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화장품 원료에 의해 유도되는 미세 피부반응에 대한 기기적 평가 연구

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Instrumental Assessments of Sub-clinical Skin Reactions induced by Cosmetic Ingredients

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요 약: 인체피부에서 화장품이나 화장품 원료의 안전성시험은 대체로 육안평가로 이루어져왔다. 하지만, 피부반응 초기의 미세한 변화에 대해서 육안으로는 감지하지 못하는 경우가 많다. 따라서, 본 연구에서는 수종의 화장품 원료에 의해 유발되는 홍반반응에 대해 육안평가뿐 아니라, 레이저도플러혈류이미지(LDPI) 측정장비를 이용한 혈류변화 측정, Vapometer®를 이용한 경피수분손실량(TEWL) 측정, 분광광도계를 이용한 피부색 측정과 같은 기기평가를 병행하여 비교하였다. 30명의 건강한 여성 피험자를 대상으로 오일류 7종, 계면활성제류 6종, 보습제류 5종을 피험자의 등 부위에 24 h 폐쇄첩포(D0)하여 피부반응을 유도하였고 첩포 제거 후 30 min (D1), 24 h (D2)에 육안평가와 기기평가를 각각 실시하였다. 육안평가 결과, 프로필렌글라이콜을 제외한 모든 오일류와 보습제류는 저자극 수준의 피부반응(반응도 0+ ~ 2.9+)을 보인 반면, 프로필렌글라이콜과 모든 계면활성제류는 중자극에서 강자극 수준의 피부반응(반응도 3+ ~ 5+)을 보였다. 기기측정 결과, 육안평가에서 저자극 범주의 피부반응에 대해서 혈류량이나 피부색보다 경피수분손실량이 가장 민감하게 피부변화를 감지하였다.

Abstract: The safety of cosmetics or cosmetic ingredients on human skin is generally evaluated by visual assessment but some early subtle skin changes may not be noticed by the naked eyes. Thus, the present study was conducted to detect skin reactions induced by mildly irritating cosmetic ingredients by using a laser Doppler perfusion imager (LDPI) method that measures blood flow, a vapometer® that measure strans epidermal water loss (TEWL), and a spectrophotometer that measures the skin color as the erythema values (a*). Visual assessment showed that all tested oils and humectants except propylene glycol belong to the low skin irritation ranges (grades 0+ to 2.9+) while all tested surfactants and propylene glycol belong to the moderate-to strong-skin irritation ranges (grades 3+ to 5+). Among three instrumental methods, TEWL assessment appeared to be more sensitive than spectrophotometric or LDPI method and suitable for the detection of subtle skin response invisible to the naked eye (grades 0+ to 2.9+). Skin reactions of grade 3+ to 5+ could be detected by all three instrumental methods. In conclusion, the current study suggested that the sub-clinical skin reactions due to mild irritants contained in cosmetics can be best assessed by TEWL measurements.

Keywords: *sub-clinical skin reaction, mild irritants, transepidermal water loss*

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1. Introduction

Skin reactions induced by irritants or allergens show diverse clinical symptoms such as erythema, infiltration, vesiculation, dryness, fissuring, and hyperkeratosis, as well as subjective stimulation reactions such as tugging, itching, stinging, and burning[1]. Therefore, skin reactions caused by cosmetics or the raw materials of cosmetics include not only visible phenomena, but also subtle physiological and morphological skin changes that cannot be detected by the naked eye[2-4]. Thus skin reactions should be evaluated in diverse ways to truly ensure the safety of human skin. The most general methods to evaluate the safety of cosmetics on human skin are visual evaluation[5]. With these methods, the level of skin irritation is determined by the level of erythema. However, in regard to raw materials of cosmetics that cause mild irritation, it is difficult to visually evaluate skin reactions that have not been shown to be erythema or prior to the appearance of erythema. Physiological changes that occur early in the process of irritation, such as changes in cutaneous blood flow, moisture content, and pH, would be expected to occur before any reaction is visible[6]. Therefore, when irritation reactions can be seen by the naked eye, it is already too late to assess the early changes in the skin's physiology[7]. These early changes may be the key to our ability to distinguish subtle skin effects, and therefore to support future product-development efforts. Various instruments have been used in the evaluation of diverse physiological and structural skin changes [4,8]. In previous studies, the deterioration of the skin barrier function from damage to the keratin layer or lipid loss have been evaluated by transepidermal water loss (TEWL), water content, and water retention rate[9]. In regard to reactions developed in response to the dilation of blood vessels within the dermis and the release of inflammatory substances, the vascular blood flow rate has been measured with laser Doppler Perfusion Imaging (LDPI). Skin color changes caused by erythema have been measured through the use of a spectrophotometer[10,11]. Altered skin thickness due to skin reactions to irritants has been measured by ultrasonography. Micro-morphological changes of the skin have been evaluated by using a skin surface replica or an image analyzer[12]. LCPI easily measures a change in blood flow when erythema is detected by the naked eyes[13]. However, physiological changes that occur early in the process of irritation, such as changes in blood flow, moisture content, pH, etc., would be expected to occur before any reaction is visible. As in the cases associated with hyperkeratosis or scale caused by inflammaory reactions, the reations may affect the temperature, the skin color (a*) and the blood flow rate[14,15]. This study was conducted instrumental assessments to detect weak (sub-clinical) skin irritations that could not be observed by visual assessments. Skin reactions to mildly irritating cosmetic ingredients were monitored using a LDPI method that measures blood flow, a vapometer® that measures TEWL, and a spectrophotometer that measures the skin color as the erythema values (a*).

2. Materials and Methods

2.1. Materials

Cosmetic ingredients used in this study were presented in Table 1. As commonly used in cosmetics, they comprised oils, surfactants and humectants and complied with the International Cosmetic Ingredient Dictionary[16]. The oils provide emolliency, moisturizing, grooming and acting as solvents and vehicles to carry other agents[17]. The surfactants create their dispersion between two substances normally immiscible[18]. The humectant is a hygroscopic substance. It is often a molecule with several hydrophilic groups, most often hydroxyl groups, but amines and carboxyl groups, sometimes esterified, can be encountered as well; the affinity to form hydrogen bonds with molecules of water is crucial here. Since hygroscopic substances absorb water from the air, they are frequently used in desiccation or for humidity buffering[16].

2.2. Subjects

Thirty healthy female volunteers between ages 20 and 49 participated in this study, with a mean age of

Table 1. Information of Test Materials

Classification	Test materials (Abbreviations)	Manufactures	Conc.
Oils	Hydrogenated polydecene (HP)	ExxonMobil Chemical Co. (USA)	As is
	Meadowfoam seed oil (MS)	Corda International plc. (UK)	As is
	Cetyl Ethylhexanoate (CE)	Kokyu Alcohol Kogyo Co., Ltd. (JAP)	As is
	Cyclopentasiloxane & Cyclohexasiloxane (CC)	Dow Corning Corp. (USA)	As is
	Dimethicone (D)	Dow Corning Corp. (USA)	As is
	Pentaerythrityl Tetraethylhexanoate (PT)	Stearinerie Dubois Fils	As is
	Phyto-squalalne (PS)	Sophim (FRA)	As is
Surfactants	C14-22 Alcohols & C12-20 Alkyl Glucoside (AAM)	Seppic Inc. (FRA)	10 % in DW
	Cetearyl Alcohol & Cetearyl Glucoside (CCG)	Seppic Inc. (FRA)	10 % in DW
	Polysorbate 60 (P-60)	ICI Chemicals & Polymers Ltd. (UK)	10 % in DW
	Sorbitan Stearate (SS)	ICI Chemicals & Polymers Ltd. (UK)	10 % in DW
	Glyceryl Stearate & PEG 100 Stearate (GP)	ICI Chemicals & Polymers Ltd. (UK)	10 % in DW
	Polyglyceryl-3 Methylglucose Distearate (PMD)	Goldschmidt Chemical SEA Pte Ltd (GER)	10 % in DW
Humectants	Glycerin (G)	LG Houshold & Health Care Ltd. (KOR)	As is
	Glycereth 26 (G-26)	INKOS. Co., Ltd. (KOR)	As is
	Polyethylene Glycol 400 (PEG-400)	BASF SE (GER)	As is
	Propylene Glycol (PG)	ICI Chemicals & Polymers Ltd. (UK)	As is
	Dipropylene Glycol (DPG)	ASAHI GLASS Co., Ltd. (JAP)	As is

33.9 ± 8.0 years. Volunteers' exclusion criteria were current skin diseases, pregnancy, breast-feeding, and medications such as oral contraceptives, anti-histamines, and anti-inflammatories. All subjects were explained in detail about the test procedures and signed an informed consent. Participation was completely voluntary. This study was conducted in compliance with the principles of Good Clinical Practice described in the Declaration of Helsinki[20].

2.3. Instruments

In this study, the skin reactions were measured using the following three instruments for instrumental assessments. First, TEWL was measured with a VapoMeter® (Delfin, Technologies Ltd., Finland) to quantify the barrier status of the skin. The result of this measurement is given in units of $g/m^2 \cdot h$. Second, skin color was assessed by colorimetric measurements made with a Spectrophotometer (CM2500d, Minolta, Japan), an instrument for use with well-reflected skin color. Measurement values were represented in L*, a*, and b*. The selected a* value represents the color range from red (positive values) to green (negative values)[21]. Last, microcirculatory activity at the test site was measured with a LDPI (PeriScan PIM Ⅱ, Perimed AB, Sweden). Based on the well-known laser Doppler principle, it collects back-scattered light without touching the tissue, and generates color-coded images of the spatial distribution of the tissue perfusion. The values were given in arbitrary units, volts (V)[22].

2.4. Study Design

The patch test was performed to induce skin reaction by cosmetic ingredients[23,24]. The test materials were applied as they are released by the manufacturer but, the surfactants were applied as 10% in aqueous solution due to their chemical properties. Patch test was conducted on both volar forearms of the volunteers using IQ chambers® (Chemotechnique Diagnostics AB, Sweden). Each chamber was filled with 20 μL of the test materials and fixed to the skin for 24 h under occlusive conditions. After removal of the chambers, the test sites were dried with soft paper. Evaluations of test sites were performed with visual and instrumental as-

sessments at 30 min (D1) and 24 h (D2) after patch removal. The study was carried out in a partly air-conditioned room a tatemperature of 22 ± 2 ℃. Average relative humidity was 45 ± 5 %. Before assessments, the subjects were rested for at least 20 min in the test room. The skin reactions were scored according to the system modified by the Frosch & Kligman[25] and CTFA guidelines[26]. The scoring system was as follows: 0, no visible reaction; 1, slight erythema, spotty or diffuse; 2, moderately uniform erythema; 3, intense erythema with edema; and 4, intense erythema with edema and vesicles.

2.5. Statistical Analysis

Visual scoring compared between mean values of skin reaction grades that were calculated as the mean of each material on days 1 and 2 (D1 and D2). The equation of means was as follows:

$$Mean = \sum \frac{Grade \times No.\ of\ Responders}{4(Maximum\ Grade) \times n(Total subjects)} \times 100 \times \frac{1}{2}$$

The rate of increase was analyzed as the different values calculated from the baseline (D0) and the arithmetical mean values (D1 and D2). Significant differences were determined by the Repeated Measures ANOVA using SPSS program (ver. 13, IBM Corp., USA).

3. Results

3.1. Visual Evaluation

As a result of the visual assessment (Table 2), the tested cosmetic ingredients could be divided into non-irritation (no reaction, N. R), mild-irritation (1+ to 2.9+ grade), moderate-irritation (3.0+ to 4.9+ grade) and severe-irritation groups (above 5+ grade). Non-irritation group included three oils (HP, MS and CE) and three humectants (G, C-26 and PEG-400). And mild-irritation group included four oils (CC, D, PT and PS) and one humectant (DPG). Moderate-irritation group included two surfactants (AAM and CCG) and severe-irritation group included four surfactants (P-60, SS, GP and PMD) and one humectant (PG). Except for PG, oils and humectants exhibited non- or mild-irritation that is invisible to the naked eye. In contrast, surfactants tested as 10 % in aqueous solution caused moderate- or severe-irritation. Also, distilled water (DW) caused itself mild-irritation.

Table 2. Results of Visual Assessments

Classification	Test materials	D1	D2
Oils	HP	N.R	N.R
	MS	N.R	N.R
	CE	N.R	N.R
	CC	2.50	N.R
	D	2.50	N.R
	PT	1.67	N.R
	PS	1.67	N.R
Surfactants	AAM	4.17	1.67
	CCG	3.33	N.R
	P-60	5.83	N.R
	SS	5.83	N.R
	GP	6.67	N.R
	PMD	5.00	N.R
Humectants	G	N.R	N.R
	G-26	N.R	N.R
	PEG-400	N.R	N.R
	PG	8.33	2.50
	DPG	2.50	0.83
Vehicle control	DW	2.30	N.R

N.R; no reaction

3.2. Instrumental Evaluation

Many test materials caused significant increases in TEWL and a* values on D1 (at 30 min after patches removal) and blood flow on D2 (Table 3, Figure 1). Particularly, some oils (HP, MS and CE), which were included in the non-irritation group by visual assessments, showed significant increases in TEWL and a* values on D1. Two of them (HP and CE) also increased blood flow on D2. Humectants of non-irritation group also exhibited significant increases in TEWL value (G-26) or a* value (G and PEG-400) on D1. Two of them (G and G-26) increased blood flow on D2. Among the materials of mild-irritation group, four materials (CC, D, PT, and DPG) increased TEWL and

Table 3. Results of Instrumental Assessments

Classification	Test materials	TEWL (g/m^2 · h)			LDPI (V)			Spectrophotometer (a* value)		
		D0	D1	D2	D0	D1	D2	D0	D1	D2
Oils	HP	4.857	6.400*	4.413	0.637	0.646	0.669*	5.284	5.746*	5.297
	MS	4.897	6.017*	4.627	0.637	0.642	0.665	4.916	5.458*	4.882
	CE	5.253	6.450*	4.690	0.642	0.656	0.684*	4.710	5.343*	4.637
	CC	4.953	6.423*	4.870	0.672	0.683	0.695	4.724	5.382*	4.776
	D	4.913	7.317*	5.460	0.677	0.687	0.704	5.089	5.571*	5.083
	PT	5.523	7.060*	5.263	0.712	0.722	0.749*	5.218	5.958*	5.145
	PS	5.800	6.550	5.523	0.591	0.606	0.625*	6.170	6.407	5.887
Surfactants	AAM	5.383	9.240*	5.670	0.607	0.664	0.640*	5.651	6.553*	5.852
	CCG	4.927	8.067*	5.010	0.666	0.705	0.696	5.830	7.155*	6.009
	P-60	5.480	7.677*	5.190	0.685	0.736	0.724	5.861	7.442*	6.085
	SS	5.467	8.497*	5.540	0.716	0.767	0.754*	5.842	7.470*	6.129
	GP	4.913	7.473*	5.203	0.570	0.657*	0.605*	5.668	7.328*	5.888
	PMD	4.720	6.947*	5.053	0.591	0.656*	0.623*	5.484	6.924*	5.543
Humectants	G	4.990	4.890	5.163	0.622	0.635	0.660*	5.516	6.044*	5.409
	G-26	5.307	6.140*	5.073	0.671	0.674	0.709*	5.615	5.736	5.526
	PEG-400	5.130	5.347	5.323	0.678	0.688	0.705	4.859	5.332*	4.816
	PG	5.197	9.107*	6.377*	0.682	0.726	0.728*	4.560	6.610*	5.859*
	DPG	5.563	7.193*	5.743	0.698	0.722	0.740*	4.530	5.792*	5.084*
Vehicle control	DW	5.380	8.743*	5.760	0.712	0.754	0.733	4.706	6.622*	4.880

Data are shown as median values. *Significant different from the D0 ($p < 0.05$)

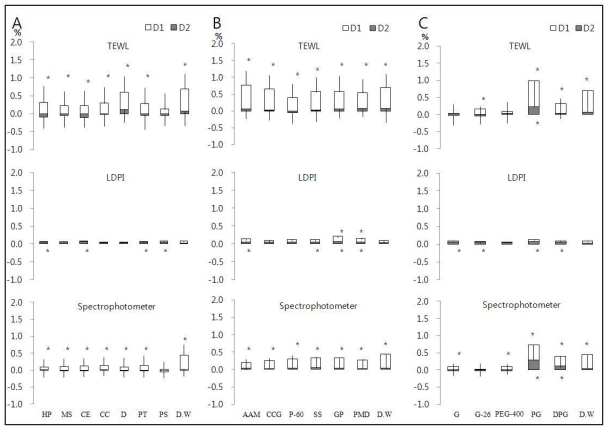

Figure 1. TEWL (Vapometer®, g/m^2 · h), cutaneous blood flow (LDPI) (V) and skin color (Spectrophotometer) (a* value) values in patch testing with oils, surfactants and humectants and exposure for 24 h. Data are shown as increasing rate of different time-point of measurement. A: oils, B: surfactants, C: humectants. *$p < 0.05$ versus baseline (D0).

a* values on D1 compared to the baseline D0. But no significant changes were seen with PS. Tree materials (PT, PS and DPG) induced increases of blood flow on D2 while CC and D did not show such effects. All materials of moderate- or severe-irritation group (AAM, CCG, P-60, SS, GP, PMD and PG) caused changes in TEWL and a* values on D1, but only changes of blood flow were seen with GP and PMD on D1. In short, TEWL and a* values were changed more sensitively than blood flow by cosmetic ingredients (Figure 2).

4. Disccussion and Conclusion

Toxicology tests are important in helping to ensure the safety of human skin against new materials or products used generally in everyday life[27]. In particular, mild irritants are abundant in cosmetics and skin irritation reactions caused by cosmetics are difficult to

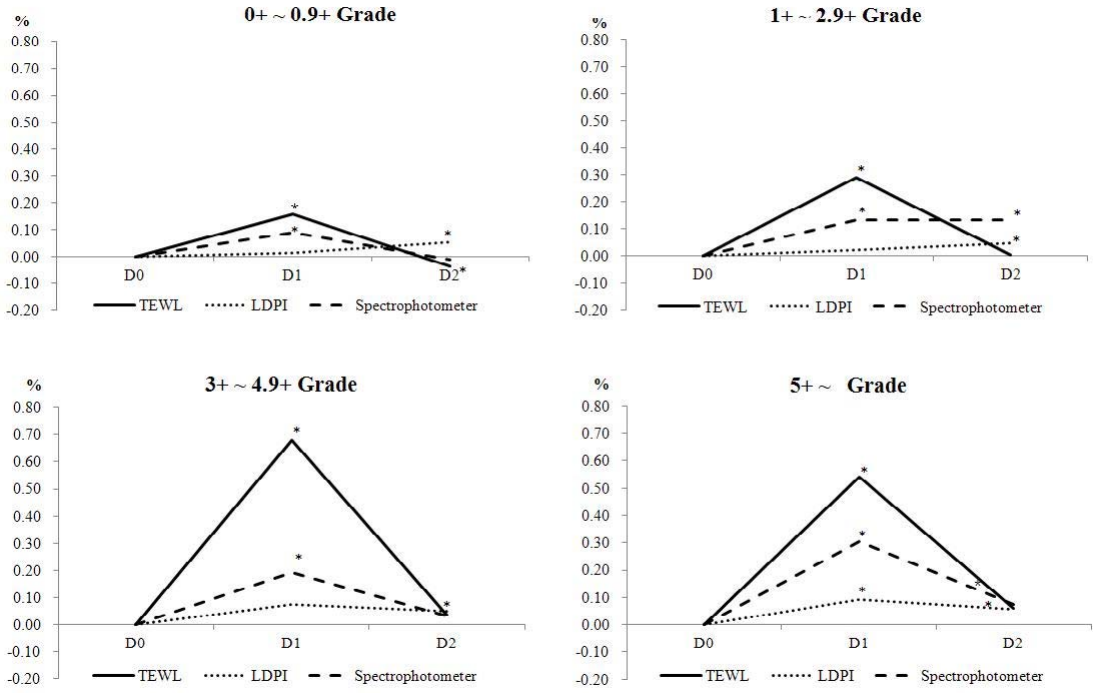

Figure 2. Increasing rate of LDPI, SPEC and TEWL values depended on visual assessments. Data are expressed as increasing rate (%) according to instrumental measurement values when compared to before the patch application (D0). *p < 0.05 versus D0.

detect with the naked eye. Similarly, early skin inflammatory reactions are also difficult to detect. Thus, together with visual assessments of morphological changes, various instrumental methods have so far been used. Appropriate methods have been developed to measure various skin parameters such as skin hydration, superficial pH, skin color (colorimetry and chromametry), skin blood flow (LDPI) and skin barrier function (TEWL).

The used raw materials in this study primarily used for cosmetics (Table 1), instrumental assessments of TEWL allowed detection of weak (sub-clinical) skin reactions in response to mild-irritation. The early inflammatory reactions could also be monitored by using a spectrophotometer that measures skin color change due to erythema. LDPI measurements successfully revealed the blood flow increases caused by inflammatory reactions.

Three oils (HP, MS and CE) appeared to belong to non-irritation group in visual assessments, whereas the others showed 1.6+ to 2.5+ grade of irritation (Table 2). Generally, oils were thought to protect skin barrier by supplying oily layer on the skin. However, the results of this study indicated significant differences in irritation potential between the oils. Although oils such as HP, MS and CE showed non-irritation in visual assessments, they significantly increased TEWL and a* values (Table 3), indicating the high sensitivities of these instrumental methods.

TEWL measurement was considered to be useful for the evaluation of the irritants causing skin barrier disruption. The method would be particularly useful to detect sub-clinical damage that could not be detected by the naked eye. The increasing rates of TEWL values correlate with the intensity of inflammatory reactions (Figure 2). The other method of evaluating inflammatory skin reactions, LDPI, could not detect weak inflammatory reactions. Only strong inflammatory reactions could be detected (Table 3, Figure 2).

According to the results, surfactants were assigned to moderate- to strong-irritation group (higher than grade 3+). Surfactants are known to be one of the ma-

jor cosmetic raw materials that damage the barrier function of the stratum corneum, thereby inducing irritation[28]. In these experiments, the visually detectable skin irritation levels of surfactants were shown to be high in comparison with other test substances. However, because water caused itself mild-irritation, the observed irritation levels of surfactants should be corrected for the water effects to obtain the net values for surfactants. In addition, TEWL measurement doesn't seem to be perfectly ideal for the safety evaluation of aqueous solutions of surfactants.

Although LDPI and a* values increased in response to irritants although their increasing rates were lower than that of TEWL values (Figure 2). Therefore, LDPI and spectrophotometric methods would be useful if TEWL measurements are not appropriate.

The current study also identified several ingredients that caused a significant irritation. For example, PG showed the strongest irritancy among the humectants and GP was the strongest irritants among the surfactants. The mechanism for these phenomena is of interest but requires further studies. Nonetheless it was suggested that use of these ingredients in cosmetics should be avoided or carefully controlled. In conclusion, this study demonstrated that instrumental methods may be useful to detect sub-irritation skin reactions developed by the mild irritants generally contained in cosmetics. TEWL assessment is considered to be the best way to evaluate skin reactions of oils and humectants. LDPI and spectrophotometric methods may also be useful when accurate TEWL measurements are hampered by the water contained in aqueous solutions of surfactants.

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