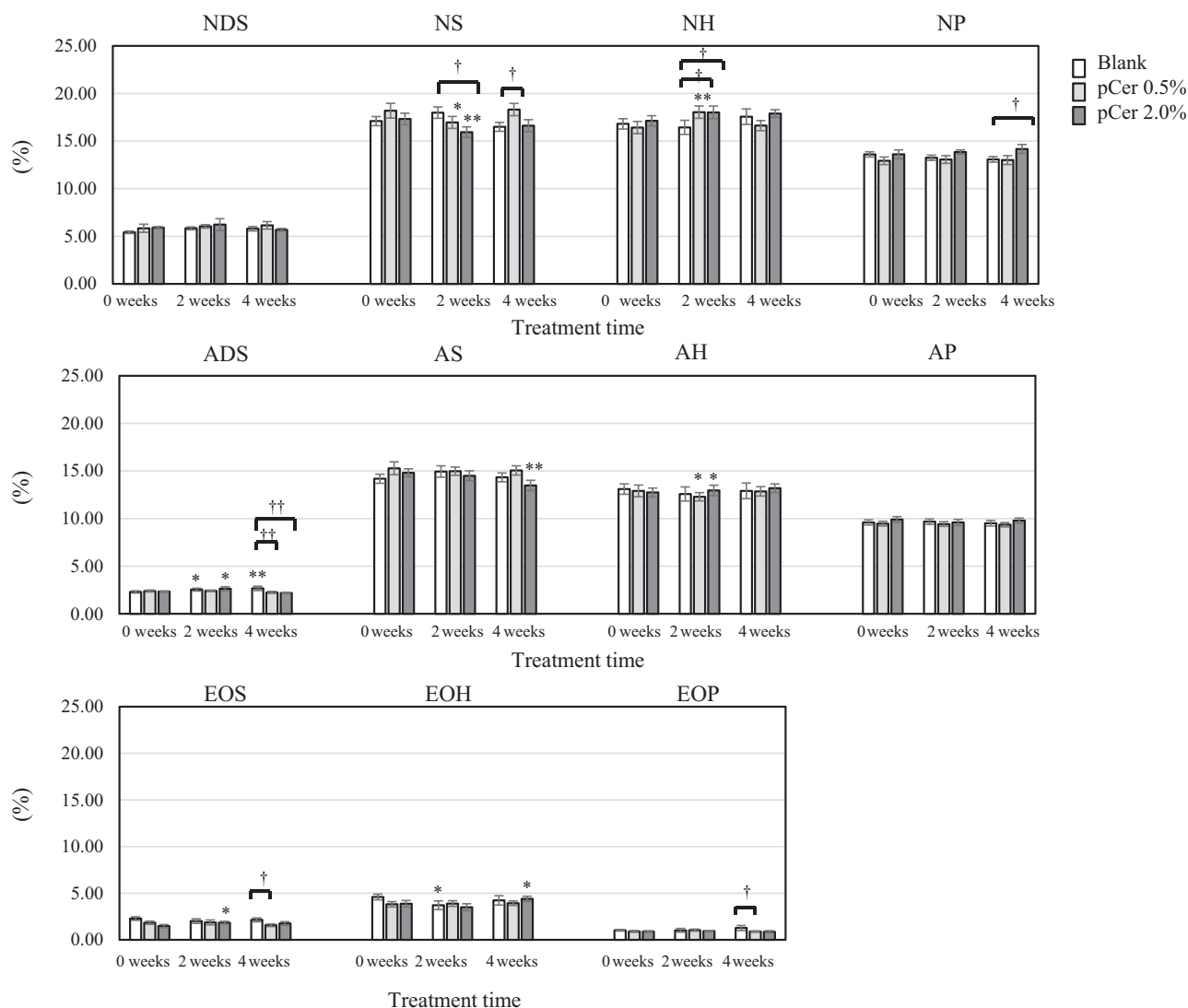


Figure 5 Changes in ceramide profile composition after continuous use of each test sample, (** $P < 0.01$, * $P < 0.05$, vs. 0 Weeks, †† $P < 0.01$, † $P < 0.05$, vs. the Blank, Bonferroni's test).

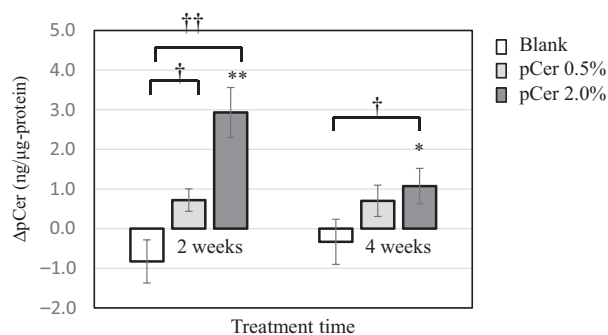


Figure 6 Changes in amount of pCer absorption into the SC after continuous use of each sample. (** $P < 0.01$, * $P < 0.05$, vs. 0 Weeks, †† $P < 0.01$, † $P < 0.05$, vs. the Blank, Bonferroni's test).

thought that changes in ceramide composition were most likely to appear. The following changes were obtained by the continuous use of pCer: CER[NS], which is found in low barrier areas such as the lips and in the barrier-disrupted skin of patients with atopic dermatitis, decreased. CER[NH], which has a high correlation with lip roughness, tended to increase. CER[NP], which had the highest correlation with lip roughness, increased in comparison with the Blank. These results suggest that the continuous use of pCer improved the ceramide profile so that the lips were less likely to become rough.

As for the pCer absorption, it may be natural that the higher the pCer content, the easier the absorption. However, since the absorption amount of pCer after 2 weeks was much larger than after 4 weeks, pCer is likely to be absorbed if the skin is rough, and if the roughness improves, the absorption may settle down.

Ishida et al. [12] verified the improvement of skin by the continuous use of pCer in the SC of patients with atopic dermatitis, and

reported that pCer penetrating into the SC also changes the ceramide profile of the skin itself to an improved condition. It has been clarified that the ceramide profile of the lips is similar to that of the skin of patients with atopic dermatitis. In this study, we considered that pCer was absorbed into the SC and then the ceramide profile was improved in a positive direction.

For the first time, the effect of the synthetic pseudo-ceramide pCer to improve the roughness of the lips was shown excluding the effect of occlusiveness derived from the formulation. The water content of the SC, which had the highest correlation with lip roughness, was improved by the samples containing pCer, but it was also improved by the Blank after 2 weeks. Therefore, the improvement of capacitance was not considered to be an effect of pCer alone. However, the improvement of TEWL was observed, and it became clear that pCer has an effect to improve the barrier function. Since pCer is absorbed more after 2 weeks than after 4 weeks, we concluded that pCer was first absorbed in rough lip areas, improved the ceramide profile and consequently restored the barrier function.

We have previously reported that high occlusive formulations of lip film, which do not depend on a specific care agent, have an effect to improve the roughness of lips [10]. Therefore, the use of the synthetic pseudo-ceramide pCer in a formulation having a highly occlusive formulation improves the roughness of the lips, improves the capacitance of the SC and TEWL, and provides an excellent lip care agent.

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Ethical approval

The study protocol for subjects was approved by the Ethical Committee of the Kao Corporation (#16-43).

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