Original Article

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Exploratory study on the effect of ferulic acid derived from rice bran on dull skin

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SUMMARY: Ferulic acid (FA) from rice bran is known to possess strong antioxidant properties. Furthermore, its multifunctional properties, such as skin whitening, anti-glycation, and anti-inflammatory effects, have attracted attention. We undertook an exploratory study to examine whether FA might be associated with improvements in skin dullness. A single-blind study was conducted on 24 healthy women, in which a cream containing 1% FA was applied to one side of the face and a placebo cream was applied to the other side to verify the effects. To explore the molecular mechanism, RNA-seq was performed on HEKn keratinocyte and Hs68 fibroblast, followed by qRT-PCR. In the human clinical trial, the melanin index significantly decreased in the area where the FA cream was applied, and a questionnaire also subjectively confirmed the improvement of dark and yellowish dullness by FA cream. RNA-seq analysis of HEKn cells treated with FA revealed significant changes in the expression of FGF-2 (basic fibroblast growth factor) promoting melanocyte proliferation, and CD36 (cluster of differentiation 36), related to the removal of advanced glycation end-products (AGEs). qRT-PCR confirmed that FA significantly downregulated FGF-2 and upregulated CD36 expression. These results suggest that FA may exert its effects by regulating the expression of genes involved in melanogenesis and glycation, contributing to the suppression of melanin production and the reduction of AGEs for the improvement of dull skin.

Keywords: Dull skin, ferulic acid, glycation, melanin, thickened stratum corneum

1. Introduction

Ferulic acid (FA), a phenolic compound is known to act as a scavenger and possesses strong absorption in UV (*1-3*). The structure of FA is shown in Supplementary Figure S1 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*). With the growing interest in health and beauty, much attention has been paid to the other functions of FA, such as antioxidant, anti-inflammatory, and anti-bacterial activity (*1,4,5*).

Dull skin is one of the most common skin problems among various racial and cultural backgrounds. In addition to intrinsic factors, such as darkening of skin tone due to melanin accumulation and changes in the blood flow, dullness might be related to extrinsic factors, such as reduced light reflectance and roughness of the skin. Furthermore, the visual impression of dullness is exacerbated by the loss of skin transparency with aging (*6,7*).

Advanced glycation end-products (AGEs) accumulated in the skin with aging and amplified by exogenous factors, such as UV, cause dull and yellowish skin (*8*). The Japan Cosmetic Industry Association states that one of the causes of dullness is a decrease in transparency due to thickening of the stratum corneum.

Common treatments for dull skin include arbutus and vitamin C derivatives for whitening and exfoliation treatment such as peeling (*9,10*). Although these methods have shown certain effects, they tend to rely on a single mechanism of action and there are issues, such as variation in effects among individuals and irritation and deterioration of the barrier function of the skin with long-term use (*11*). In this study, we examined the effects of a cream containing 1% FA (FA cream) and a placebo cream in a human clinical trial. Moreover, using human epidermal keratinocytes (HEKn cells) and dermal fibroblast cells (Hs68 cells), the expression levels of genes related to melanin production and glycation were examined by quantitative real-time PCR (qRT-PCR).

2. Materials and Methods

2.1. Reagents

The FA used in this study was provided by Tsuno Food Industry Co., Ltd. (Wakayama, Japan), and its purity was more than 98% (Lot number: F09883).

2.2. Stability tests of FA

Placebo and FA creams were formulated with FA concentrations of 0, 0.5, 1.0, 1.5, and 2.0%. The composition is shown in Supplementary Table S1 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*). These formulations were stored at 40°C for 30 days. After the storage, the creams were evaluated for discoloration and odor in Supplementary Figure S2 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*).

2.3. Human clinical trial

This clinical trial was approved by the ethics committee of Tsuno Food Industry Co., Ltd. (Rin 24-002) and conducted in accordance with the Declaration of Helsinki. This trial was registered in the UMIN Clinical Trials Registry (UMIN000058687) and performed from fall to winter. Twenty-four healthy Japanese women 30 to 50 years old received a cream containing 1% FA and placebo cream to apply twice a day for 12 weeks. They were distributed to subjects once a month for the three months. Each subject applied FA cream to one side of the face and placebo cream to the other. The number of subjects applying the test product to the right side and the number of subjects applying the product to the left side were assigned equally. Assignments as to which cream was applied to which side were kept confidential until all subjects had completed the study (single-blind study). Prior to the measurement, subjects were acclimatized for 30 min in a constant temperature and humidity chamber (22°C, 50% humidity) after washing their faces. Using a Mexameter MX18 (Courage+Khazaka electronic GmbH, Cologne, Germany), the melanin index was measured on both sides of the face at week 0 and every 4 weeks thereafter, for a total of four measures. We used the VISIA Evolution system (Canfield Scientific, Parsippany, NJ, USA) to photograph subjects' faces. At least three measures were performed on both sides of faces. Imaging was conducted under standardized and reproducible conditions in accordance with the manufacturer's guidelines. Lighting conditions were controlled using the system's built-in cross-polarized and UV illumination modes. Camera settings were kept constant across all measurements. In addition, subject positioning was standardized using chin and forehead stabilizers to ensure consistency with previously analyzed images. We utilized VISIA Evolution system to measure the spots in their faces and analyzed using the included software. Using Corneometer CM825 (Courage+Khazaka electronic), the stratum corneum water content was measured. This was based on the capacitance measurement of a dielectric medium and the hydration level could be detected in this system. At least three measurements were performed on both sides of each subject's face.

We performed the Visual Analog Scale (VAS) to obtain subjective evaluation of skin changes and feeling including touch and appearance after utilization. Subjects were asked to answer a questionnaire using a VAS at week 0 and 12. The degree of skin condition that they felt was recorded on a 10 cm (0 cm = very bad, 10 cm = very good). This allowed participants to express their perception on a continuous scale between two extremes.

In this study, the handling of participants' personal information and the protection of privacy regarding the publication of study results were thoroughly explained during a pre-study briefing. Signed informed consent was obtained from each participant to ensure respect for their privacy. We obtained additional informed consent regarding the publication of the facial images. To protect the participants' privacy, all images were anonymized.

2.4. Cell culture

Normal human epidermal keratinocytes isolated from neonatal foreskin (HEKn) were cultured in EpiLife™ Medium containing 10% Human Keratinocyte Growth Supplement (HKGS) (Gibco™, Thermo Fisher Scientific, Waltham, USA), 100 U/mL penicillin and 60 μM $CaCl_2$ at 37°C under 5% CO_2. Human dermal fibroblast cells (Hs68) were also cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 10 mg/mL streptomycin (Fujifilm Wako Pure Chemical, Osaka, Japan). HEKn and Hs68 cells were suspended in 24-well plates (WATSON, Tokyo, Japan) at 1×10^5 and 2×10^5 (cells/well), respectively, and precultured overnight at 37ºC.

2.5. Measurement of cell viability

FA was added to HEKn and Hs68 cells at 0 - 5 mM, followed by incubation for 24 h at 37°C. After treatment, the culture medium was removed and replaced with serum-free DMEM containing 10% tetrazolium salt reagent (cell counting kit-8, Dojindo Laboratories, Kumamoto, Japan). Cells were then incubated for 2 h and cell viability was assessed by the absorbance at 450 nm (Tecan, Kanagawa, Japan). Relative viability was calculated by normalizing to the untreated group.

2.6. RNA sequencing (RNA-seq) for profiling of gene expression

HEKn and Hs68 cells were incubated with FA at 0.2 and 1 mM, and 0.4 and 2 mM, respectively, for 24 h at 37°C and total RNA was extracted. A TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA) was utilized to construct the library. For RNA-seq, next-generation sequencing (NGS) was performed using NovaSeq6000 (Illumina).

2.7. Expression levels of melanogenesis-related genes

HEKn cells were incubated with FA (0.5, 1, and 1.5 mM) for 4 h at 37°C and total RNA was obtained. cDNA was synthesized from total RNA (250 ng) using PrimeScript RT reagent (TAKARA BIO, Shiga, Japan). Using TB GreenR Premix Ex Taq™ II, qRT-PCR was performed. Each gene expression level was calculated relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences for GAPDH, basic fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF), stem cell factor (SCF), and neuregulin 1 (NRG1) were designed from the following literature (*12-16*) and shown in Supplementary Table S2 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*).

2.8. Expression levels of glycation-related genes

Expression levels of CD36 were measured by qRT-PCR, as well as that of melanogenesis-related genes. HEKn cells were incubated with FA (0.5, 1, and 1.5 mM) for 24 h and treated with Isogen II to obtain total RNA. cDNA was obtained using the PrimeScript RT reagent. The primer sequences for CD36 and GAPDH were designed following the cited literature and shown in Supplementary Table S2 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*) (*12,17*).

2.9. Statistical analysis

Statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC, USA). A paired *t*-tests were used for the VAS questionnaire, while Dunnett's test was applied to the melanin index measurements, cell viability, and gene expression. All data were shown as means ± SEM. The significance level for each test was set at 5% or less.

3. Results

3.1. Changes in color and smell of FA cream

In Supplementary Figure S2 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*), obvious discoloration and odor change were observed in creams containing more than 1.5% FA compared to placebo cream. Neither odor nor color change were observed for creams containing 0, and 0.5% FA creams, as well as placebo cream. In creams containing 1% FA, a slight change in discoloration was observed, but no change in odor was observed at 40°C. Consequently, 1% FA cream was utilized and new creams were provided to subjects once a month in the human clinical trial.

3.2. Subject grouping in the human clinical trial

Subjects were assigned to two groups to ensure that the mean baseline values of the primary outcome (melanin content) and the secondary outcomes (stratum corneum water content and spot score) were equivalent (Figure 1A and 1C). No significant differences were observed in the baseline values of these parameters between FA-treated and placebo groups. The study design for the topical application of FA was summarized in Figure 2. Twenty-nine subjects were evaluated for eligibility, but as one of them did not meet the selection criteria of spot score, only the remaining 28 subjects were randomized into two groups. One group applied FA cream to the right side of the face, while the other applied FA cream on the left side. During the trial, four subjects withdrew due to personal reasons. Finally, 24 subjects, ranging in age from 30 to 50 years old, with a mean age of 41 years, completed the 12-week treatment.

3.3. Human clinical trial

The primary end-point of the change in the melanin index was shown in Figure 3 as zero before placebo and FA application. In the area where FA cream was continuously applied, the melanin index significantly decreased eight weeks after the start of application, with a change of − 8.6 ± 1.53 compared to week 0. Although there was no statistically significant difference between FA and placebo groups at week 8, the reduction in melanin index in the FA group was approximately 1.35 times greater than in the placebo group.

As shown in Supplementary Figure S4A (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*), the amounts of stratum corneum water content in the placebo group decreased significantly between week 4 and week 12 from −6.11 ± 2.04 to −11.44 ± 1.95 (week 12, $p < 0.0001$). In contrast, in the FA group, the stratum corneum water content decreased significantly between week 8 and week 12 from −10.14 ± 1.82 to −8.75 ± 1.88 (week 8, $p < 0.0002$; week 12, $p = 0.0014$). As shown in Supplementary Figure S4B (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*), spot score in the placebo group and the FA group at week 4 after the application were −1.50 ± 0.71 (week 4, $p = 0.159$) and −1.28 ± 0.90 (week 4, $p = 0.335$), respectively. From week 4 to week 8, the score of the placebo group continued to improve, while that of the FA group increased and returned to pre-application levels. At week 12, the spot scores of the two groups crossed.

The VAS questionnaire survey that was conducted

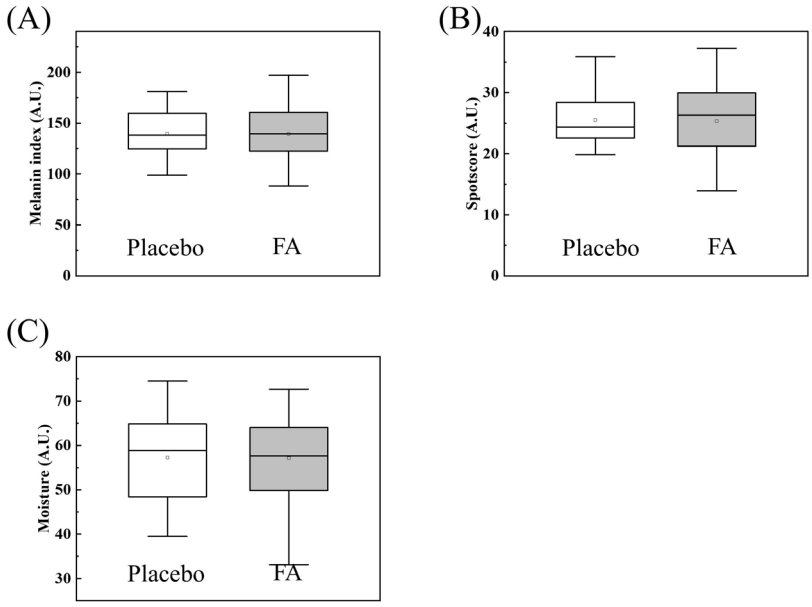

Figure 1. Box plot. A box plot for each measurement item after allocation is shown. The white bar shows the values of sides that were treated with placebo cream, while the gray bar shows the values of sides that were treated with FA creams. **(A)** Vertical axis is melanin value (A.U.). **(B)** Vertical axis is spot score (in arbitrary units) relative to number of spots. **(C)** Vertical axis is stratum corneum water content (A.U.).

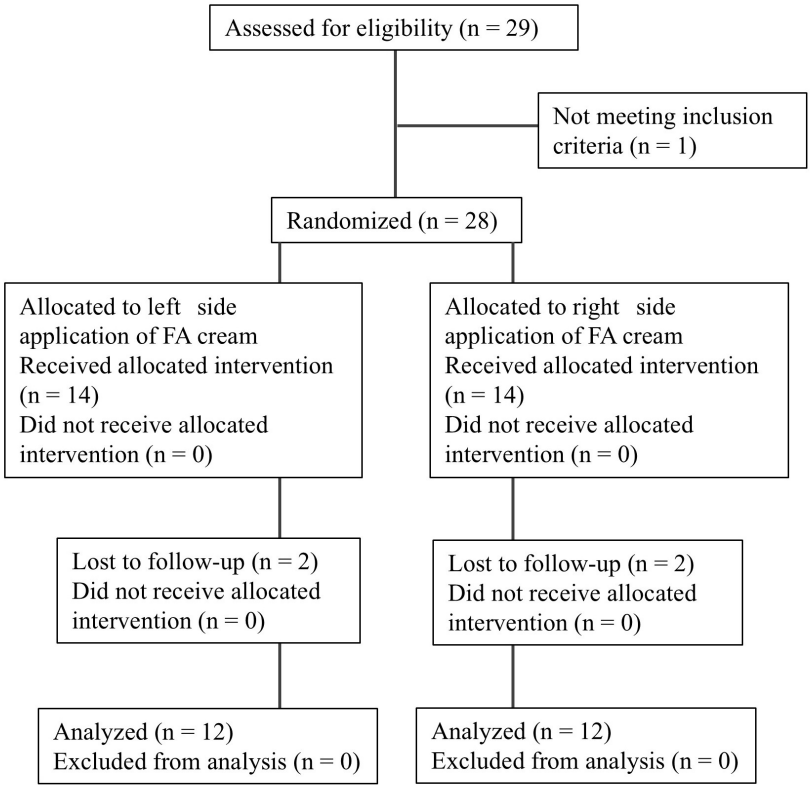

Figure 2. A total of 29 subjects were assessed for eligibility. One subject was excluded for not meeting the inclusion criteria based on spot score. The remaining 28 subjects were randomly assigned to two groups: one group (n = 14) applied FA cream to the right side of the face, and the other group (n = 14) to the left side. During the study period, four subjects withdrew due to personal reasons or health-related issues. A total of 24 subjects (mean age: 41 years) completed the 12-week treatment.

as an evaluation of the application experience was performed from week 0 to week 12 and these results of the evaluation items were shown in Figure 4. In the item related to evaluating the brownish dullness caused by melanin production, the FA result was 0.44 ± 0.51 points at week 12, a slight increase compared to that of the placebo group (Figure 4A). In the categories of moisture and dark dullness, the FA group showed significant improvements, with changes of 0.75 ± 0.42 and 0.73 ± 0.50 points, respectively (Figure 4B and 4C). The evaluation item related to yellowish dullness caused by glycation at week 12 in the FA group was observed to change to 1.16 ± 0.49 points (Figure 4D). There were also significant improvements in the overall ratings for skin dullness and skin translucency in the FA group that were also observed at week 12, with changes of 0.78 ± 0.24 and 0.65 ± 0.24 points, respectively (Figure 4E and 4F).

Figure 5A and 5B showed facial images of two subjects at pre- (week 0) and post-application (week 12) taken with the VISIA Evolution. In both subjects, there was an overall improvement in skin lightness compared to pre-application, with a reduction in dullness, particularly in the cheek area.

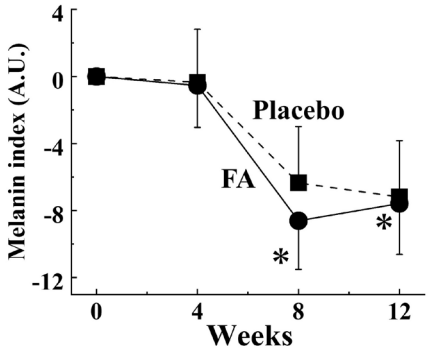

Figure 3. Effects of FA on dull skin. The dotted line shows placebo treatment sides, and the straight line shows the FA treatment sides (FA group, $n = 24$; placebo group, $n = 24$). Amount of change of skin melanin index from prior to cream application (week 0) is shown. The vertical axis is the skin melanin index, and the horizontal axis is the number of weeks from the start of the application. Data are shown as mean ± SEM and were analyzed by paired *t*-tests between the FA and placebo groups and by Dunnett's test to compare week 0 in each group. No significant difference was observed between the placebo and FA groups (week 8, $p = 0.320$). In the FA group, significant decreases were observed at week 8 ($p = 0.0149$) and week 12 ($p = 0.0314$) compared to week 0. *$p < 0.05$.

3.4. Measurement of cell viability

FA at various concentrations was administered to HEKn and Hs68 cells, and cell viability was assessed. For HEKn cells, FA up to 0.5 mM promoted cell proliferation. In contrast, 5 mM FA significantly reduced cell viability to 42.9%, consistent with cytotoxicity. From

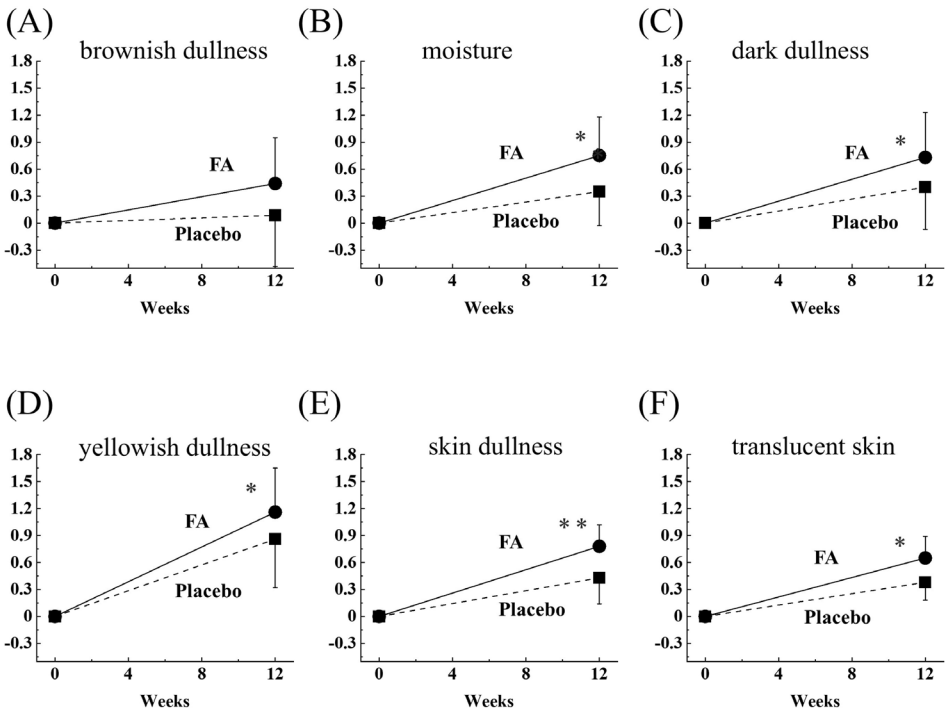

Figure 4. Self-assessment of skin condition throughout the treatment period. (A) Subjective improvement in brown dullness. **(B)** Subjective improvement in moisture. **(C)** Subjective improvement in dark dullness. **(D)** Subjective improvement in yellowish dullness. **(E)** Subjective improvement in overall dullness of the skin. **(F)** Subjective improvement in skin translucency. Data are expressed as means ± SEM ($n = 24$). Data were analyzed by paired *t*-tests. *$p < 0.05$, **$p < 0.01$ *vs.* significantly different from FA week 0. AU, arbitrary units. At week 12, paired t-tests were used to compare the placebo group and FA group (brownish dullness, $p = 0.093$; moisture, $p = 0.134$; dark dullness, $p = 0.242$; yellowish dullness, $p = 0.197$; skin dullness, $p = 0.83$; translucent skin, $p = 0.215$).

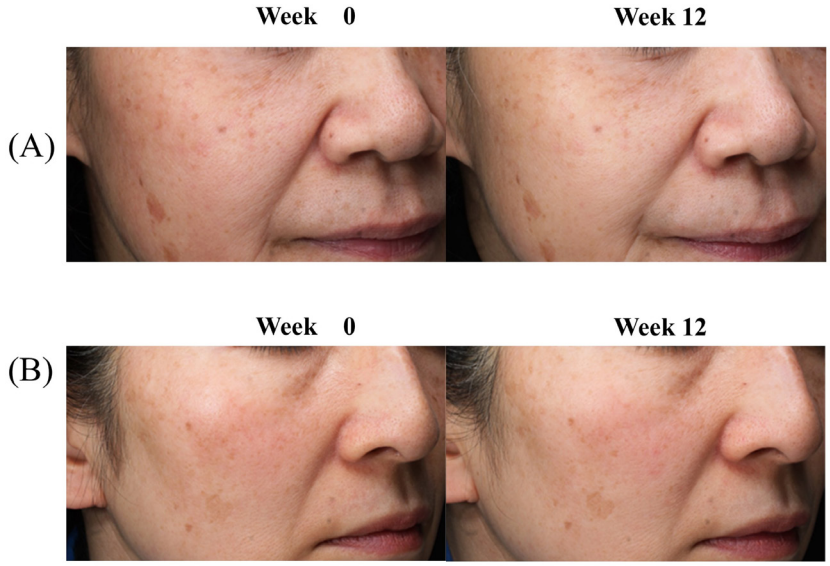

Figure 5. Facial images of two subjects (A and B) at pre- (week 0) and post-application (week 12), were taken with the VISIA Evolution. (A) Upper left: before application (week 0); upper right: after 12 weeks of FA cream application. **(B)** Lower left: before application (week 0); lower right: after 12 weeks of FA cream application.

these findings, the upper limit of a safe concentration of FA was determined to be 1.5 mM, maintaining at least 90% cell viability (Supplementary Figure S3A, *https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*). In contrast, for Hs68 cells, cell viability was maintained at about 90% with 2.5 mM treatment while a decrease in cell viability to 76.4% was observed with 5 mM FA (Supplementary Figure S3B, *https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*).

3.5. Suppression of melanin production-related genes

The main pathways in melanogenesis are thought to involve G protein-coupled receptors (GPCRs) and tyrosine kinase receptors (*18*). Using HEKn and Hs68 cells, we performed RNA-seq analyses and observed little change in gene expression related to melanin production with FA treatment in Hs68 cells at all concentrations. In contrast, a significant decrease in expression of *FGF-2* gene was observed with FA treatment in HEKn cells. Since we wanted to examine the relationship between FA treatment and the tyrosine kinase signaling pathway involving FGF-2, SCF, HGF, and NRG1, we quantitatively analyzed their gene expression in HEKn cells by qRT-PCR (Figures 6A-6C). The expression of FGF-2, SCF, and NRG1 were significantly reduced after 1.0 and 1.5 mM FA treatment for 4 h, while *HGF* gene could not be detected. In the case of FGF-2, 60% inhibition was observed at 1.5 mM FA. For SCF and NRG1, 65% and 21% inhibition were observed at 1 mM FA, respectively.

3.6. Increased expression of genes that remove glycation

The accumulation of AGEs is thought to be due to glycative stress. It also affects the skin and contributes to a dull, yellowish appearance that is mediated by multiple AGEs receptors, such as RAGE (receptor for advanced glycation end products), AGE-R1, AGE-R2, and AGE-R3. RAGE and AGE-R2 particularly cause inflammation (*19*). In contrast, AGE-R1 and AGE-R3 are thought to be involved in the degradation and removal of AGEs, as also are CD36 and SR-A (scavenger receptor class A) (*20*). We performed RNA-seq analysis using HEKn cells treated with FA, examined the genes related to glycation, and observed only a change in CD36 gene expression. No significant changes in the expression of other genes related to glycation were observed. Therefore, we quantitatively analyzed the gene expressions of CD36 in HEKn cells by qRT-PCR and found an approximately three-fold increase in *CD36* gene expression with 1.5 mM FA treatment (Figure 6D).

4. Discussion

Several human clinical trials of FA have been reported previously. When FA, vitamin C, and vitamin E were combined, an increase in solution stability, reduction of UV-induced skin damage, and the photoprotective effects of FA were observed (*21*). Similarly, in a human clinical trial of FA alone on patients with photoaging symptoms, there was a significant improvement observed in melanin levels, erythema, and skin moisture content (*22*). To the best of our knowledge, human clinical trials examining the effect of FA on dull skin have not been reported. Therefore, we performed human clinical trials using FA by itself and exhibited the effectiveness of FA on dull skin in this study. In addition, the mechanism of FA on

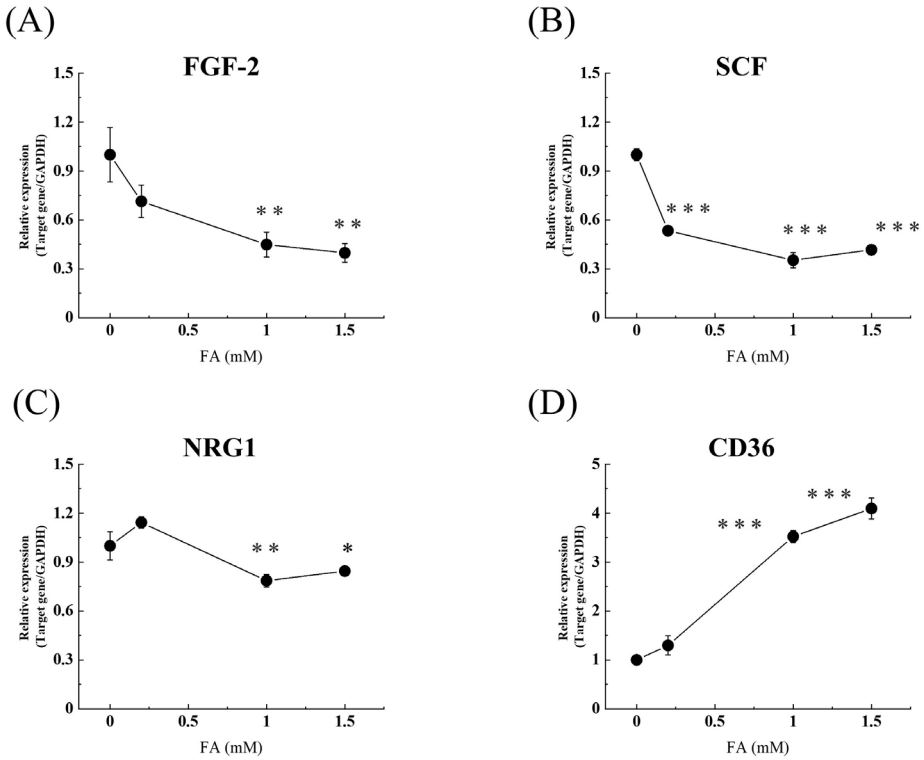

Figure 6. Results of the gene expression analysis. In HEKn cells, FA inhibited FGF2, SCF, and NRG1 expression, while FA facilitated CD36 expression. **(A)** Evaluation of FGF2 gene expression levels. **(B)** Evaluation of SCF gene expression levels. **(C)** Evaluation of NRG1 gene expression levels. **(D)** Evaluation of CD36 gene expression levels. The horizontal axis is the gene expression level and the vertical axis is the concentration of FA treatment. The expression level of each gene is given as a ratio to that of GAPDH. Data are expressed as means ± SEM (n = 4) and were analyzed by Dunnett's test. *p < 0.05, **p < 0.01 ***p < 0.001 (*vs.* FA-untreated samples). (FGF-2 in 1 mM FA, p = 0.002; FGF-2 in 1.5 mM FA, p = 0.001; SCF in 0.2 mM FA, p < 0.00001; SCF in 0.2 mM FA, p < 0.00001; SCF in 1 mM FA, p < 0.00001; SCF in 1.5 mM FA, p < 0.00001; NRG1 in 1 mM FA, p = 0.009; NRG1 in 1.5 mM FA, p = 0.039; CD36 in 1 mM FA, p < 0.00001; CD36 in 1.5 mM FA, p < 0.00001).

skin dullness at the genetic level was investigated.

Although FA has been widely noted as a cosmetic ingredient, there are stability issues due to high sensitivity to light and oxygen (*23*). These issues limit the range of FA applications in beauty and skin care products. In this study, stability tests were conducted for FA (Supplementary Figure S2, *https://www.ddtjournal.com/action/getSupplementalData.php?ID=307*) and the FA concentration for the clinical study was set at 1%. In Figure 3, a decrease in melanin levels was observed in both groups after week 4. One factor contributing to this might be that this examination was conducted from September to December when UV radiation was greatly reduced. Previous studies have reported that reduced UV exposure leads to a natural reduction in melanin production (*24*). Even in such an environment, our results suggest that the amount of melanin, one of the main causes of dull skin, was reduced in the group of subjects who continuously applied FA cream for 8 weeks, compared to week 0. However, a comparison between groups showed no statistically significant differences. This is presumably due in part to the relatively low concentration of 1% used in this study. On the other hand, from week 8 to 12, a phenomenon was observed in which the melanin indexes of the FA and placebo groups crossed. One reason for this might be winter-specific environmental factors such as low temperature and moisture in addition to the decrease in UV exposure. It has been reported that the skin barrier function tends to be impaired in winter environments such as low humidity and cold temperatures, and that external stimuli such as dryness and friction can cause inflammation (*25*). Such inflammation may cause post-inflammatory hyperpigmentation (PIH), which may slightly alter melanin levels (*26*).

A melanin suppression effect of FA was supported not only by objective indicators but also by subjective evaluation (VAS questionnaire). In the subjective evaluation, the improvement effect was confirmed in the subjects' perception of their own skin for all the other evaluation items, except for the brownish dark category. Specifically, improvement was recorded in the evaluation items of moisture, dark dullness resulting from skin dryness and stratum corneum thickening, yellowish dullness due to glycation, as well as skin dullness and translucent skin. Significant improvements were observed at week 12 in the FA group compared to week 0 (Figure 4B-4F). The results of the assessment of subjects were consistent with the changes in facial images in two representative subjects shown in Figure 5A and 5B. After the application of FA cream, the evaluation and VISIA Evolution of both subjects showed a significant

improvement in the overall dullness of the skin and an improvement in the brightness of the skin tone. These results suggested that FA might be effective in improving skin tone by acting in a complex manner against various causes of dark spots.

Melanogenesis, the cause of brownish dullness, is thought to be regulated by multiple signaling pathways, involving primarily MITF (microphthalmia-associated transcription factor) which regulates the expression and activity of melanogenic enzymes. Main signaling routes include GPCRs (*e.g.* melanocortin receptor, endothelin receptor B and frizzled receptor), tyrosine kinase receptors (*e.g.* SCF/KIT, FGF-2, and HGF signaling pathways) (*18*). To analyze the efficacy of FA at the molecular level, we performed RNA-seq using HEKn and Hs68 cells. The results indicated fluctuations in gene expression of FGF-2. We further investigated the effects of FA on the melanogenic pathway involving tyrosine kinase signaling such as SCF, FGF-2, HGF, and NRG1 using qRT-PCR. Although the *HGF* gene was not detected, decreases in gene expression of the *SCF*, *FGF2*, and *NRG1* genes were observed. FA may inhibit the melanogenic pathway *via* tyrosine kinase-type receptors (Figure 6A-6C). Furthermore, FGF-2 has been shown to activate the MAPK/ERK and PI3K/Akt pathways *via* the FGF receptor and promote keratinocyte proliferation. This proliferation might thicken the epidermis and stratum corneum thereby contributing to the dark appearance of the skin by increasing diffuse light reflection (*27,28*). Decrease in *FGF2* gene expression by FA may be involved in the suppression of stratum corneum thickening through regulation of keratinocyte growth and survival. These changes at the molecular level were also consistent with the improvement in darkening caused by stratum corneum thickening observed in human clinical trials.

AGEs were thought to cause dull, yellowish skin, and other skin problems (*8*). As shown in Figure 6D, FA treatment significantly enhanced gene expression of CD36, a receptor that degrades and removes AGEs. This result was consistent with the previous studies, indicating that FA may remove AGEs produced in glycation reactions and prevent the development of yellowish dullness (*29*).

In summary, FA demonstrates multifunctional potential in both the prevention and amelioration of different forms of dull skin, brownish dullness, yellowish dullness, and dark dullness. Thus, it may be that FA would be extremely useful as a comprehensive skincare material for improving dull skin and enhancing skin clarity. Future studies on the practical use of FA will be needed to improve the stability of the formulation for higher concentrations of FA, combined with other whitening ingredients and anti-glycation/anti-aging ingredients. Further studies should be done in different season (spring, summer). The applicability to different skin types and age groups should also be investigated.

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