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Effects of vitamin C on dark circles of the lower eyelids: quantitative evaluation using image analysis and echogram

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Background/aims: The pathogenesis of dark circles of the lower eyelid (DCLE) has been considered to involve stasis and hyperpigmentation of the eyelids. We have already reported that dermal thickness of lower eyelid skin may represent another factor that affects the appearance of DCLE. The aim of this study was to evaluate the efficacy of vitamin C, which is known to increase collagen, on DCLE through a clinical trial.

Methods: Fourteen subjects with DCLE applied either 10% sodium ascorbate (ANa) or ascorbic acid glucoside (AG) lotion in split-face fashion (opposite side: vehicle only) for 6 months. Melanin index (MI), erythema index (EI), thickness and echogenicity of the dermis at bilateral lower eyelids was measured during this trial.

Results: Change in EI was significantly smaller on the ANa-treated side than on the vehicle-treated side. Dermal thickness tended to be thicker for the ANa-treated side than for the vehicle-treated side, although no significant difference was seen. Both EI and dermal thickness tended to change in parallel manner. On the other hand, no significant differences in changes of EI, MI, and dermal thickness were found between AG- and vehicle-treated sides.

Conclusion: ANa may improve DCLE by thickening the eyelid dermis and concealing dark coloration due to congested blood.

Key words: dark circles of the lower eyelid – EI – dermal thickness – vitamin C lotion

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DARK CIRCLES of the lower eyelid (DCLE) represent an esthetic problem that has been attributed to hyperpigmentation and congestion of the eyelids (1). In addition, we have reported that dermal thickness of lower eyelid skin may represent a substantial factor affecting the appearance of DCLE through *in vivo* and *in vitro* study (2). DCLE may become less obvious in the presence of a thicker dermis, as back-scattering of incident light from full-thickness dermis will increase, while light absorption by underlying blood or orbicular muscles will decrease. We conducted a trial to investigate the efficacy of 10% vitamin C lotion on DCLE, which might increase collagen in the dermis (3, 4), and obtained positive results.

Materials and Methods

Subjects and design of the clinical trial

After obtaining informed consent from all volunteers, we performed a clinical trial using two kinds of 10% vitamin C lotion [sodium ascorbate (ANa) and ascorbic acid glucoside (AG)] from May to November 2004. DCLE was confirmed in subjects by inspection by two expert cosmetic researchers who were accustomed to clinical evaluation of facial pigmented skin lesions. Subjects comprised 14 Japanese with DCLE (six men, eight women; mean age, 38.5 years), divided into two groups (ANa, $n = 6$; AG, $n = 8$). Each group was administered either form of vitamin C in split-faced, double-blinded fashion (opposite side, vehicle only) for 6 months. All subjects were advised to apply about 0.3 mL of vitamin C lotion and vehicle by different fingers (or with different pieces of cotton) twice daily (morning and evening) during the study.

Assessment of melanin index (MI) and erythema index (EI) of the lower eyelids by image processing

All measurements were performed in a seated position under controlled conditions (temperature,

20 °C; relative humidity, 45–55%). A D-100 single lens reflex digital camera (Nikon, Tokyo, Japan) with flash lamp was used to obtain digital images of the test area in subjects. As for image analysis of DCLE, we used an image processing method using Image J freeware (2). After splitting a brightness-adjusted original color image into RGB channel images, the (log R − log G) image was defined as the EI image and the inverse log R image was defined as the MI image, based on the optical properties of hemoglobin and melanin (5, 6). Brightness intensity was uniformly multiplied by 4, as the image obtained using this formula was too dark to see.

All facial images were taken under the same conditions to avoid variations in brightness levels of images. In addition, a piece of Casmath (Dai Nippon Printing, Tokyo, Japan) was always placed in each image as a standard for color and brightness to ensure that image brightness was kept constant. Regions of interest (ROIs) were selected at the same lower eyelid area in MI and EI images, and mean brightness values within respective ROIs were adopted as MI and EI for the eyelid.

Assessment of dermal thickness and echo density of lower eyelids in subjects with DCLE

B-mode ultrasound images were obtained using a UX-02 ultrasonic diagnostic system (Rion, Tokyo, Japan) at 30 MHz. Dermal thickness and echo density were measured using the ROI function of IP Lab image analysis software (Scanalytics, Fairfax, VA, USA). After tracing the border of the dermis using a trackball and selecting this as an ROI in the image (7), mean length of seven lines drawn in parallel at regular intervals perpendicular to the long axis of the ROI was determined as dermal thickness. Echo density, as mean brightness intensity (0–255 gray level) of echo in the ROI, was automatically calculated using the software (2).

Statistical analysis

For every parameter, the difference between ANa- and vehicle-, or AG- and vehicle-treated sides were compared with determine changes from baseline (before trial) using two-way repeated analysis of variance (ANOVA). Statistical analysis was conducted using JMP 5.1 software (SAS Institute, Tokyo, Japan) and values of $P < 0.05$ were considered statistically significant.

Results

Assessment of MI and EI of the lower eyelids by image processing

Despite a lack of significant difference in change of MI between vitamin C- and vehicle-treated sides (data not shown), significant difference in change of EI (from 2 to 6 M) was seen between ANa- and vehicle-treated sides (Fig. 1). On the other hand, no significant difference in change of EI was seen between AG- and vehicle-treated sides (data not shown).

Measurement of dermal thickness and echo density of lower eyelids in subjects with and without DCLE

A similar trend, although not significant, was found in change of dermal thickness between ANa- and vehicle-treated sides (Fig. 2). No significant difference in dermal echo density were noted (data not shown). Furthermore, no significant differences in changes of dermal thickness

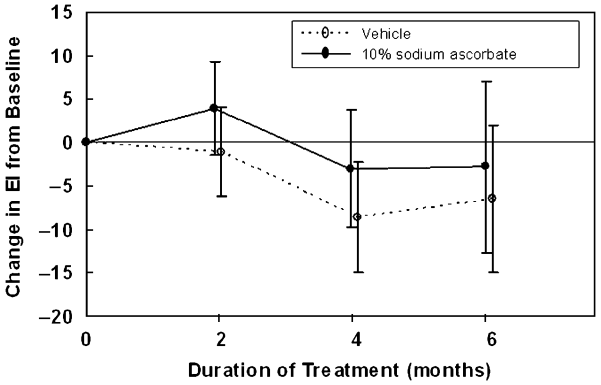

Fig. 1. Change in mean (± SD) erythema index (EI) measured at lower eyelids during treatments with 10% sodium ascorbate (ANa) and vehicle (P < 0.05: ANa vs. vehicle).

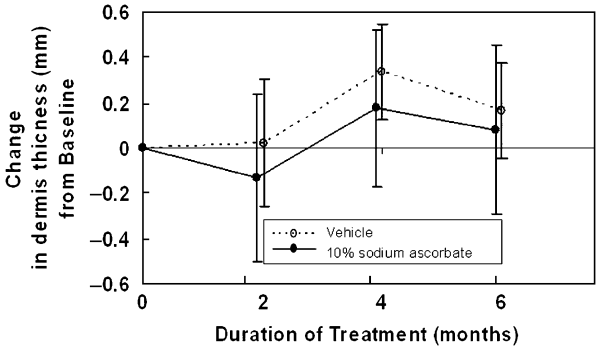

Fig. 2. Change in mean (± SD) dermal thickness measured at lower eyelids during treatments with 10% sodium ascorbate (ANa) and vehicle (NS: ANa vs. vehicle).

and echo density were seen between AG- and vehicle-treated sides (data not shown).

Discussion

Hyperpigmentation and stasis of the eyelids have been considered as possible causes of DCLE, in addition to accentuated shadows created by complex light reflection from a concave, sunken orbital area (1). Anti-DCLE cosmetics have thus generally been designed to improve blood circulation and/or reduce melanin (8). In addition to both quality and quantity of hemoglobin in lower eyelid skin, dermal thickness is likely to be involved in the appearance of DCLE, as our experiments using an *in vitro* model showed that reflectance of the model increased when the collagen gel layer over the blood layer became thicker (2). If thickness or density of the dermis increases, the appearance of DCLE is expected to improve. We therefore performed a clinical trial using two kinds of 10% vitamin C lotion to change the characteristics of eyelid dermis. Vitamin C has been shown to have various influences on the skin. Vitamin C and derivatives, such as magnesium ascorbyl phosphate and AG, have a long history as topical skin lightening agents and are known to inhibit melanogenesis in human melanocytes (9). In addition, vitamin C has also been shown to regulate collagen production (1, 10). Indeed, vitamin C displays biochemical properties on collagen synthesis and regulation *in vitro* (11, 12). In this clinical trial, we expected regulation of collagen synthesis by vitamin C in addition to inhibition of melanogenesis, as vitamin C may promote collagen production and conceal the color of blood stasis, which would improve the appearance of DCLE. In humans, Fitzpatrick and Rostan (3) demonstrated an increase of Grenz zone collagen and mRNA for type I collagen synthesis of the cheek following topical application of 10% vitamin C. Humbert et al. (4) reported that topical application of 5% vitamin C cream over a 6-month period by healthy female volunteers improved both clinical appearance and ultrastructure of photodamaged skin compared with vehicle-treated area. However, absorption of ascorbic acid is limited by instability and water solubility in terms of penetration into the lipophilic stratum corneum (3). To solve the instability of vitamin C, ANa and AG are widely used by cosmetic manufacturers. In this clinical trial, significant difference was seen in EI between ANa- and vehicle-treated sides, indicating that ANa may influence cutaneous circulation or properties of the eyelid dermis and reduce EI. As values of EI and dermal thickness tends to change in parallel, the latter factor (effect on the dermis) seems more likely. In our previous analysis of DCLE using image analysis (2), we reported both MI and EI of the lower eyelid were significantly higher in subjects with DCLE than in subjects without (2). However, the present results of EI change, but no MI change, suggest that the effect of vitamin C is mainly due to collagen production rather than decreased melanogenesis. Vitamin C may also influence damage to the dermis by UV exposure. This clinical trail was performed from May to November, and results may thus have been partly due to the influence of UV exposure in the summer season. According to Darr et al. (13), vitamin C prevents or decreases inflammation after UV exposure. UV-irradiation increases matrix metallo-proteinase (MMP) activity, which in turn promotes damage to dermal collagen fibers (14, 15). Cho et al. showed that oral administration of a mixture of antioxidants (vitamins C and E) significantly suppressed UVB-induced expressions of MMPs and enhanced expression of type I procollagen (16). Vitamin C may also influence circulatory conditions in the eyelid. Mean transcutaneous oxygen tension ($TcPO_2$) value is known to be significantly lower in patients receiving hemodialysis than in normal subjects. Oral supplementation with a combination of vitamin C and vitamin E for 6 months to these patients ($TcPO_2$ <50 mmHg) reportedly improves $TcPO_2$ values markedly (17). Therefore, in addition to effects on the dermis, ANa might improve stasis or congestion of blood in the eyelid. Unfortunately, we could not measure $TcPO_2$ of the lower eyelid in the present study. However, considering these findings from the literature and the results of our clinical trial, ANa seems likely to improve DCLE through the reduction of damage to the dermis and acceleration of collagen production (1, 3, 4, 10–12, 16), in addition to positive effects on local circulation in the eyelid.

We concluded that ANa may improve DCLE, mainly by thickening of the eyelid dermis and improving stasis or congestive state in the eyelid. Long-term topical application of ANa may become an effective treatment for esthetic problems due to DCLE.

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