Skin color varies depending on the genetics, age, race, seasonal change, UV irradiation, hormonal influences and the presence of pigmentary disease. Constitutive skin color is the genetically determined level of skin pigmentation in the absence of direct or indirect influences such as solar radiation and other environment factors. In contrast, facultative skin color is characterized by the increase in melanin pigmentation above the constitutive level and is brought about by ultraviolet(UV) rays, so called suntanning1. The increase in melanin after an exposure to UV rays results from the activation of tyrosinase in melanocytes and the increase in the number of active melanocytes2. The best way to avoid this UV-induced tanning or pigmentation is the usage of materials that screen out UV rays. Nevertheless, the materials that inhibit

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**Whitening Effect of Cosmetics Containing Magnesium L-Ascorbyl-2-Phosphate(VC-PMG, Vitamin C Derivatives) Assessed by Colorimeter**

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**Backgrounds**: An inhibitory effect of magnesium L-ascorbyl-2-phosphate(VC-PMG, a stable derivative of ascorbic acid) on melanogenesis has been described. Furthermore, glabridin in licorice is known to have inhibitory effects on melanogenesis and widely used for raw materials for depigmenting agents.

**Objective**: The purposes of this study are to provide objective data by measuring the visual clinical effects of VC-PMG with the colorimeter and to promote the development of depigmenting agents.

**Methods**: 20 volunteers joined the study. With an artificial UVB irradiation, eight tanned areas were made on the inner side of the forearm. During two months, each tanned area was treated with five whitening cosmetics with 3% VC-PMG and increasing concentration of licorice from 0% to 3%. Darkness degree of each area was measured weekly by the colorimeter and the visual assessment.

**Results**: For all cosmetics, whitening effect was measured by colorimeter and visual assessment. The cosmetic containing VC-PMG 3% and licorice 1% had more whitening effect than any other cosmetics of different concentrations. Moreover, VC-PMG 3% alone also had whitening effect in some volunteers.

**Conclusion**: VC-PMG was clinically found to have whitening effect.

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**Key Words**: VC-PMG Whitening Colonmeter.
tyrosinase and melanin production and have selective melanocyte toxicity or nonselective suppression of melanogenesis may also have depigmenting effects on the already tanned skin or the pigmentary conditions such as postinflammatory hyperpigmentation, lentigines, and melasma. Phenol/catechol compounds such as hydroquinone, monobenzyl ether of hydroquinone (MBEH) are also known as whitening materials. In addition, it is reported that trans-retinoic acid, azelaic acid, kojic acid, arbutin and ascorbic acid have effects on melanogenesis. Furthermore, natural extracts such as paper mulberry and glabridin are in development to utilize in whitening cosmetics.

Ascorbic acid (AsA) inhibits melanin production by reducing o-quinones so that melanin cannot be formed by the action of tyrosinase until all AsA is oxidized. Melanin can be changed from jet black to light tan by AsA by the reduction of oxidized melanin. However, it is rapidly oxidized and decomposes in aqueous lotion and thus is not generally useful as a depigmenting agent. To resolve this problem, magnesium-L-ascorbyl-2-phosphate (VC-PMG) was synthesized. It is stable in water, especially in neutral or alkaline solution containing boric acid or its salt. VC-PMG is hydrolyzed by phosphatases of live or skin to AsA and thus exhibits vitamin C-reducing activity. Moreover, VC-PMG in cream base can suppress melanin formation by tyrosinase inhibition. Therefore, with the additive effect of L1-methyleneglycol-bis, it is effective in reducing skin pigmentation.

Skin-color measurement is the common clinical method to determine whitening effects of cosmetics. In the past, the way to determine skin color was mostly to see with naked eyes. This method, however, had much room for intervening subjective opinions and disabled one from explaining skin color exactly to others. Much research on methods for measuring skin color have been carried out till now. Among these measuring methods, colorimeter is now widely used in the methodology, for example, for research on cosmetics, because this method is not based on examiner’s subjective feeling but makes it possible to measure skin colors objectively and scientifically.

In this study, we aimed to measure the visual clinical whitening effects of VC-PMG via the colorimeter, a new objective methodology.

Table 1. Volunteer profile

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of volunteer</th>
</tr>
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<tbody>
<tr>
<td>20-29</td>
<td>3</td>
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<tr>
<td>30-39</td>
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<td>40-49</td>
<td>7</td>
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<tr>
<td>50-59</td>
<td>1</td>
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Table 2. MEDs and irradiation doses of volunteers

<table>
<thead>
<tr>
<th>volunteer</th>
<th>MED</th>
<th>irradiation amount</th>
<th>volunteer</th>
<th>MED</th>
<th>irradiation amount</th>
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<tr>
<td>3</td>
<td>35</td>
<td>52.5</td>
<td>13</td>
<td>75</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>75</td>
<td>14</td>
<td>30</td>
<td>60</td>
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<td>67.5</td>
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<td>120</td>
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</tr>
<tr>
<td>10</td>
<td>40</td>
<td>80</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

mean 42.5 75.75

Unit: (mJ/cm)
MATERIALS AND METHODS

Subjects and Materials
20 Korean volunteers (20 males, the mean age of 37) joined the study. All gave written consents after explanation of the experimental procedure. Examinees are healthy people over 18 years old and those were excluded who had experience of UV irradiation during the last three months or had a history of photosensitive disease or took a photosensitive medicine (Table 1).

Methods

A. Determination of MED
We evaluated minimal erythemal doses (MEDs) of each subject after 16 to 24 hours from causing identifiable erythema with irradiating UVB ray on the back. YS UVB-400 with FS 72 T12-UVB-HO lamp (Philips, Holland), was used as the source of UVB ray and the wavelength of lamp was 290-325nm and its maximum at 301 and 302nm. Radiometer measured the UVB strength by using IL 700 (International Light Inc.). During irradiation, the radiation intensity of lights was made to increase in equal ratio and the size of the region to which UV rays are applied was more than 0.5cm². Skilled experts assessed MEDs after 16 to 24 hours from irradiation of UVB rays and finally determined an arithmetic means of them as MEDs. It was found that MEDs of 20 volunteers were 10-75mJ/cm² (42.5mJ/cm² on the average) (Table 2).

B. Tanning
A relatively clean region in the inner side of the forearm was chosen as the region for this study because of its accessibility. We first measured L-values in the internal side of forearm by using colorimeter, CR-300 (Minolta camera Co., Ltd, Osaka, Japan), and then patched square templates (3cm²) used in the light patch test. Then, by using the same UVB lamp for measuring MEDs, we irradiated 1.5MED to make 8 tanned areas. Erythema appeared after 24 hours and pigmentation appeared within 1 to 3 days after irradiation. After 9 days, for 11 out of 20 volunteers, tanned patches almost disappeared. Therefore, for 10 out of these 11 volunteers, 2MED was applied again to the inner side of the opposite forearm and for the remaining one, 6MED was irradiated to gain the final tanned areas (Table 2).

C. Whitening Cosmetics
Five cosmetics were used for whitening effect.
SW-27: Control (VC-PMG 0%, Licorice 0%)
CJ enprani lotion
SW-28: VC-PMG 3%, Licorice 0%
SW-29: VC-PMG 3%, Licorice 1%
SW-30: VC-PMG 3%, Licorice 2%
SW-31: VC-PMG 3%, Licorice 3%

VC-PMG: magnesium-L-ascorbyl-2-phosphate (Nikkol, the degree of purity 85%)
Licorice: licorice extracts
(all of them from CJ enprani, Korea)

D. Application of Whitening Cosmetics
After irradiation for tanning, there was a stabilizing period (a time for the pigmentation) for about 7 days. One control and four whitening cosmetics were used in this double-blind vehicle-controlled study. Each examinee was given five cosmetics and asked to evenly apply them twice a day by using a swab not to mix with one another. Each application order for each examinee was different. Examinees were told to apply five cosmetics for 2 months on 5 out of 8 tanned patches, nothing on 1 out of remaining 3 patches and two out of five cosmetics were applied again on the remaining 2 patches for reliability. Therefore, two out of five cosmetics were applied respectively on two different patches for each examinee.

E. Measurement of Whitening Effect
Whitening effects were evaluated at regular intervals based on visible assessment, photography and L-value of chromameter (CR300, Minolta Camera Co., Ltd., Osaka, Japan).

a) Visible Assessment
Two examiners measured the degree of pigmentation via visible assessment and recorded a mark in accordance with the following grades. (0; undetectable, 1; mild pigmented, 2; moderately pigmented, 3; highly pigmented)

b) Photography
An examiner took a photograph by using Nikon camera with ISO100 Kodak film on each measurement.
An examiner measured L-value three times for each tanned patch by using colorimeter (CR300, Minolta Camera Co., Ltd., Osaka, Japan). L-value on the colorimeter indicates a numerical expression of color difference on black/white axis. Thus, the higher L-value implies a color closer to its whiteness degree.

After tanning, this measurement was taken at weekly intervals for two months.

### Statistics

We conducted 'Mixed Procedure with SAS System' to determine the differences of whitening effects among whitening cosmetics and 'Repeated measures ANOVA test' to ascertain the significance of

<table>
<thead>
<tr>
<th>Cosmetics</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.75</td>
</tr>
<tr>
<td>SW-27 (VC-PMG 0%, Licorice 0%)</td>
<td>1.714286</td>
</tr>
<tr>
<td>SW-28 (VC-PMG 3%, Licorice 0%)</td>
<td>1.75</td>
</tr>
<tr>
<td>SW-29 (VC-PMG 3%, Licorice 1%)</td>
<td>1.892857</td>
</tr>
<tr>
<td>SW-30 (VC-PMG 3%, Licorice 2%)</td>
<td>1.857143</td>
</tr>
<tr>
<td>SW-31 (VC-PMG 3%, Licorice 3%)</td>
<td>1.821429</td>
</tr>
</tbody>
</table>

p<0.05

**Table 3. Difference between Visible Assessment Grades for Each Cosmetic**
measurement interval.

RESULTS

1. Whitening Effect by colorimeter

For five cosmetics named SW-27, SW-28, SW-29, SW-30 and SW-31, the higher TIME*CREAM Estimate (the increase ratio of L-values measured by using colorimeter before and after application), means the greater whitening effect. The result is as follows: SW-29 > SW-28 > SW-27 > SW-31 > control > SW-30 in the order of the greatness of whitening effect. Differences between them, however, were not statistically significant (data are not shown).

2. Whitening Effect for Each Measurement Interval

For all whitening cosmetics, measurements showed statistically significant differences through 56-day measurement period, except that of the interval between 42 and 49 days.

3. Reliability

Two out of five cosmetics were applied respectively on two different patches for each examinee. As a result, most of examinees proved to be statistically reliable. While two of them showed low reliability, the other 18 examinees turned out to be highly reliable (included in the visible assessment).

4. Visible Assessment

Through visible assessment for every measurement, each mark was given in accordance with 4 grades: 3 for highly pigmented, 2 for moderately pigmented, 1 for mildly pigmented and 0 for undetectable. Differences between visible assessment grades on the 56th day after application and at the time of tanning in the beginning were measured for each volunteer by each group of the cosmetics. Then, the means of the differences for each volunteers were evaluated by each five group of cosmetics to elucidate the visual whitening effects of the cosmetics (table 3).

As for SW-27 where only the vehicle was applied, there seemed to have less whitening effect, compared to the control on which nothing was applied according to visible assessment. The other four cosmetics containing VC-PMG 3% and licorice of different concentrations appeared to have better whitening effect than control. The result is as follows: SW-29 > SW-30 > SW-31 > SW-28 in the order of the greatness of whitening effect (Fig.1). Therefore, SW-29 showed the greatest differences in the degree of whitening.

Although the degree of differences in whiteness was greatest in SW-29, the number of examinees who experienced whitening effect, even a slight difference, were slightly different. The number of examinees who experienced whitening effect of each cosmetic is as follows respectively: SW-28 (6 out of 20), SW-29 (5/20), SW-30 (5/20), SW-27 (4/20), SW-31 (3/20). Thus according to visible assessment, the cosmetic of SW-28 turned out to have whitening effect on the most examinees.

5. Photography

We continuously observed whitening effects by taking a photograph for each measurement, and compared them with those of visible assessments (Fig. 2 A,B)

DISCUSSION

This study deals with the whitening effect of cosmetics. There are several materials which are known for having whitening effects. Compounds of catechol and phenol, such as hydroquinone, N-acetyl-4-S-cysteaminylphenol and monobenzylether of hydroquinone (MBEH), are known as whitening materials. In addition trans-retinoic acid, azelaic acid and ascorbic acid - both of them are saturated dicarboxylic acids, Kojic acid (5-hydroxy-4-pyran-4-one-2-methyl), 4-hydroxyanisole, and arbutine also fall under whitening materials. Besides, natural extracts such as mulberry extract (paper mulberry), licorice extract (glabridin), bearberry extract, A. patula and A. viscida and green tea extract are also known as whitening materials.

AsA affects collagen synthesis, drug-metabolizing enzyme, production of interferon, antiviral, antibacterial, and antioxidization and photoprotective actions. AsA does not possess intrinsic absorptive capacity for UV radiation and is believed to act after UV radiation has penetrated the skin and interacted with resident skin chromophores to generate reactive oxygen radicals species. It has been proposed that the influence of AsA on the monopherase activity of tyrosinase is
from its ability to reduce the enzymatically generated o-quinones, so that melanin cannot be formed by the action of tyrosinase until AsA is oxidized. However, the disadvantage of AsA is its instability, especially in aqueous solution. Magnesium-L-ascorbyl-2-phosphate (VC-PMG) was synthesized to overcome this problem. VC-PMG is stable in water, especially in neutral or alkaline solutions containing boric acid. VC-PMG is hydrolyzed by phosphatases of liver or skin to AsA and thus exhibits Vit C-reducing activity. Moreover, VC-PMG in cream base can suppress melanin formation by tyrosinase inhibition. VC-PMG directly or indirectly suppresses melanin formation catalyzed by tyrosinase. Tyrosinase related proteins (TRP1) had been suggested to have a variety of catalytic functions, including low levels of those enzymatic activities ascribed to tyrosinase. TRP1 has also been reported to possess 5,6-dihydroxyindole-2-carboxylic acid oxidase activity, an activity blocked by AsA. AsA probably suppresses melanin formation at various oxidative steps of melanin formation, such as 5,6-dihydroxyindole oxidation. It is suggested that VC-PMG is absorbed percutaneously, stays in the skin, and inhibits tyrosinase activity of melanocytes. Therefore, with the additive effect of L-methyleneglycol-bis, it is effective in reducing skin pigmentation. Kameyama et al. reported clinically significant lightening effect in 19 out of 34 patients with melasma and solar damage induced by UVB irradiation. Also, application of 10% VC-PMG cream to the skin of patients with hyperpigmented disorders, such as ephelides, chloasma, or senile freckles lightened the pigmentation is some. Furthermore, VC-PMG also produced the same lightening in normally pigmented healthy skin in the same report.

Licorice extract is also known for whitening effect and widely used for raw materials for cosmetics. Glabridin, one constituent of licorice, has been reported to inhibit melanogenesis. Glabridin inhibits tyrosinase activity of melanocytes without any cytotoxicity. They further showed that UVB-induced pigmentation and erythema was inhibited by topical application of 0.5% glabridin. The anti-inflammatory properties of glabridin were attributed to inhibition of superoxide anion production and cyclooxygenase activity.

In this study, five whitening cosmetics were used. Among them, SW-27 was a vehicle which did not contain VC-PMG and licorice. SW-28, 29, 30, 31 contained VC-PMG (Nikko, the degree purity 85%) 3% and contained 0%, 1%, 2%, 3% licorice respectively.

The results of visible assessment for the differences in the degree of whiteness revealed the greater whitening effect in the following order: SW-29, SW-30, SW-31, SW-28 and SW-27. However, the number of examinees who experienced whitening effect of each cosmetic is as follows respectively: SW-28(6 out of 20), SW-29(5/20), SW-30(5/20), SW-27(4/20), SW-31(3/20). This suggests that SW-28 showed better whitening effect in general and yet the degree of visual whitening effect was greater in SW-29 whoever showed the differences. Thus, the result may propose that VC-PMG by itself has better whitening effect, but licorice, which is known as a depigmenting agent may enhance the degree of whiteness in some patients.

Objective color measurement, as is nowadays possible using different instrumental techniques, has proven very useful for measurements at different time intervals. The eye is very sensitive for the discrimination of colors very close to each other but is rather poor in remembering colors, making comparison of color at different time intervals very difficult and subjective. Hence, instrumental numerical quantification of colors seems to be well suited for the evaluation of pre- and post treatment colors. In the cosmetic field measuring skin color is an important tool for evaluating the efficacy of whitening products. Various types of colorimeters are introduced in the cosmetic field, but objective comparison of their accuracy and sensitivity was necessary to choose a proper instrument in each experimental design.

According to the results of colorimeter, whitening effects were greater compared to the control patch on which nothing was applied in the following order: SW-29, SW-28, SW-27, SW-31, SW-30. However, the measurements of the whitening effects by colorimeter were statistically insignificant.

In general, according to the results of visible assessment and colorimeter, SW-29(VC-PMG 3%, licorice 1%) had more whitening effect than the other four cosmetics of different concentrations. Moreover, we evaluated the number of examinees who demonstrated whitening effect through the visible assessment for each cosmetic. There was
better whitening effect in SW-28(VC-PMG 3%, licorice 0%, 6 out of 20 volunteers) than the control group. Despite the lack of consistency, cosmetics containing licorice also demonstrated whitening effects. It can be interpreted as this unclear correlation between licorice concentration and whitening effect was due to some sort of reactions occurring when VC-PMG and licorice is mixed which is yet to be found.

The reason for the insignificant measurements the colorimeter is, first, that colorimeter is so sensitive that very little pressure could change values and thus deviation of measured values was great. In fact, according to a paper reported previously, colorimeter used in this research is more likely to produce statistically insignificant results than other methods such as maxameter and dermaspectrometer. Therefore, the possibility cannot be ruled out that other instruments may have produced different results. Secondly, this research chose the forearm as the region of examination. This region has relatively less hair but the degree of hairiness could vary according to individuals. An examinee may have hair on the forearm, which could cause colorimeter to produce darker results.

CONCLUSIONS

1. This study is to apply whitening cosmetics on the inner side of the forearm and to observe whitening effects.
2. VC-PMG and licorice extract were used as whitening materials.
3. VC-PMG was clinically found to have whitening effect. The cosmetic with greatest whitening effect turned out to be SW-29(VC-PMG 3%, licorice 1%) and in case of SW-28(VC-PMG 3%, licorice 0%), whitening effects were found in 6 out of 20 examinees.

REFERENCES