Clinical evaluation of the brightening effect of cationic liposome-based formulation on facial skin

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Abstract

Background: Liposomes are applied to various products for effective skin delivery of ingredients. However, while many studies have been conducted on the skin penetration of liposome, studies on the actual efficacy of ingredients contained in liposomes are insufficient.
Methods: In previous studies, we have studied the skin penetration capacity of liposomes according to the surface charge of liposome particles [1]. As a result, cationic liposomes showed the best skin permeability. Therefore, we prepared a chitosan-based cationic liposome containing niacinamide (NA), a brightening ingredient. A clinical study was performed to evaluate cationic liposome help the efficacy of the niacinamide.
Results: The clinical evaluation was conducted for 4 weeks with 21 female subjects. The clinical outcomes were evaluated by measuring skin brightness (L-value), melanin index, and skin melasma (affected areas, mm^2). As a results, a essence containing cationic liposome showed significant improvements like increased skin brightness, decreased melanin index and skin melasma compared to a liposome-free essence.
Conclusion: This clinical study demonstrated the efficacy of cationic liposome on skin brightening effect in cosmetic formulation. Because cationic liposomes enhanced skin absorption of active ingredients, active ingredients performed effectively in human skin. Thus, cationic liposome can be an effective delivery system of active ingredients that enhance skin efficacy.

Keywords: cationic liposome; clinical evaluation, skin delivery system; skin brightness

Introduction.

The skin is a tissue present in the outermost layer of the body and consists of the epidermis, dermis, and subcutaneous fat layers. The most important functions of this skin are physical protection from the external environment and maintenance of homeostasis [2]. The outermost keratin layer of the epidermis is known as the "Brick and Mortar" model, which consists of keratinocytes (Brick) and keratinocytes (Mortar) filling between them. Intercellular lipids, which consist of hydrophobic components such as ceramide, cholesterol, and free fatty acids, play a key role in skin barriers that defend against skin penetration of external substances through the formation of unique lamellar structures. Accordingly, active ingredients, especially hydrophilic components, have limitations in skin absorption. In other words, skin barriers caused by the dense structure of hydrophobic keratinocytes cause contradictory situations that prevent skin absorption of active ingredients [3].

In order to overcome this problem, lipid enhancer technology has recently attracted a lot of attention. This technology is a skin carrier manufactured using such as lecithin. Typical examples include nanoemulsions, liposomes, and lipid nanoparticles [4]. These lipid-based skin carriers are widely used in various fields because they do not damage the skin barrier and effectively help absorb active ingredients. In particular, liposomes are the oldest commercial technology among these lipid carriers and are the most effective and highly likely to mass-production.

Liposomes composed of double layers can simultaneously support hydrophilic and hydrophobic active ingredients. The physical properties, stability, and skin absorption effects of liposomes, such as particle size, surface charge, pH, and multilayer structure, may vary depending on the composition [5, 6].

We recently showed that cationic liposomes containing NA effectively facilitated the migration of melanosomes to the epidermis in 3D skin models, thereby enhancing the brightening effects of NA [1]. In comparison to the untreated control, melanin transfer was decreased by 53% when cationic liposomes were employed for the delivery of NA, while a 42% and 43% decrease was observed for neutral and anionic liposomes, respectively. The 3D skin model contains a stratum corneum with charged groups, such as keratin and other

sulfated glycosaminoglycans, resulting in electrostatic interactions between cationic liposomes and the skin.

In this work, we investigate the clinically evaluated the percutaneous delivery of NA using chitosan-based liposomes and examined the resulting clinical outcomes on skin brightening and melanin transfer in human subjects.

Materials and Methods.

Materials

Niacinamide (LASONS, India), choleth-24 (NIHON EMULSION, Japan), chitosan (degree of deacetylation ≥ 85%) (CHIBIO, China), hydrogenated lecithin (LIPOID, Germany), cholesterol (ACTIVE CONCEPTS, USA), ceramide NP (DOOSAN, Korea), butylene glycol (KYOWA, Japan), glycerin (EMERY, Malaysia), dipropylene glycol (SKC, Korea), xanthan gum (CP KELCO, USA), triethanolamine (DOW CHEMICAL, USA), carbomer (LUBRIZOL, Belgium), hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer (SEPPIC, France), sorbitan isostearate (SEPPIC, France), polysorbate 60 (SEPPIC, France) and ammonium acryloyldimethyltaurate/VP copolymer (CLARIAN, Spain) were commercially obtained.

Formulation Preparation

Preparation of chitosan-based cationic liposome containing niacinamide

The preparation of the cationic liposome is shown in Table 1. Phase B were dissolved at 70 ~ 80 ℃. After that, it is slowly added to the phase A at room temperature, and stirred for 5 minutes at 2,000 ~ 3,000 rpm using a agi-mixer. And then, finally liposomes were prepared using a high pressure homogenizer (MN400BF, Micronox, Korea) conditions 1,000 bar, 3 cycle. The liposome base contains 10% of niacinamide, as a brightening ingredient.

Table 1. Composition of cationic liposome formulations

Phase	Ingredients	Composition (%)
A	Water	to 100
	Butylene glycol	10.00
	Niacinamide	10.00
	Chitosan	0.05
	preservative	q.s [a]
B	Hydrogenated lecithin	1.00
	Cholesterol	0.50
	Ceramide NP	0.01
	Dipropylene glycol	10.00

[a] q.s = quantum sufficit

Preparation of cosmetic formulation containing cationic liposome

We prepared two cosmetic formulations, control and test, to conduct the clinical evaluation of the brightening efficacy. In the control formulation, 2% niacinamide was added to the aqueous phase. In the test formulation, 20% of the cationic liposome base (Table 1) was incorporated (Table 2). Thus, both essence formulations contained 2% niacinamide as the active ingredient, with the control containing it in the aqueous phase, and the test formulation containing it in the liposomes.

Table 2. Ingredients and compositions of cosmetic formulations

Phase	Ingredient	Composition (%, w/v)	
		Control	Test
A	Cationic liposome base	-	20.00
	Water	To 100	To 100

	Butylene glycol	10.00	8.00
	Glycerin	5.00	5.00
	Xanthan Gum	0.05	0.05
	Triethanolamine	0.08	0.08
	Carbomer	0.10	0.10
	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer	0.25	0.25
	Sorbitan Isostearate	0.02	0.02
	Polysorbate 60	0.02	0.02
	Ammonium Acryloyldimethyltaurate/VP Copolymer	0.10	0.10
	Niacinamide	2.00	-
	preservative	q.s [a]	q.s [a]
B	Dipropylene glycol	5.00	3.00
	Choleth-24	0.30	-
	Fragrance(Parfum)	q.s.	q.s.

Physicochemical characterization of cationic liposome

To investigate the stability of the niacinamide-loaded cationic liposomes, particle size and zeta potential changes were monitored at room temperature for 28 days. The particle size and zeta potential were measured using dynamic light scattering (SZ-100, Horiba, Japan). And, the morphology study was performed by a cryogenic transmission electron microscopy (Cryo-TEM). Cationic liposomes were loaded onto a carbon lacey film on a Cu grid and immersed in liquid ethane to freeze them rapidly. Frozen samples were observed by cryo-

TEM at an acceleration voltage of 200 kV (Tecnai F20, FEI, Hillsboro, OR, USA) at KIST (Seoul, Republic of Korea).

Clinical evaluation

Subjects

The clinical study was conducted by Global Medical Research Center in Seoul, Republic of Korea, from September 13 to October 13, 2021. A total of 21 female Korean subjects, aged between 30 and 60 years old (mean age 50.048 years) with melasma on the face. The number of test subjects was conducted by selecting 20 or more people based on the regulations on the functional cosmetics review of the Ministry of Food and Drug Safety (MFDS). All subjects were included based on a preliminary interview that consisted of inclusion and non-inclusion criteria for written informed consent. Subjects who were pregnant, lesion at the test site, and with a history of medical treatment on the concerned skin area were excluded from the study.

Subjects used samples after washing their face in the morning and evening. They applied the control formulation on the left side (vertical half) of the face, and test formulation on the right side of the other side for 4 weeks.

In vivo skin brightening study

To evaluate the brightening efficacy of control and test formulation, three assessment (L-value, melanin index (M.I), melasma area) were measured. Measurements were conducted triplicate before the treatment and at 2 and 4 weeks after the treatment.

Skin birghtness measurement (L-value)

For skin brightness measurements, the front of the face was photographed using Mark-Vu equipment (PSIPLUS Co., LTD, KOREA). Images taken under normal light were used for the evaluation by analyzing the skin brightness (L-value) of the test sites (left and right cheeks) using the I-Max Plus program. The L-value increases as the skin brightness improves.

Melanin index measurement (M.I.)

Melanin values, representing skin color, were measured using a spectrometer (Mexameter MX18, Courage Khazaka Electronic, Germany), and the test sites (hyper-pigmented area) were measured three times. The measurement uses three wavelengths: 568 nm (green), 660 nm (red), and 880 nm (infrared), corresponding to melanin and erythema using the absorption principle, and measures the reflectance of light emitted from the probe. The M.I. value decreases as skin melanin index improves.

Melasma area measurement

For skin melasma measurement, the test sites (left and right cheeks) were analyzed using imaging equipment (Antera 3D CS,Miravex, Ireland), and the analysis mode used to assess the affected area (mm^2) of melanin-hyperconcentration. A light emitting diode (LED light source) was used to analyze the surface image of the skin, the data extracted from the three-dimensional shape image of the built-in program, and the appearance of the skin digitized. The affected area (mm^2) value decreases as the blemishes improve.

Data analysis

Statistical analyses were conducted using IBM SPSS 25.0 software program. Significance before and after product use was confirmed with a hypothetical mean difference of 5% ($p<0.05$). The significance of within-group comparisons was evaluated through Repeated measures ANOVA (parametric method) or Friedman test (non-parametric method) depending on whether the normality test was satisfied. The difference at each time point was tested using Bonferroni's method. For comparison between groups, the significance was evaluated through paired samples t-test (parametric method) or Wilcoxon signed rank test (non-parametric method) depending on whether the normality test was satisfied.

Results.

Physicochemical characterization of cationic liposome

The appearance of liposome was observed using cryo-TEM. Cryo-TEM measurement showed that the liposomes were present as bi- or multi-layer nanoparticles (Figure 1). Liposome stability was monitored using a dynamic light scattering device. The liposomes

showed excellent stability in terms of particle size and zeta potential and maintained a positive charge, when stored for 28 days at room temperature (Figure 2). Along with visual evaluation, we determined that the cationic liposomes were stable without any loss of integrity.

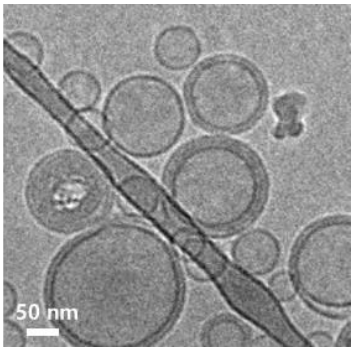

Figure 1. Morphology of chitosan-based cationic liposome was characterized by a cryo-TEM.

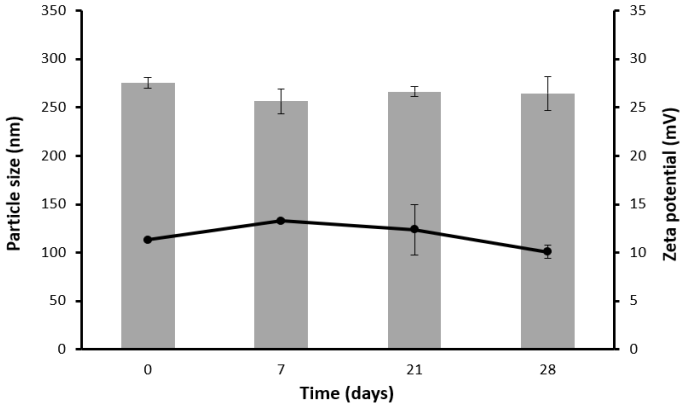

Figure 2. Characterization of chitosan-based cationic liposome. Hydrodynamic size and zeta potential of liposome was measured for 28 days.

In vivo skin brightening study

To compare *in vivo* skin brightening efficacy of cosmetic formulations with or without cationic liposome containing niacinamide, subjects' cheek area were analyzed by L-value, M.I., Melasma area before and after 2 and 4 weeks of usage.

Skin brightness measurement (L-value)

Both the control and test formulations of niacinamide resulted in an increase in skin brightness after 2 and 4 weeks compared with that before treatment. More importantly, test formulation containing cationic liposome exhibited statistically significant increase in skin brightness parameters compared to control formulation (Figure 3). This result shows a better skin efficacy enhancement effect by using cationic liposome.

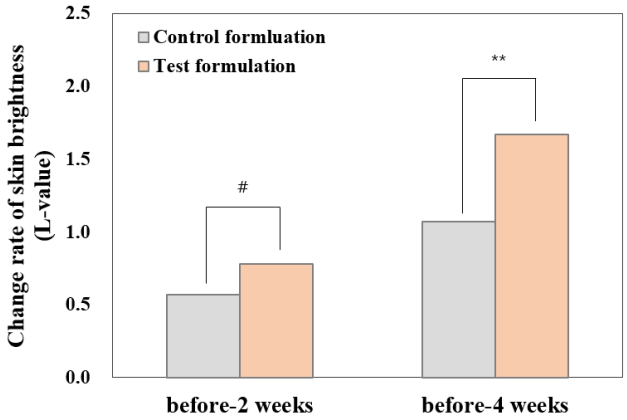

Figure 3. Measurement result of change rate of skin brightness between groups (#: $p<0.05$ by Wilcoxon signed rank test, **: $p<0.05$ by Paired samples t-test).

Melanin index measurement (M.I.)

Control and test formulations both significantly ($p<0.05$) decreased the melanin index after 2 and 4 weeks than before use (Figure 4). Moreover, the decrease in the melanin index of the test formulation site was more than that observed in the melanin index at the control site ($p<0.05$). Specifically, the test formulation showed a 1.92- and 1.53-fold decrease in melanin index compared with that of the control formulation after 2 and 4 weeks, respectively.

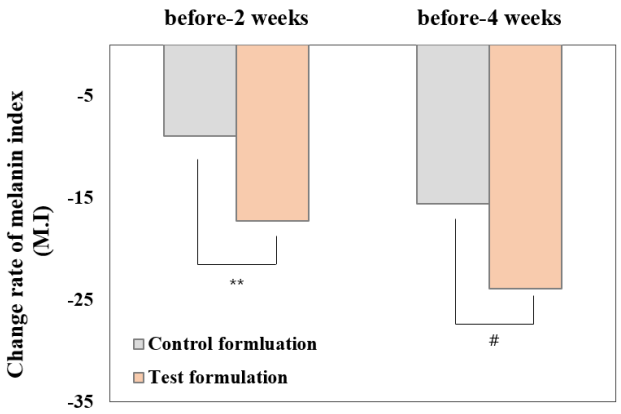

Figure 4. Measurement result of change rate of melanin index between groups (#: $p<0.05$ by Wilcoxon signed rank test, **: $p<0.05$ by Paired samples t-test).

Melasma area

Images of the subjects' melasma area were obtained before and after 2 and 4 weeks of usage as shown in Figure 5A. Both melasma area significantly decreased after 2 and 4 weeks of treatment with both the control and test formulations ($p<0.05$) (Figure 5B). The degree of change in melasma area was higher in the test formulation site and showed significantly better improvement than the control formulation site, with 2.08- and 1.83-fold lesser melasma areas than with the control formulation after 2 and 4 weeks, respectively.

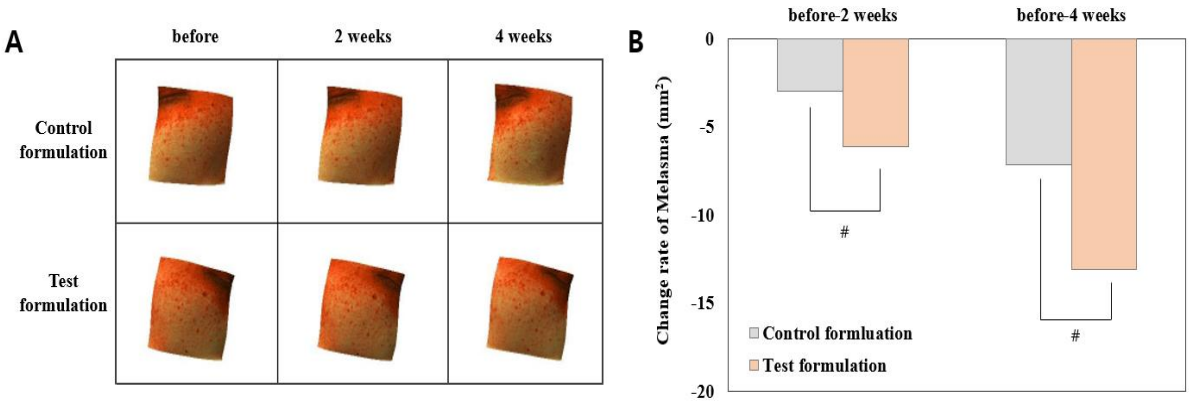

Figure 5. (A) Representative images of the subjects' cheek area, (B) Measurement result of change rate of Melasma area between groups (#: $p<0.05$ by Wilcoxon signed rank test).

There were no reports of adverse reactions from the participants. Therefore, the formulation used in this clinical evaluation study were considered safe.

Discussion.

Penetration of hydrophilic materials, such as niacinamide or adenosine, into the hydrophobic stratum corneum is not easy. However, using cationic liposomes can overcome these limitations. Because cationic liposomes presumably increased the propensity to adhere to the negatively-charged keratin molecules through electrostatic interactions [7]. In our previous study, we used DOTAP (Dioleoyl-3-trimethylammonium propane) to develop cationic liposome. However, DOTAP cannot be applied to cosmetic products owing to supply and regulation of cosmetic ingredients. Therefore, in this study we employed chitosan originated from mushrooms, a biocompatible cationic ingredient, as an alternative to DOTAP. Also, we prepared chitosan-based cationic liposomes using a high-pressure homogenizer-method rather than film hydration-method. Eventhough liposomes manufactured by the high pressure homogenizer-method were confirmed through cryo-TEM that their unique bi- or multi-structure was well formed. And zeta potential measurements clearly indicated that the prepared liposomes were cationic.

Since cationic liposomes have excellent skin permeability, it was verified through clinical evaluation to help the effectiveness of the supported efficacy ingredients. However, since this study was a comparison of cationic liposomes not applied or applied, it is considered that comparative clinical studies on anionic liposomes, excluding chitosan, should be conducted in the future. Nevertheless, this study is meaningful in verifying that cationic liposomes can help improve the performance of cosmetics products.

Conclusion.

Chitosan-based cationic liposomes were prepared by high-pressure homogenization and applied to cosmetic formulation. As a result of clinical evaluation, test formulation, containing cationic liposome, enhanced percutaneous delivery of NA. Test formulation enhanced various indices related to skin brightness, such as L-value, M.I., and melasma area, in comparison to the control formulation after 2 and 4 weeks of treatment. Therefore,

chitosan-based cationic liposome would be helpful to improve skin penetration of cosmetic ingredients and also skin efficacy enhancement effect.

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Conflict of Interest Statement. NONE.

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