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5′-CMP (5′-Cytidylic Acid), a Fundamental Nucleotide Enhancing Skin Resilience

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1. Introduction

Nucleotide is a minimum building block comprising DNA/RNA and is thought to be conditionally essential nutrient under various stress conditions, getting insufficient particularly upon the biological events accompanying vigorous cellular proliferation [1]. Among nucleotides, 5'-CMP (5'-cytidylic acid) is presumed to become insufficient most severely and is contained in human breast milk at a significantly higher concentration than other nucleotides [2], supporting the development of the intestinal epithelium of infants [3]. One of the reasons for this is thought to be that 5'-CMP is not only a raw material for RNA but also an essential raw material for the biosynthesis of phospholipids, which are the main components of every biological membranes, including cell membranes. In this study, the functionality of 5'-CMP towards the skin was addressed using various techniques of dermatological science. First, *in vitro* evaluations using monolayer cells and 3D-cultured epidermal model elucidated that 5'-CMP strongly suppressed inflammatory responses of UVB-irradiated epidermal keratinocytes and promoted type I collagen production of dermal fibroblasts via the action of keratinocytes. Transcriptome analysis of keratinocytes followed by lipidomics analysis of 3D-cultured epidermal model revealed that 5'-CMP stimulated the phospholipid biosynthetic pathway and increased the contents of phosphatidylcholine, ceramide, and sphingomyelin and improved epidermal barrier function, suppressing DNA-damage responses upon UVB irradiation and a cellular damage signal molecule, sphingosine 1-phosphate (S1P) [4], which impairs type I collagen production by dermal fibroblasts [5]. Thus, 5'-CMP treatment might enhance skin resilience and make skin tissue less susceptible to damages, which is thought to be a part of the mechanism underlying suppression of epidermal inflammation and promotion of dermal type I collagen production. Finally, double-blind RCT human studies for oral administration or topical application of 5'-CMP were conducted and revealed that 5'-CMP improved skin sagging, spots, and redness when taken orally, and also wrinkles when applied topically as shown below.

2. Materials and Methods

Inflammation suppression assay using UVB-irradiated 3D-cultured epidermal model: The 0.05 ml of sample solutions (PBS containing 0, 0.125, 0.25, 0.5 or 1% 5'-CMP) were applied to 3D-cultured epidermal models (LabCyte EPI-MODEL24, J-TEC) from the stratum corneum side and incubated for 24 hours. After removal of the sample solution, the epidermal models were irradiated with 600 mJ/cm^2 UVB and incubated for another 24 hours with the new sam-

ple solutions applied from the stratum corneum side. The amount of IL-6 and and IL-8 in the culture supernatant was determined by ELISA.

Evaluation of type I collagen production by dermal fibroblasts via the epidermal keratinocytes: Normal human epidermal keratinocytes (NHEK, Kurabo) were seeded into 96-well plates using HuMedia KG2 (KG2, Kurabo) at a density of 2.5×10^4 cells/well and cultured at 37°C for overnight. After replacing the culture medium with HuMedia KB2 (KB2, Kurabo) containing 5'-CMP at a concentration of 0, 1.25, 2.5 or 5 mM for 24 hours, the cells were incubated for 24 hours and the culture supernatant was collected. Normal human dermal fibroblasts (NHDF, Kurabo) were seeded into 96-well plates using DMEM medium with 5% FBS at a density of 2×10^4 cells/well and cultured at 37°C for overnight. After replacing the culture medium with the NHEK culture supernatant obtained above (referred to as “K-CM”), or the culture medium containing 5’-CMP at concentrations of 0, 1.25, 2.5 or 5 mM (referred to as “direct treatment”), the cells were incubated for 24 hours. The culture supernatant was collected and type I collagen content was determined by ELISA. The procedure was performed in quadruplicate and the average value was taken.

Ex vivo evaluation using dermatomed human skin discs: Fresh dermatomed human skin discs (10 mm diameter, Biopredic) were used for the study. Each 0.03 ml sample solution (PBS containing 0%, 1% or 2% concentration of 5’-CMP) was applied to the fresh skin discs from the stratum corneum side and incubated for 24 hours. The sample solution was replaced with a new one every 24 hours for a total of 72 hours of treatment. Skin tissue sections were prepared and collagen/elastin fibers were visualized by Elastica van Gieson staining.

DNA microarray analysis of UVB-irradiated epidermal keratinocytes: NHEK were seeded at a density of 30×10^5 cells/well in 6-well plates with KG2 and incubated overnight at 37°C. After replacing the culture medium with KB2 with or without 0.8 mg/ml 5’-CMP, the plates were incubated for 24 hours. After removal of the samples, the cells were irradiated with 0 or 20 mJ/cm^2 UVB. After UVB irradiation, the cells were incubated with the sample-free medium for an another 6 hours. The cells were harvested and RNA extracted from the cells was used for DNA microarray analysis (Clariom S Human Array, Thermo). Analysis was performed in triplicate, and genes with significantly ($p< 0.05$) altered expression were extracted.

Comprehensive phospholipid analysis of 3D-cultured epidermal model by lipidomics: The 0.1 ml of sample (PBS containing 5’-CMP) was applied to the 3D-cultured epidermal model (LabCyte EPI-MODEL24 6D, J-TEC) from the stratum corneum side and incubated for 7 hours. After removal of the sample solution, the epidermal models were incubated for another 17 hours. Culture was continued for 5 days, repeating the sample treatment described above. The epidermal models were collected and subjected to lipidomics analysis (Multiphospholipid Analysis, LIPIDOME LAB). The concentrations of 5'-CMP in the samples were 0, 0.25, and 0.5 w/v%. The procedure was performed in triplicate and the average value was taken.

Evaluation of the barrier function of 3D-cultured epidermal model by trans-epidermal water loss (TEWL): The 3D-cultured epidermal model (LabCyte EPI-MODEL24 6D, J-TEC) was acclimated at 37°C for 48 hours and subjected to TEWL measurement (Day 0) using VAPOSCAN (AS-VT100, ASCH JAPAN). After 0.05 ml of sample (PBS containing 5’-CMP) was applied from the stratum corneum side and incubated for 7 hours, the samples were removed and incubated for another 17 hours followed by TEWL measurement (Day 1). The culture was continued for 4 days, with the sample treatment and TEWL measurement repeated as described above (Day 2-4) as described above. The concentration of 5'-CMP in the

samples were 0, 0.25, and 0.5 w/v%. The procedure was performed in triplicate and the average value was taken.

A double-blind RCT human study for 12 weeks of oral administration of 5'-CMP: A randomized, placebo-controlled, double-blind, parallel-group comparison study was conducted for 36 healthy Japanese men and women who are aware of dry skin (7 men, 31 women; age range 25-54 years, UMIN000051832). The study was conducted in accordance with the declaration of Helsinki and ethical guidelines for medical and biological research involving human subjects, and written informed consent was provided by all subjects. Participants were randomized and assigned into two groups to receive placebo capsules and active capsules containing 300 mg 5'-CMP daily for 12 weeks. At two observation points, 0 week and 12 weeks, skin elasticity was analyzed using Cutometer MPA580 (Courage & Khazaka) and VISIA analysis was performed using VISIA Evolution (Canfield Scientific).

A double-blind RCT human study for 12 weeks of topical application of 5'-CMP to eye corner wrinkles: A split-face, randomized, placebo-controlled, double-blind comparison study was conducted for 33 healthy Japanese women with Grade 1-3 eye wrinkles (age range 36-54 years, UMIN000053406). The study was conducted in accordance with the declaration of Helsinki and ethical guidelines for medical and biological research involving human subjects, and written informed consent was provided by all subjects. Participants were randomized and assigned into group-A (16 women, age range 38-54, mean wrinkle grade 3.11±SD0.22) and group-B (17 women, age range 36-53, mean wrinkle grade 3.13±SD0.27). Group-A participants received placebo serum and the serum containing 0.25 w/v% 5'-CMP, and group-B participants received placebo serum and the serum containing 0.5 w/v% 5'-CMP. Participants applied 0.5 g of one sample serum to one half of the face around the eye corner and another sample serum to the other half of the face daily for 12 weeks. At three observation points, 0 week, 9 weeks and 12 weeks, visual evaluation of eye wrinkle grade and wrinkle replica image analysis were performed.

3. Results and Discussion

5'-CMP suppresses inflammatory responses of UVB-irradiated 3D-cultured epidermal model.

Epidermal keratinocytes exposed to exogenous stress, including UVB-irradiation, secrete inflammatory mediators such as IL-6, and IL-8, which in turn activate various skin aging signals. Previously we investigated the effect of pretreatment of normal human epidermal keratinocytes (NHEK) with each nucleotide (5'-AMP, 5'-GMP, 5'-CMP, 5'-UMP) on the secretion of the inflammatory mediators, IL-6 and IL-8, induced by UVB-irradiation (20 mJ/cm^2) and found that 5'-CMP was the most potent suppressor of their secretion among the

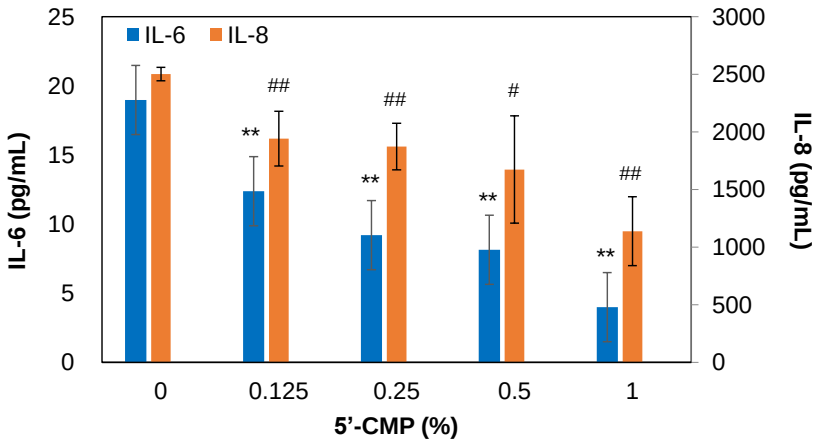

Figure 1. Amount of IL-6 and IL-8 secreted by 3D-cultured epidermal model treated with 5'-CMP followed by UVB-irratiation; Student's t-test, $^{**}p<0.01$ vs. 0% (IL-6), $^{\#}p<0.05$; $^{\#\#}p<0.01$ vs. 0% (IL-8).

nucleotides, followed by 5'-UMP, whereas 5'-AMP and 5'-GMP had no effect.

The 3D-cultured epidermal model was treated with 0.125 to 1% 5’-CMP solution from the stratum corneum side and UVB-induced secretion of IL-6 and IL-8 was evaluated. A statistically significant suppression of both IL-6 and IL-8 secretion was observed (Figure 1). Thus, 5’-CMP was found to markedly suppress the inflammatory responses of skin epidermis upon UVB-irradiation.

Ex vivo evaluation of 5'-CMP using dermatomed human skin disc.

To investigate the effect of 5'-CMP on the dermis, freshly dermatomed human skin discs were treated with a PBS solution containing 1% or 2% concentration of 5'-CMP from the stratum corneum side for 3 days, and the skin sections were subjected to the Elastica van Gieson stain, figuring a significant increase in density of dermal collagen fibers (Figure 2). Inasmuch as 5’-CMP is a highly hydrophilic compound and unlikely penetrate into the dermal layer, these phenomena observed in the dermis were thought to be effects via the epidermis.

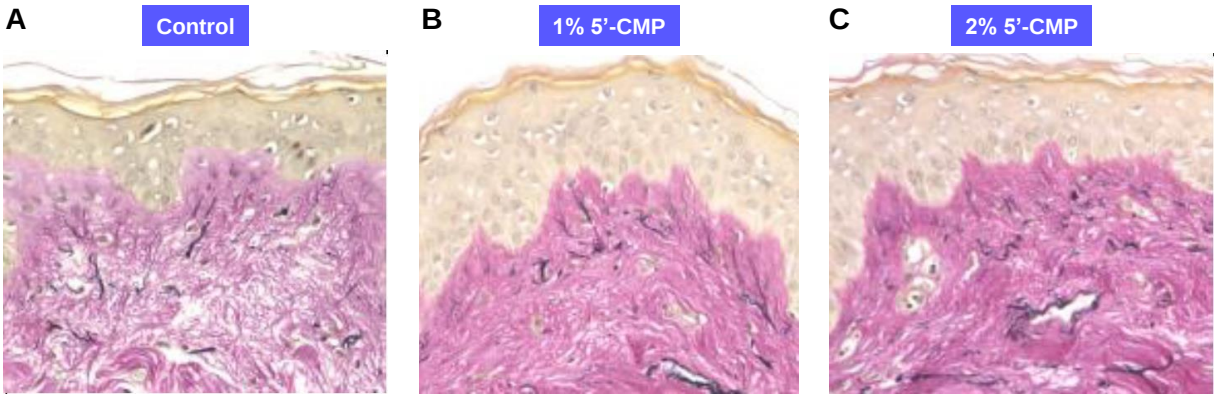

Figure 2. The skin sections of the dermatomed human skin discs treated with 0% (A), 1% (B), or 2% (C) 5’-CMP from the stratum corneum side, visualized by the Elastica van Gieson staining.

5’-CMP promotes type I collagen production by dermal fibroblasts through the action of epidermal keratinocytes.

To investigate the mechanism for the effects of topically applied 5’-CMP on the dermal layer of dermatomed human skin discs as described above, a mode of action of 5’-CMP on dermal fibroblasts was analyzed. When normal human dermal fibroblasts (NHDF) were directly treated with 5’-CMP at various concentrations (0, 1.25, 2.5 or 5 mM), type I collagen production was not affected (“direct treatment” in Figure 3). Similarly, direct treatment of NHDF with its intradermal metabolites such as cytidine, uridine and uracil had no effect (data not shown). On the contrary, when the culture medium of NHEK treated with 5’-CMP at various concentrations (0, 1.25, 2.5 or 5 mM) was applied to NHDF, the production of type I collagen was statistically significantly increased (“K-CM” in Figure 3). These results

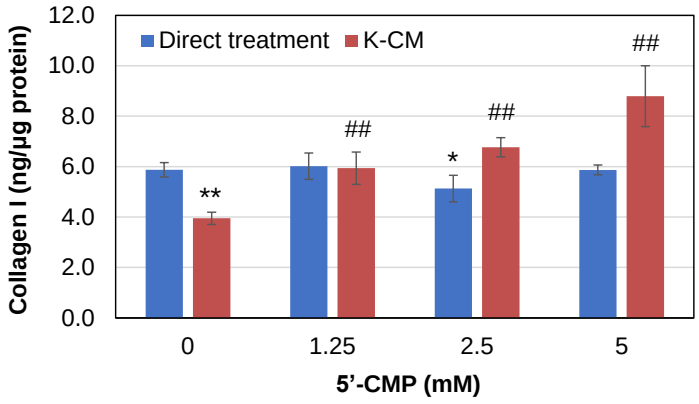

Figure 3. Type I collagen production by NHDFs cultured with 5’-CMP directly (Direct treatment) or culture supernatant of NHEK treated with 5’-CMP (K-CM). Student's t-test (n=4), *p<0.05, **p<0.01 vs. 0 mM (direct treatment), $^{\#}p$<0.05, $^{\#\#}p$<0.01 vs. 0 mM (K-CM).

indicate that 5’-CMP promotes type I collagen production by dermal fibroblasts through the action of the epidermis, which may explain the enrichment of the collagen fibers in dermal layer of dermatomed human skin discs topically applied with 5’-CMP.

Transcriptome analysis of NHEK treated with 5’-CMP: 5’-CMP induces large-scale gene expression changes toward accelerated biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) and phospholipids.

To find clues for clarifying the molecular mechanism underlying the suppression of inflammation toward the epidermis and aslo the promotion of type I collagen production by dermal fibroblasts through the epidermis, DNA microarray analysis (Clariom S Human Array, Thermo) was performed using NHEK treated with 5’-CMP followed by UVB-irradiation.

1,525 genes were found to be statistically significantly upregulated more than 2-fold by UVB irradiation, of which 155 genes were significantly improved (decreased) by 5'-CMP treatment. Among the 155 genes, many genes related to inflammatory response stood out, including IL1B (IL-1β, Probe Set ID: TC0200013916.hg.1), IL6 (IL-6, TC0700006890.hg.1), IFNK (IFN-κ, TC0900006892.hg.1), and the NF-κB related genes NFKBIA (TC1400008940.hg.1) and NFKBIZ (TC0300013855.hg.1), as well as IRF1 (Interferon regulatory factor 1, TC0500012017.hg.1), supporting the anti-inflammatory effects of 5'-CMP described above.

In addition, many genes related to DNA damage repair such as DDIT3 (DNA damage inducible transcript 3, TC1200010968.hg.1), DDX5 (DEAD box helicase 5, TC1700011449.hg.1), DNAJC25 (DnaJ homolog, subfamily C, member 25, TC0900012159.hg.1), SERPINB2 (serpin peptidase inhibitor, clade B, member 2, TC1800009242.hg.1), SFR1 (SWI5-dependent homologous recombination repair protein 1, TC1000008798.hg.1), SIAH1 (Siah E3 ubiquitin ligase 1, TC1600011535.hg.1), and also apoptosis related genes BCL2L11 (BCL2-like 11, TC0200008870.hg.1) and PLK3 (polo-like kinase 3, TC0100008105.hg.1) were statistically significantly downregulated by 5’-CMP treatment, suggesting that 5'-CMP treatment suppresses DNA damage by UVB-irradiation itself and possibly thereby suppresses inflammation.

On the other hand, 2,493 genes were found to be significantly downregulated less than half-fold by UVB-irradiation, of which 147 genes were significantly improved (upregulated) by 5'-CMP treatment. Among these, a large number of genes from the solute carrier family stood out, including SLC2A1 (glucose transporter GLUT1, TC0100013908.hg.1), SLC7A1 (amino acid transporter CAT1, TC1300008487.hg.1), SLC38A1 (amino acid transporter SNAT1, TC1200010516.hg.1), SLC38A2 (amino acid transporter SNAT2, TC1200010518.hg.1), SLC30A4 (zinc transporter ZNT4, TC1500009298.hg.1) and SLC39A14 (zinc transporter Zip14, TC0800006980.hg.1), suggesting that 5’-CMP treatment may stimulate various intracellular metabolic pathways.

In addition, genes involved in fatty acid synthesis including ACSS2 (acyl-CoA synthetase), ACACA (acetyl-CoA carboxylase), ELOVL6 (ELOVL fatty acid elongase), HACD4 (3-hydroxyacyl-CoA dehydratase), and SCD5 (stearoyl-CoA desaturase), were upregulated by 5’-CMP treatment. The former 2 genes are responsible for the supply of malonyl-CoA, the elongation unit of the fatty acid chain, and the latter 3 genes are involved in fatty acid chain elongation and synthesis of long-chain polyunsaturated fatty acids (LC-PUFA) as shown in the upper block of Table 1.

Further, Kennedy pathway and Lands circuit genes involved in phospholipid synthesis, were upregulated by 5’-CMP treatment, including ACSVL4 (acyl-CoA synthetase, very long chain), SERINC5 (serine incorporator for phosphatidylserine shynthesis), LCLAT1 (lysocardiolipin acyltransferase), LPCAT4 (lysophosphatidylcholine acyltransferase) as

shown in the lower block of Table 1. Flippases ATP8B1 and ATP10D, which function as phospholipid transporters, were also upregulated.

Taken together, it was suggested that 5'-CMP treatment stimulates the synthetic pathway of fatty acids, particularly LC-PUFA, using acetyl-CoA produced by the metabolism of glucose and amino acids, and further stimulates the phospholipid synthetic pathway from LC-PUFA.

Probe Set ID	Signal Intensity (log2)				Gene Symbol	mRNA - Description	Function
	UVB(-)		20 mJ/cm² UVB				
	Control	5'-CMP	Control	5'-CMP			
	mean (n= 3)	mean (n= 3)	mean (n= 3)	mean (n= 3)			
TC2000007202.hg.1	**10.74**	**11.18**	**9.55**	**10.12**	ACSS2	Acyl-CoA synthetase short-chain family member 2	Acetyl-CoA synthesis
TC1700010488.hg.1	**13.55**	**13.50**	**11.64**	**12.13**	ACACA	Acetyl-CoA carboxylase alpha	Malonyl-CoA synthesis
TC0400012956.hg.1	**13.02**	**13.01**	**10.77**	**11.47**	ELOVL6	ELOVL fatty acid elongase 6	Fatty acid elongation
TC0900009685.hg.1	**9.77**	**9.74**	**8.67**	**9.23**	HACD4	3-hydroxyacyl-CoA dehydratase 4	Unsaturated fatty acid synthesis
TC0400011180.hg.1	**8.32**	**8.21**	**6.70**	**7.50**	SCD5	Stearoyl-CoA desaturase 5	Unsaturated fatty acid synthesis
TC0900008860.hg.1	**8.83**	**9.23**	**7.78**	**8.39**	ACSVL4	Acyl-CoA synthetase very long-chain family member 4	Acyl-CoA synthesis from LC-PUFA
TC0500011260.hg.1	**11.62**	**11.61**	**10.12**	**11.30**	SERINC5	Serine incorporator 5	Phosphatidylserine synthesis
TC0200007144.hg.1	**11.54**	**11.43**	**10.26**	**10.87**	LCLAT1	Lysocardiolipin acyltransferase 1	Phospholipid synthesis (Lands cycle)
TC0200016626.hg.1	**13.36**	**13.58**	**11.91**	**12.37**	LPCAT4	Lysophosphatidylcholine acyltransferase 4	Phospholipid synthesis (Lands cycle)
TC1800008781.hg.1	**11.67**	**11.80**	**10.51**	**11.30**	ATP8B1	ATPase, aminophospholipid transporter, class I, type 8B, member 1	Phospholipid transport (Flippase)
TC0400007422.hg.1	**10.84**	**10.58**	**9.54**	**10.18**	ATP10D	ATPase, class V, type 10D	Phospholipid transport (Flippase)

Table 1. Among the genes whose expression was significantly ($p<0.05$) decreased by half or more by UVB irradiation, the genes related to lipid metabolism whose expression was significantly ($p<0.05$) increased by 2-fold or more by 5'-CMP treatment.

Lipidomics analysis of 3D-cultured epidermal model treated with 5'-CMP: 5'-CMP increases the amount of phospholipids and sphingolipids with LC-PUFAs and decreases the cellular damage signal, sphingosine 1-phosphate (S1P).

According to the result of the transcriptome analysis, comprehensive phospholipid analysis by lipidomics was performed using 3D-cultured epidermal model. The epidermal model was treated with 0.25% or 0.5% 5'-CMP from the stratum corneum for 5 days followed by lipidomics analysis (Multiphospholipid Analysis, LIPIDOME LAB). The results showed that several phospholipids including a major plasma membrane phospholipid phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol 1-phosphate, phosphatidylinositol 2-phosphate and phosphatidylinositol 3-phosphate were increased by 5'-CMP in a dose-dependent manner. Sphingolipids including major intercellular lipids, sphingomyelin, ceramide, glucosyl/galactosylceramide and lactosylceramide were also increased. Analysis by fatty acid chain length showed that phosphatidylcholine increased in molecular species with a wide range of fatty acid chain lengths, with a statistically significant increase in molecular species with long chains of C38 or more (Figure 4A). In addition, increases in molecular species with LC-PUFAs such as C34:2, C36:2, C38:2 and C40:2 were prominent (Figure 4A). A similar trend was observed for ceramide and sphingomyelin, with a notable increase in molecular species with long fatty acid chains such as C22 and C24, as

well as a prominent increase in molecular species with unsaturated fatty acids such as C22:1 and C24:1 (Figure 4B). The results of the transcriptome analysis described above suggested that 5'-CMP treatment stimulated the biosynthetic pathway of LC-PUFAs and phospholipids containing LC-PUFAs as fatty acid chains, which is consistent with this lipidomics analysis. Since ceramides containing long-chain fatty acids are thought to be particularly important for epidermal barrier function [6], it was suggested that 5'-CMP might enhance the epidermal barrier by increasing their production.

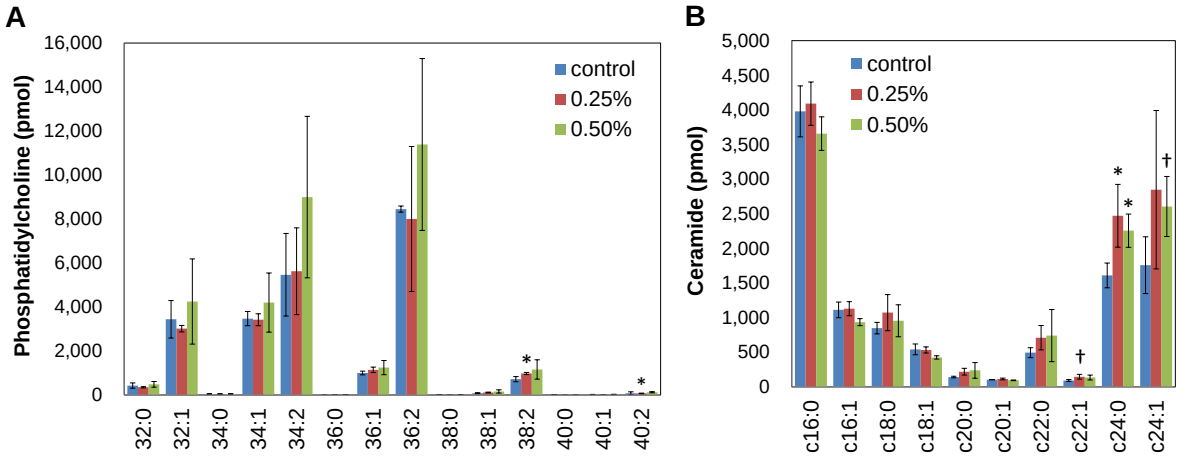

Figure 4. Content of phosphatidylcholine (A) and ceramide (B) in 3D-cultures epidermal model treated with 0% (control), 0.25% or 0.5% 5'-CMP. Student's t-test (n=3), $^{\dagger}p<0.1$, $^{*}p<0.05$ vs. control.

On the contrary, the content of some lipid species was decreased by 5'-CMP treatment. In particular, the content of sphingosine, a degradation product of sphingomyelin, and sphingosine 1-phosphate (S1P), a lipid mediator derived from sphingosine and known as a cellular damage signal molecule [4], were drastically decreased in a dose-dependent manner by 5'-CMP, in stark contrast to the increase in sphingomyelin (Figure 5).

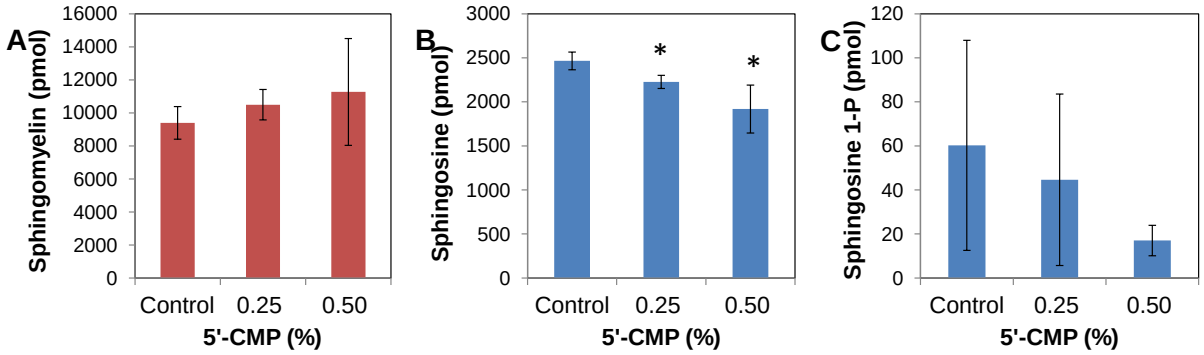

Figure 5. Content of sphingomyelin (A), sphingosine (B) and sphingosine 1-phosphate in 3D-cultures epidermal model treated with 5'-CMP. Student's t-test (n=3), $^{*}p<0.05$ vs. control.

Inasmuch as it was known that degradation of sphingomyelin occurs to produce the cellular damage signal molecule S1P when the tissue is damaged [4], the drastic decrease in S1P by 5'-CMP treatment suggests that cell damage itself was suppressed. This story is well consistent with the suppression of the UVB-induced DNA damage response by 5'-CMP treatment observed in the transcriptome analysis described above. Furthermore, S1P is known to repress type I collagen production by dermal fibroblasts [5], suggesting that the drastic reduction in S1P normalized and enhanced dermal type I collagen production.

5'-CMP enhances barrier function of 3D-cultured epidermal model.

The lipidomics analysis described above revealed that 5'-CMP treatment of the 3D-cultured epidermal model increased phosphatidylcholine, a major cell membrane phospholipid, and ceramide and sphingomyelin, major intercellular lipids, especially with a significant increase in molecular species containing long-chain fatty acids, which are thought to be particularly important for barrier function [6]. We then evaluated the actual barrier function of a 3D-cultured epidermal model treated with 5'-CMP. The epidermal model was treated with 0.25% or 0.5% 5'-CMP from the stratum corneum and subjected to daily trans-epidermal water loss (TEWL) measurement for 4 days. In the control epidermal model, the stratum corneum maturation progressed from Day 1 to Day 4, and a decrease in TEWL was observed over time (Figure 6). In the epidermal models treated with 0.25% or 0.5% 5'-CMP, TEWL remained lower than the control, and at the 0.5% concentration, it was statistically significantly lower than the control on Day 2, and the trend toward statistically lower values continued until Day 4 (Figure 6). At the 0.25% concentration, there was also a trend toward lower levels compared to the control on Day 4. Thus, 5'-CMP was found to enhance actual epidermal barrier function as suggested by the result of the lipidomics analysis.

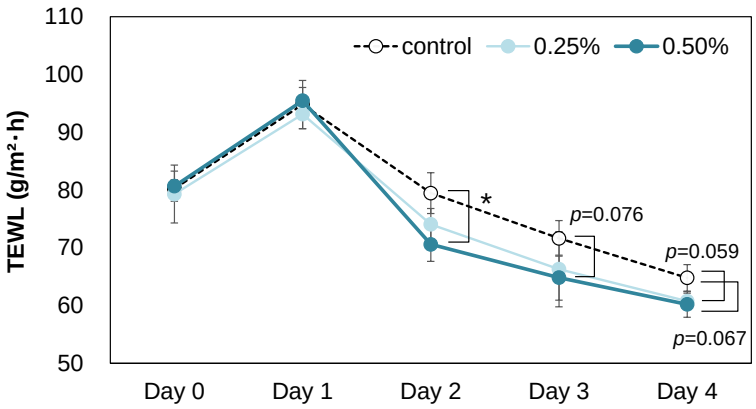

Figure 6. Evaluation of TEWL of 3D-cultured epidermal model treated with 0% (white), 0.25% (light blue), or 0.5% (blue) 5'-CMP. Student's t-test (n=3), *p<0.05 vs. control.

Taken together, it is presumed that 5'-CMP improves epidermal barrier function by increasing the production of major cell membrane phospholipids and intercellular lipids, particularly molecular species containing long-chain fatty acids that are important for barrier function, thereby enhancing resilience to various external stresses including UVB radiation, and reducing cellular damage (S1P production), resulting in suppression of epidermal inflammation and promotion of dermal type I collagen production.

Effect of oral administration of 5'-CMP on skin condition.

To evaluate the effect of oral administration of 5'-CMP, a randomized, placebo-controlled, double-blind, parallel-group comparison study was conducted for 36 healthy Japanese men and women who are aware of dry skin (7 men, 31 women; age range 25-54 years). Participants were randomized to receive 300 mg of 5'-CMP or placebo daily for 12 weeks.

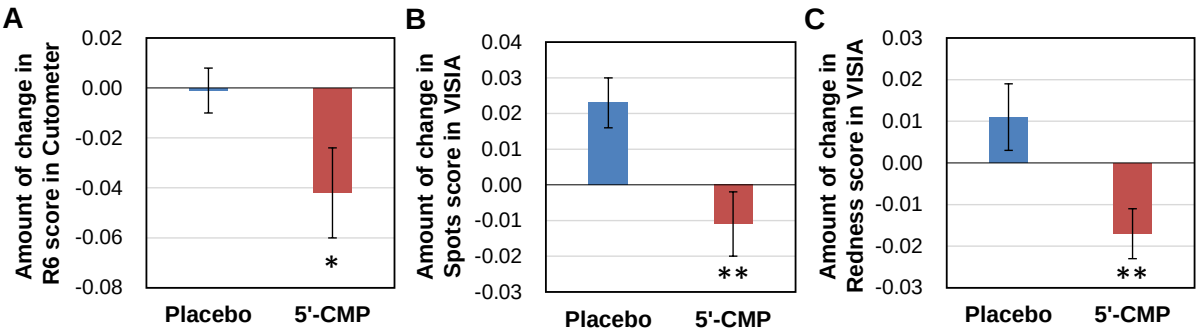

Figure 7. Results of the double-blind RCT human study for 12 weeks of oral administration of 5'-CMP; Amount of pre/post change in R6 score (an index of sagging) in Cutometer (A), spots score (B) and reddness score in VISIA analysis(C). Student's t-test, *p<0.05, **p<0.01 vs. placebo group.

The result showed that the R6 score (an index of sagging) in Cutometer (Figure 7A), the spots score (Figure 7B) and the reddness score in VISIA analysis (Figure 7C) were statistically significantly improved in the 5'-CMP intake group compared with the placebo group.

Effect of topical application of 5'-CMP to eye corner wrinkles.

To evaluate the effect of topical application of 5'-CMP to eye corner wrinkles, a split-face, randomized, placebo-controlled, double-blind comparison study was conducted for 33 healthy Japanese women with Grade 1-3 eye wrinkle (age range 36-54 years). Participants were randomized and assigned into group-A (placebo serum vs. 0.25% 5'-CMP serum) and group-B (placebo serum vs. 0.5% 5'-CMP serum) and each serum was applied to half of the face around eye corner for 12 weeks. The result showed that the maximum depth of maximum wrinkles in replica analysis decreased over time at 9W and 12W in the 0.25% 5'-CMP serum applied groups, and this change was statistically significant compared to before application. On the other hand, no decrease in maximum wrinkle depth was observed in the placebo serum applied group, and the values in the active group were statistically significantly lower than those in the placebo group at both the 9W and 12W time points (Figure 8A). Essentially the same results were also obtained in the 0.5% 5'-CMP serum applied group, but the improvement was even more remarkable than with the 0.25% concentration (Figure 8B).

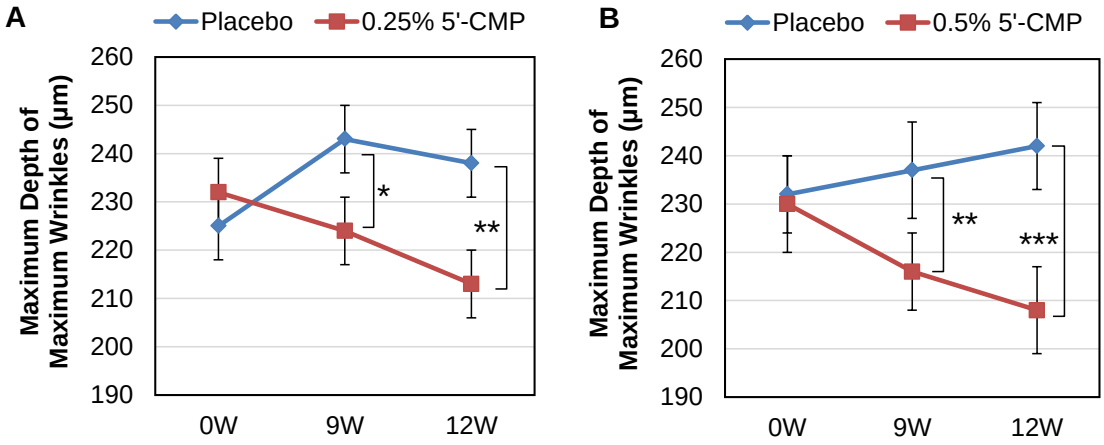

Figure 8. Results of the double-blind, split-face, RCT human study for 12 weeks of topical application of 5'-CMP to eye corner wrinkles; the maximum depth of maximum wrinkles in replica analysis for (A) placebo serum vs. 0.25% 5'-CMP serum, (B) placebo serum vs. 0.5% 5'-CMP serum. Student's t-test, *p<0.05, **p<0.01, ***p<0.001 vs. placebo group.

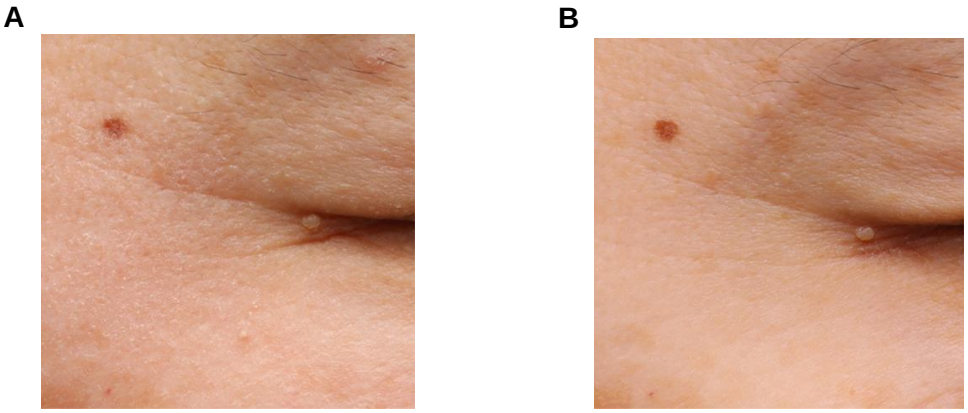

Figure 9. Photographs of the eye-corner before and after the study of a case of significant efficacy (female, age 47 years) with the 0.5% CMP serum applied; (A) 0W, (B) 12W.

Photographs of the eye corner before and after the study of a case of significant efficacy with the 0.5% CMP serum applied are shown in Figure 9, in which the eye corner wrinkles are improved, as well as the overall condition of the skin.

Thus, 5'-CMP improved skin sagging, spots, and redness when taken orally, and also wrinkles when applied topically, possibly by enhancing skin resilience to external stresses and reducing cellular damage, thereby suppressing epidermal inflammation and promoting dermal type I collagen production.

4. Conclusion

The fundamental nucleotide, 5'-CMP, increased a major cell membrane phospholipid, phosphatidylcholine, and the major intercellular lipids, ceramide and sphingomyelin, particularly molecular species containing long chain fatty acids, and improved epidermal barrier function, which may enhance skin resilience to external stresses including UVB-irradiation, resulting in a reduction in cellular damage and thereby epidermal inflammation, and an increase in dermal type I collagen production (Figure 10). Possibly through this mechanism, 5'-CMP significantly improved skin sagging, spots and redness when taken orally, and eye corner wrinkles when applied topically, complementing each other (Figure 10). Thus, 5'-CMP is a plausible material for both internal and external cosmetic applications based on the elaborated evidences obtained by molecular dermatological science.

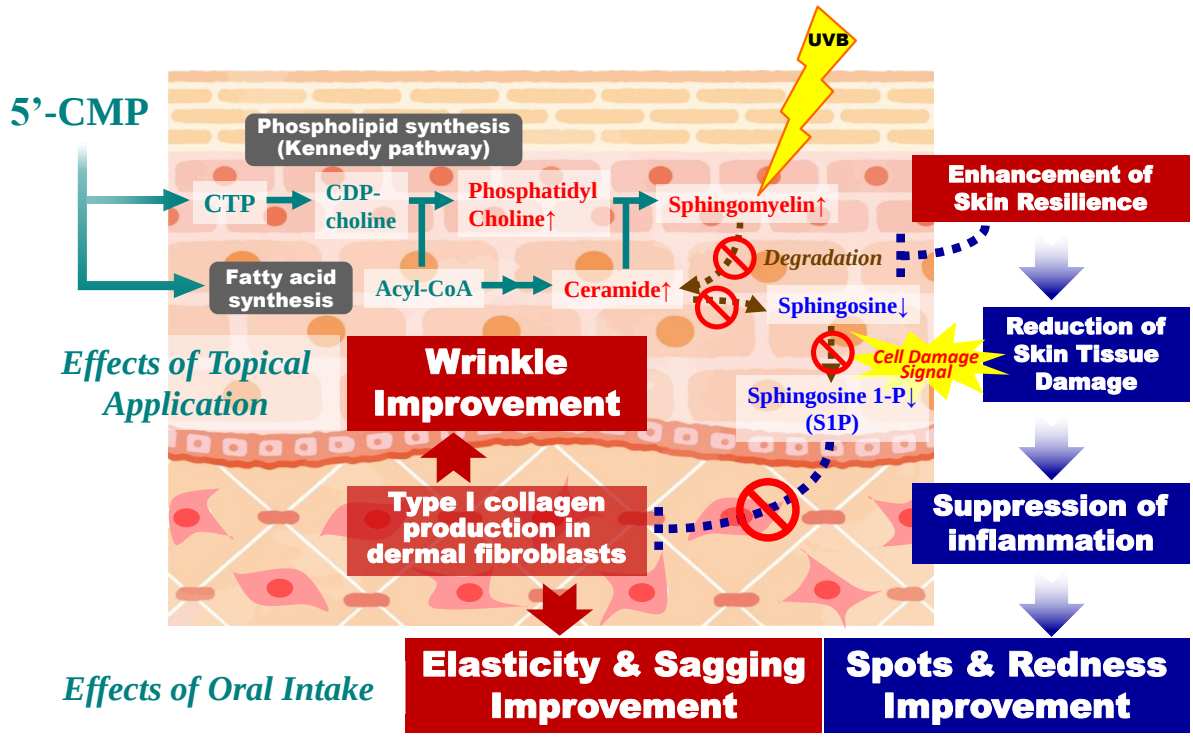

Figure 10. Proposed scheme for the mechanism of action of 5'-CMP on skin

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