Efficacies of a novel gemini compound “2-(Dimethyldocosylammonio)ethyl octadecyl ethyl phosphate” as a cosmetic ingredient

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Abstract

We examined the efficacies of the gemini compound 2-(dimethyldocosylammonio)ethyl octadecyl ethyl phosphate (DOEP) bearing a phosphorylcholine-like moiety as a cosmetic ingredient. All evaluations were performed in comparison with hydrogenated lecithin (PC), which has a similar structure to DOEP. We observed samples of PC and DOEP in water by transmission electron microscopy, and both compounds exhibited self-association and formed nanoparticles. Partially lamellar structures were observed in nanoparticles of PC; in contrast, DOEP consisted entirely of clear lamellar structure. In evaluations for skin care effect, DOEP tended to express higher efficacies about improvement of moisturizing effect, smoothness, and penetration-enhancing comparing with PC. In comparing these two compounds, we hypothesize that the skin care effects arise from self-assembled layers on the skin; thus DOEP, having stronger self-association (from long alkyl chains on both ends that drive vesicle formation), expresses better skin care effects compared to PC. The results indicate that DOEP is useful as a cosmetic ingredient, and it has greater efficacies than PC, an existing ingredient.

Keywords: gemini compounds, phosphoryl choline-like moiety, lecithin, moistness, MIU, penetration-enhancing effect

1. Introduction

A gemini compound is a generic term for when multiple hydrophilic and hydrophobic groups are contained in a single molecule. This class of compound has attracted the attention of chemists because of its rare structure, and various instances of gemini compounds have been synthesized in this decade. Functional evaluations of gemini compounds indicate unique properties not found in existing compounds, including high surface activity and high self-association(3). Meanwhile, a phosphorylcholine moiety is a polar group of phospholipids that constitute the biological membranes in a cell. 2-methacryloyloxyethyl phosphorylcholine (MPC) is a monomer bearing a phosphorylcholine moiety, and polymers containing MPC demonstrate excellent biocompatibility; such polymers have found application as surface coating agents for artificial organs(2). Moreover, the biocompatibility and safety of the polymers are being considered during applications as cosmetic ingredients. It has been reported that the polymer exerts some useful properties like high moisture retention and protection from surfactant toxicity(4). The phosphorylcholine moiety is quite useful in the life sciences industry because of its human origin. With such a background, syntheses and physical property analyses of dialkyl-gemini compounds bearing a phosphorylcholine-like moiety were reported by Mengar et al.(5). The report described that those gemini compounds self-assemble into various forms when in water (e.g., micelles, vesicles, and gels) depending on variations in terminal alkyl chain length(6,7). We hypothesized that these gemini compounds would be extremely useful, having both advantages of the gemini compound and the phosphorylcholine moiety. We synthesized gemini compounds for which alkyl chain length and type were varied, and screened them for their safety and efficacies as cosmetic ingredients. The results showed exceptional characteristics for 2-(dimethyldocosylammonio)ethyl octadecyl ethyl phosphate (DOEP). DOEP self-assembles into nanoscale multi-lamellar vesicles in water, and provides several beneficial effects as a cosmetic ingredient(8). DOEP would be a useful cosmetic ingredient, though its effects have only been compared to non-
treatment or water treatment in previous evaluations and never against an existing cosmetic ingredient. In this study, in order to examine the skin care effects of DOEP, we compared it to a hydrogenated lecithin (PC) that is a major cosmetic ingredient having a similar structure to DOEP.

2. Materials and Methods

2.1 Reagents

DOEP and PC (Fig. 1) used for the study were Vinoveil®-BS-100P and COATSOME® NC-21 (phosphatidylcholine content: >90%), respectively, manufactured by NOF Corporation (Japan). All other reagents were commercial analytical grade unless specified otherwise.

Fig. 1 Chemical structure of DOEP (Top) and PC (Bottom)

2.2 Preparation of samples

Water dispersions of DOEP (DOEP-Dis) were prepared by adding ion-exchange water into DOEP adjusting to 1 wt% concentration, and stirring with a homomixer (70 °C, 3000 rpm, 10 min). Water dispersions of PC (PC-Dis) were prepared in the same way, except with PC instead of DOEP.

2.3 Transmission electron microscopy (TEM) observations

The underwater association of PC-Dis and DOEP-Dis was observed with a transmission electron microscope (JEOL Ltd. (Japan), JEM2000EX). Each sample was diluted 5 times with ion-exchange water. Diluted samples were uniformly dispersed on a grid and then negative stained at 80 °C with tungstophosphoric acid to be used as observation specimens.

2.4 Safety assessment

Toxicity of PC and DOEP was assessed by an in vitro neutral red staining method using rabbit cornea-derived cells (SIRC). This method has been developed as an alternative to the Draize test to predict eye irritation. The amount of viable cells is inversely correlated to the toxicity of a sample; since only viable cells can take up the dye, the toxicity of a sample can be determined by the amount of the dye.

Cell
SIRC (JCRB9122)

Medium
Dulbecco’s modified Eagle medium containing 10% fetal bovine serum and 0.01% antibiotic-antimycotic solution. All reagents were products of Sigma-Aldrich Co.

Method

Individual wells of a 96-well plate were inoculated with 200 μL of medium containing 10^3 cells, and the plate was incubated in a cell incubator (37 °C, 5% CO_2) for 24 h. Various concentrations of PC-Dis and DOEP-Dis were added to the well and incubated for 24 h. Total solution in the well was removed, then 100 μL medium containing 0.1 mg/mL of neutral red as the dye agent was added and incubated for 3 h. Medium with the dye agent was removed completely and rinsed gently with PBS; the dye that was taken up by cells was then extracted with a solution (water/ethanol/acetic acid = 50/49/1 (v/v/v)). The amount of dye was measured by an absorption spectrophotometer at 550 nm. The viability was calculated with the following formula, where the non-treated control cells were assigned as 100% viability:

Viability (%) = (absorbance of sample-treated cell) / (absorbance of control cell) × 100(%)

2.5 Evaluation of effect on skin

Evaluating for skin efficacies, we examined the following three tests in this study. Volunteers who attended this study were healthy men and women aged 20-50 years old. Prior to each experiment, they were conditioned in a room at constant temperature and humidity (20 °C, 40% RH) for 15-30 min.

2.5.1 Moisturizing properties

To evaluate the moisturizing properties of DOEP, transepidermal water loss (TEWL) and water content of the stratum corneum (WSC) were evaluated to examine barrier effects and water retention, respectively. Water, PC-Dis, and DOEP-Dis were applied to the medial part of forearm at 10 μL/cm². TEWL and WSC were measured before sample application and 2 h after sample application. Similarly, TEWL and WSC of non-application sites were measured as blanks. TEWL and WSC were measured with a TEWAMETER TM210 (Courage+Khasaka Electric GmbH (Germany)) and a SKICON-200EX (IBS Co., Ltd. (Japan)), respectively. Relative values were calculated from the measurements, with the pre-application values set to 100%.

2.5.2 Friction coefficient (MIU)

MIU was examined to evaluate smoothness of the skin surface. Water, PC-Dis and DOEP-Dis were applied to the medial part of forearm at 10μL/cm². MIU of non-application sites were measured as blanks. MIU values were measured before sample application and 2 h after sample application with a friction coefficient tester designed for arms (KES-SE friction tester, Kato Tech Co., Ltd. (Japan)). Relative values were calculated from the measurements, with the pre-application values set to 100%.

2.6 Evaluation of penetration-enhancing effect

Magnesium ascorbyl phosphate (VCP-Mg, Fig. 2) was used as a model drug, and concentration-dependence was evaluated for penetration-enhancing effect of DOEP with three-dimensional cultured human skin (TESTSKIN™ LSE-high, Toyoobo Co., Ltd. (Japan)) as a skin model.
2.6.1 Preparation of evaluation samples
Preparation of drug-containing PC/DOEP samples:
PC or DOEP was added to saline adjusting to concentrations of 0.1-1 wt%, and samples were homogenized by sonication for 15 min at 40W (Sonifer® 250, Branson Ultrasonics Corporation (USA)). VCP-Mg was then added to the dispersion to a concentration of 1 wt%, and it was stirred with a homomixer (3000 rpm, 10 min, room temperature) to yield the drug-containing PC/DOEP samples.

Preparation of blank sample
VCP-Mg was added to saline water at 1% concentration, and it was stirred with a homomixer (3000 rpm, 10 min, room temperature) to obtain the blank sample.

2.6.2 Method
(1) Cultured skin was taken from agar medium and both sides of the skin were washed three times with 5 mL of saline. Then, cultured skin was clipped out from the porter and attached to a vertical Franz-type cell with the epidermis side on the upper aspect. Next, the donor cell and receiver cell were filled with saline to hydrate for 1 h while being kept at 32 °C. Saline from both cells was removed, the receiver cell was filled with fresh saline, and then each sample (as described in Section 2.6.1) was added to the donor cells. After the addition of the sample, the receiver cells were kept at 32 °C while shading the light for the vertical Franz-type cells. The receiver liquid was sampled at a certain period. On that occasion, saline was added to the receiver cell to maintain the liquid volume in the cell.

2.6.3 Analysis
The sample was analyzed under the following conditions with high performance liquid chromatography (HPLC) to determine the amount of VCP-Mg in the sample.

Apparatus: CCP & 8020 series (Tosoh Corporation (Japan)).
Column: TSK-gel ODS-100V, 4.6 mm i.d. × 250 mm, partial size 5μm (Tosoh Corporation)
Column temperature: 40 °C
Moving phase: 0.9 wt% phosphoric acid water solution
Detector: UV (wavelength: 285 nm)

3. Results
3.1 Fundamental properties
Observing the physical state of DEOP-Dis and PC-Dis in water by TEM, it was found that both compounds self-assembled into particles about 100 nm in diameter; however, the structure greatly differed between the two samples. DEOP-Dis formed clear lamellar structures over the entire particle, while PC-Dis formed lamellar structures only partially (Fig. 3). With regard to safety, DOEP showed significantly higher viability than PC at high concentration (Fig. 4).
3.2 Moisturizing properties

Significant improvement was observed, both in TEWL and WSC, for PC- and DOEP-treatment sites compared to water and non-treatment sites. In addition, DOEP exerts a higher effect about these parameters compared to PC (Fig. 5, 6).

![Fig. 5 Water content of the stratum corneum (WSC)](image1)

![Fig. 6 Transepidermal water loss (TEWL)](image2)

3.3 MIU

PC- and DOEP-treatment sites had lower MIU compared to the non-treatment and water-treatment sites; hence PC and DOEP could effectively improve skin smoothness. Moreover, DOEP had a significant effect on the function compared to PC (Fig. 7).

![Fig. 7 Friction coefficient (MIU)](image3)

3.4 Penetration-enhancing effect

Both PC and DOEP exerted penetration-enhancing effects for VCP-Mg. It has been found that the effect of 1 wt% PC equals 0.5 wt% DOEP (Fig. 8).

![Fig. 8 Cumulative penetration of VCP at 4 h](image4)

4. Discussion

4.1 Comparison of basic properties

Numerous methods have been proposed for vesicle preparation, and layer structure and size vary depending on the preparation method. In this study, all evaluations were performed with a dispersion prepared by homomixer stirring to compare basic properties of the PC and DOEP. As shown in the TEM images, both compounds self-assembled into nanoparticles; however, the structures of the nanoparticles were very different. Clear lamellar structures were seen only partially in nanoparticles of PC. In contrast, nanoparticles of DOEP were entirely comprised of clear lamellar structure. It is hypothesized that DOEP is superior to PC in self-association due to the difference in hydrophobic group structures. The driving force of vesicle formation is hydrophobic interaction; thus the length of alkyl chains must strongly affect the efficacy of forming vesicles. DOEP has long alkyl chains (C = 18, 22) on both ends, and PC has a long-branched alkyl chain and a methyl group on each end; hence DOEP has more cross-link points for hydrophobic interactions to form regular vesicles compared to PC.

With regard to safety, DOEP exhibited higher cell viability in the in vitro test against PC in the high contact concentration area (>0.1%), even though PC is known to be a high safety cosmetic ingredient. While DOEP is a synthesized compound, PC is derived from natural sources. Although both compounds were well refined, the amount of impurities in DOEP is smaller than in PC, and as PC is a mixture of several kinds of phospholipids it can contain short alkyl chain compounds that tend to cause irritation.

4.2 Care effect for the stratum corneum

Skin is the largest defense organ protecting the human body from external irritation. The stratum corneum, the outermost layer of skin is composed of horny skin cells and intercellular lipids, with the intercellular lipid buried between cells forming lamellar structures. The organization of the stratum corneum has been described as...
a “brick and mortar” type structure, and this structure provides the primary functions of the stratum corneum, namely barrier function and water retention\(^{(13)}\). If the skin barrier function is suppressed, resistance against external stimulation falls, and dry skin would result from transpiration of moisture. In other words, maintenance of the stratum corneum is an essential factor to keep skin healthy.

We evaluated TEWL and WSC as care effects for the stratum corneum. TEWL is an index of skin barrier function, and is used as a performance index of skin moisturizing property together with WSC\(^{(14)}\). When skin moisturizing properties improve by betterment of skin barrier function, the TEWL value is decreased and the WSC value is increased. In the past evaluation, we note that DOEP improved skin moisturizing properties with betterment of barrier function\(^{(15)}\). In this study, both PC and DOEP could improve skin moisturizing properties and so TEWL values were decreased and WSC values were increased compared to water and non-treatment. In addition, DOEP has higher effects compared to PC even though there is no statistically significant difference.

Liposomes, vesicles of phospholipids have been applied as carriers in drug delivery systems in the pharmaceutical field, and their high moisturizing properties have been studied in the cosmetic sciences\(^{(16)}\). The mechanism of liposomes to moisturize skin is regarded as a blockade effect of its self-assembled layer on the skin surface\(^{(16)}\). We hypothesize that DOEP would improve skin moisturizing properties in the same mechanism as liposomes due to strong self-association. Further, we hypothesize that differences in homogeneity of compound layers on the skin surface are responsible for the differing levels of skin moisturizing properties between these compounds. TEM observation of the samples revealed that DOEP has a stronger tendency toward self-association than PC; therefore DOEP would form a layer on the skin that was finer and with fewer gaps than for PC. Such regularity in the layer gives a favorable blockade effect to the skin to improve skin moisturizing properties.

Verification of this hypothesis is difficult, because quantitative evaluation of compound layers on the skin surface is arduous. Preparing a cast film is one way to predict physicochemical characteristics of a layer; however, while the method is useful for polymers it is not suitable for compounds of low molecular weight. On the other hand, in evaluating hair, MIU is utilized as a parameter having high correlation with unevenness of hair surface\(^{(17)}\). Based on this, we measured MIU on a skin surface to examine the homogeneity of layers formed by PC and DOEP. It was observed that MIU of the DOEP treatment site was significantly lower than that of the PC treatment site. Since decreasing of MIU corresponds to a highly homogeneous surface, an even more highly homogeneous surface would be obtained with a strongly assembled layer. In other words, DOEP would form a layer on the skin that was finer and with fewer gaps than for PC. Such a homogeneous layer of DOEP would give a favorable blockade effect to skin, raising not only smoothness but also skin barrier effect.

### 4.3 Penetration-enhancing effect

As mentioned above, liposomes have been developed as carriers in drug delivery systems in the pharmaceutical field, and they have been used as penetration-enhancing agents for both pharmaceutical and cosmetic products. We have already indicated that DOEP can work as a penetration-enhancing agent in evaluations where hydrophilic (Antipyrine) and hydrophobic (Flurbiprofen) substances are used as model drugs\(^{(18)}\). Moreover, it has been revealed that DEOP and PC express penetration-enhancing effect for VCP-Mg at 1 wt% concentration\(^{(19)}\). In this study, we identified a DOEP concentration that presents penetration-enhancing effect at the same level of 1 wt% PC using VCP-Mg as a model drug. As a result, 0.5 wt% of DEOP has equal penetration-enhancing effect to 1 wt% PC meaning that DEOP can bring penetration-enhancing effect at half the amount as that of PC.

With regard to penetration-enhancing effects from vesicles, some mechanisms have been proposed, although authentic data has not been provided and there are several unsolved contradictions\(^{(20)}\). In the previous study, using Antipirine and Flurbiprofen as model drugs, we attempted to identify the mechanism of penetration-enhancing effect with thermodynamic analysis. The results of the analysis suggested that DEOP enhances penetration of a model drug with improvement of diffusion from a solution to the stratum corneum, but not improvement of partition in the stratum corneum. Some reports presumed that a drug in a vesicle is shifted to the stratum corneum during the process of vesicle adsorption to the skin surface\(^{(20)}\). In other words, the vesicle structure must remain intact until the vesicle absorbs into the skin; thus, stability of the vesicle is an important factor for penetration-enhancing. Regarding stability of the vesicle, DOEP has stronger association compared to PC, and we propose that this is why DOEP can exert penetration-enhancing effect in half the amount as that of PC.

### 5. Conclusions

We examined the efficacies of DOEP as a cosmetic ingredient with an evaluation of fundamental properties and skin care effects. DOEP tends to exert higher skin care effects compared to PC, and we propose that the differences arise from strength of self-association and regularity of the vesicles. Since physicochemical properties of liposomes vary greatly depending on the preparation methods, it is supposed that functions better than those of DOEP could be developed depending on preparation methods. However, in the homomixer stirring, which is a simple and easy preparation method for vesicle formation, DOEP clearly demonstrated superior function at forming vesicles with lamellar structures.

Most of the applications of gemini compounds as cosmetic ingredients are utilization for a surfactant due to its peerless high surface activity. Moreover, most of the gemini compounds used in cosmetic products are hydrophilic substances. The results show that a hydrophobic gemini compound that cannot express surface activity is a useful ingredient with excellent association
characteristics.

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References

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