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Retinyl retinoate, a novel hybrid vitamin derivative, improves photoaged skin: a double-blind, randomized-controlled trial

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Background: All-trans-retinoic acid (RA) and all-trans-retinol (ROL) are not widely used as anti-wrinkle agents due to their irritancy and photo-stability, respectively. Therefore, the safety and photo-stability in the development of RA or ROL derivatives have been an important issue.

Aim: To identify the efficacy of retinyl retinoate as an anti-aging agent of cosmetics in treating females over 30 years old with periorbital wrinkles.

Methods: The clinical study was a prospective, double-blind, randomized, and controlled study with a total of 11 Korean women. At every 4 weeks, the effectiveness was assessed with a global photodamage score, photographs, and image analysis using replicas and visiometers. The dermal distance and intensity was also evaluated using Dermascan C.

Results: A statistically significant improvement in facial wrinkles ($P < 0.05$) in eleven volunteers was observed in a clinical trial. The successive application of 0.06% retinyl retinoate cream for 3 months showed decreased depth and area of wrinkles in comparison with 0.075% retinol cream. The visual wrinkle improvement and the maximum roughness improvement rate (R2) for retinyl retinoate cream were 22% higher than that of retinol cream after 12 weeks. A statistically significant increase was observed after 8 and 4 weeks for dermal distance and dermal intensity, respectively ($P < 0.05$).

Conclusions: Retinyl retinoate had characteristic features of new anti-aging agents, and effectively improved facial wrinkle conditions.

Key words: photoaging – wrinkle – retinoic acid – retinol – vitamin

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Aging is a complex, multifactorial process resulting in several functional and esthetic changes in the skin. These changes result from intrinsic as well as extrinsic processes, such as ultraviolet radiation. Ultraviolet radiation causes breakdown of collagen, elastin, and ground substance, which, when combined with imperfect resynthesis of these components, leads to the characteristic changes seen with photodamaged skin (1, 2).

The study of cutaneous aging is of great interest for most cosmetic firms. Aging skin becomes thinner and wrinkles, lines, creases, crevices, and furrows appear, as well as a deepening and accentuation of facial expression lines (3–6). Recently, retinoids were highlighted as a highly efficient component due to the boom of high-performance cosmetics, especially as an anti-wrinkle agent. Among retinoids, retinol and retinoic acid are endogenous compounds naturally occurring in the human body, and are essential for the production, differentiation, and multiplication of epithelial tissues (7–10). Because of their ability to modulate genes involved in cellular differentiation and proliferation, retinoids are thought of as good candidates for both treating and preventing the photoaging process. However, retinoic acid causes a number of side effects such as skin irritation, and retinol is not very stable in heat, light, oxygen, and water, thereby limiting the application of retinoids as a main component for medicines and cosmetics (11–15).

In our recent studies on the development of novel vitamin derivatives, we obtained an interesting hybrid vitamin, retinyl retinoate (RetinoX8; Enprani Co. Ltd, Inchon Korea), which was an ester of all-trans-retinoic acid (RA) and all-trans-retinol (ROL). This new

synthetic hybrid of retinoid showed enhanced thermal stability and decreased photosensitivity, and exhibited decreased cell toxicity compared with that of retinol *in vitro* biological activity and dermatology test in a randomized-controlled trial (16, 17). In this report, we investigate skin wrinkle improvement on aged skin by comparison of 0.06% retinyl retinoate cream and 0.075% retinol cream.

Methods

Patients

A total of 11 generally healthy Korean women, aged 35–56 years, were selected from volunteers, and were graded at baseline (0–9 grading scales: 0 being normal skin). All subjects had grades of 5 or greater in both facial fine lines/wrinkles (primarily in the eye or the 'crow's feet' area) and texture (in the cheek area). Patients had not undergone any topical retinoidal treatment for 3 months and wrinkle removal or peeling procedures for 6 months before participating in this study. None of the women were pregnant or lactating, had atopic dermatitis, allergic diathesis, or hypersensitive skin. The study objectives, outlines, test methods, and the possible adverse effects were preinformed to all of the participants. Respective profile, Case Report Form, questionnaire, and Informed Consent Statement were obtained from all subjects. The study was conducted in accordance with the guideline for the Skin Research Center in Dermapro, which was validated 'Quality Management System Certificate' (KS A 9001:2001/ISO 9001:2000; certificate no.5855) by the KOTRIC certification center on the contact research and consulting service on human skin safety and efficacy.

Treatment regimen

The study was a prospective, double-blind, randomized clinical trial. Independent researchers listed 11 subjects in order of precedence in a computer-generated randomization list. For the selection of random permuted blocks, a randomization code was developed using a computer random number generator. The block lengths 4, 8, and 12 varied randomly. The subjects were divided into groups A and B, with women having an equal probability of assignment to the groups. Individuals in group A were tested on the left periorbital area with 0.06% retinyl retinoate cream (cream A), whereas individuals in group B were tested on the right periorbital area with 0.075% retinol cream (cream B). A regime of 12 weeks was instructed to all subjects with the application of the creams, twice daily, in the morning and at night. The retinyl retinoate was synthesized from all-trans-ROL and all-trans-RA through coupling esterification (16).

Evaluation tools

The clinical evaluations were performed at pretreatment, 4, 8, and 12 weeks. Information about the study objectives, outlines, test methods, and possible adverse effects was provided to all subjects. Approved information consent along with profile, case report form, and questionnaire were submitted by all of the volunteers.

Self-assessment questionnaire

Subjects completed self-assessment questionnaires at weeks 0, 4, 8, and 12 with the following criteria: satisfactory elasticity of the eye (Q1), fine wrinkle reduction (Q2), and wrinkle improvement (Q3). Subjects scored changes as 1, excellent; 2, good; 3, mild; and 4, no change.

Investigator's assessment

Two dermatologists evaluated the subjects' periorbital wrinkles with a double-blind test based on a photodamage score from 0 to 9 (0: no wrinkles, 1: none/mild, 2: mild, 3: mild/fine, 4: mild/moderate, 5: moderate, 6: moderate/deep, 7: moderate/severe, 8: severe, 9: very severe) at weeks 0, 4, 8, and 12. In a case when two dermatologists' evaluations were different, the low-grade's efficacy and high-grade's adverse effect were selected. The adverse effects, such as erythema, edema, scaling, itching, stinging, burning, tightness, and prickling, were analyzed on the tested skin by investigators for safety concerns.

Image analysis using replicas and visiometers

Wrinkle improvement was evaluated by measuring skin roughness and wrinkles using the skin Visiometer SV 600 (Courage & Khazaka, Koln, Germany) (18). Replicas of right and left periorbital areas were observed at test periods. In the present study, skin replicas of both crow's feet areas, obtained from a technique previously described by Grove et al. (19), were analyzed using the Visiometer software. The degree of

skin wrinkle improvement was measured ($I_{ex} = I_{in} \times e–k_d$) after analyzing light intensity based on Lambert and Beer's law. Each evaluation was performed under the same experimental conditions of location and lighting. The assessment of skin Visiometer SV 600 results was based on the following parameters: R1, skin roughness; R2, maximum roughness; R3, average roughness; R4, smoothness depth; and R5, arithmetic average roughness

Dermal distance and intensity analysis using Dermascan C

Dermal distance and intensity was evaluated using the Dermascan C (Cortex Technology, Hadsund, Denmark), a high-resolution ultrasonic image analysis. The measuring principle is based on a high-frequency transducer (20 MHz) of high resolution (Gain compensation curve: 20–25 dB, velocity: 1580 m/s, range: from the dermal–epidermal junction to the top of subcutaneous fat). Analyses were performed at weeks 0 (baseline), 4, 8, and 12.

Statistical data analysis

The changes from the baseline of wrinkle parameters were evaluated. A statistically significant difference in efficacy was achieved at the 5% level (P value of <0.05) by a paired t-test. The statistical data analysis was performed using SPSS software version 11.5.

Results

Subject self-assessments and investigator's assessment

Subject self-assessments with blinded evaluation were performed based on four questionnaires after using creams A and B, which contained retinyl retinoate and retinol, respectively, for 12 weeks. Although subjects noted an improvement with the two creams after 12 weeks, the overall satisfaction with cream A was higher than that of cream B. The percentages of a slight or a significant improvement of wrinkles (Q1), fine wrinkle reduction (Q2), and eye elasticity improvement (Q3) out of 11 subjects were 81.8%, 63.6%, and 90.9% respectively, for cream A. These values were 72.7%, 54.6%, and 81.8% respectively, for cream B. Topical cream A did not show any skin irritation responses, while, topical cream B showed adverse skin reactions of stinging and burning.

The improvement rate of visual wrinkle grade was significantly increased in both groups after 12 weeks ($P<0.05$) (Fig. 1). The mean improvement rate of photodamage score increased 8.61% for cream A (from 6.86 to 6.27, $P = 0.000$) and 7.43% for cream B (from 6.73 to 6.23, $P = 0.006$). The visual wrinkle improvement rate for cream A was 1.28% higher than that of cream B after 12 weeks. An example of facial improvement effects is shown in Fig. 2.

Image analysis using replicas and visiometers

A replica from the right and left periorbital areas was taken at week 0 (baseline), 4, 8, and 12 and analyzed based on five parameters using the Visiometer software. A statistically reduction in wrinkles by replica was observed in both creams A and B. As shown in Fig. 3, the values of R2 and R3 for cream A, which represent the maximum and the average roughness, respectively, were more improved than that of cream B. Particularly, the maximum roughness improvement rate (R2) of cream A was 22% higher than that of cream B.

Dermal distance and dermal intensity

The wrinkle improvement measured by the Dremascan C on creams A and B is shown in Fig. 4. The dermal distance and intensity increased after 4 weeks in both groups (Fig. 5a, b). The statistically significant increase was observed after 8 weeks for dermal distance and after 4 weeks for dermal intensity ($P<0.05$). The mean dermal distance increased from 1.31 to 1.43 for cream A (9.30%, $P = 0.033$) and from 1.32 to 1.43 for cream B (8.39%, $P = 0.000$). The mean dermal intensity

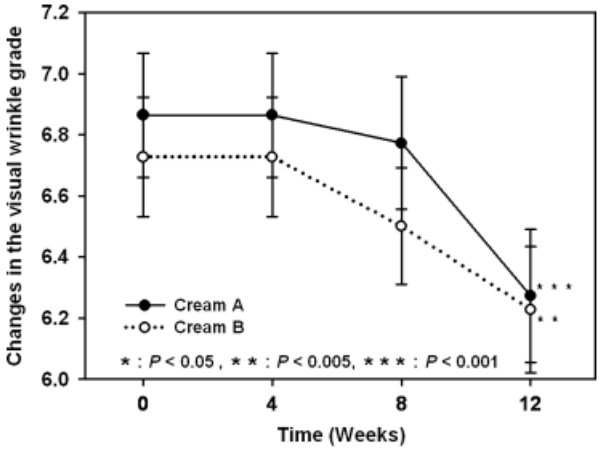

Fig. 1. Changes in the visual grade in subjects treated with 0.06% retinyl retinoate and 0.075% retinol after 12 weeks.

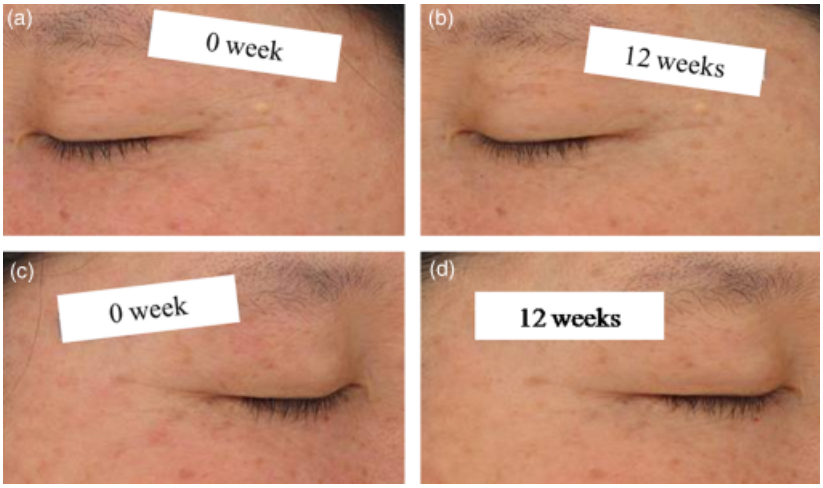

Fig. 2. A patient with wrinkles before (a, c) and 12 weeks after (b, d) treatment with retinyl retinoate (a, b) or retinol (c, d).

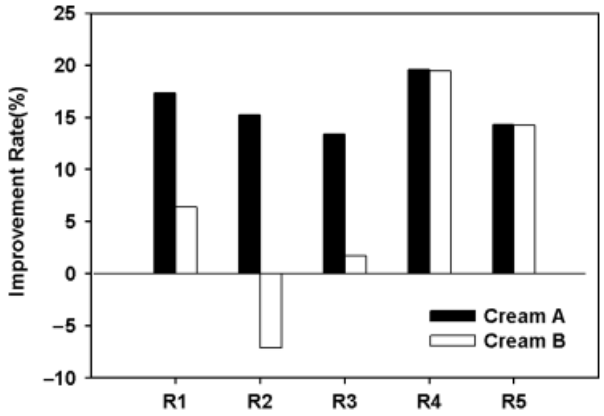

Fig. 3. Changes in wrinkle improvement were analyzed by the skin Visiometer SV 600 after 12 weeks with creams A and B. (visiometer value: R1, skin roughness; R2, maximum roughness; R3, average roughness; R4, smoothness depth; R5, arithmetic average roughness).

increased from 5.93 to 7.46 for cream A (25.88%, $P = 0.000$) and from 5.80 to 7.51 for cream B (29.40%, $P = 0.000$).

Discussion

The topical application of retinyl retinoate in women with facial photodamage demonstrated a significant improvement in the treatment of fine wrinkles in a 12-week clinical test. All the clinical data tested in this study, such as subject and investigator assessments and instrumental analysis, showed a more statistically significant fine wrinkle improvement after applying retinyl retinoate compared with the retinol application. Also, the adverse effects, such as erythema,

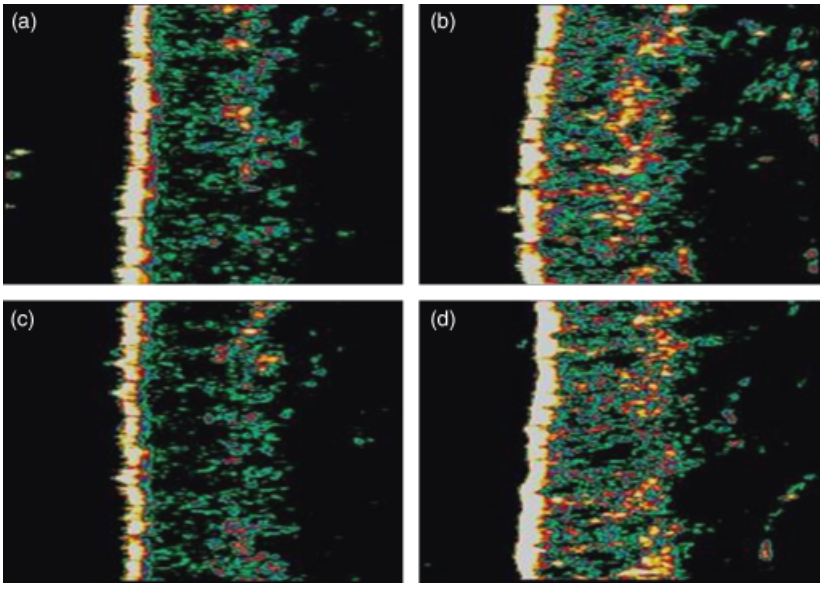

Fig. 4. Dermal distance and dermal intensity of a patient with wrinkles before (a, c) and 12 weeks after (b, d) treatment with retinyl retinoate (a, b) or retinol(c, d).

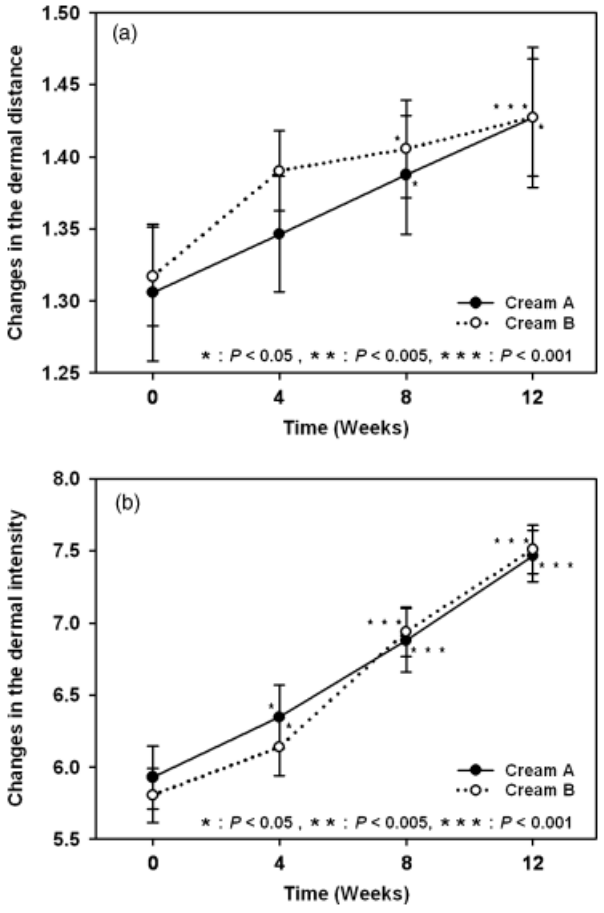

Fig. 5. Changes in wrinkles were analyzed by the Dermascan C after 4, 8, and 12 weeks of cream A and cream B on dermal distance (A) and dermal intensity (B).

edema, scaling, itching, stinging, burning, tightness, and prickling, were not reported in the 0.06% retinyl retinoate cream.

The side effects of retinoic acid and the instability of retinol in light and oxygen were the main hurdles for their application in the cosmetics industry (12–15). In this study, we showed that retinyl retinoate played a newly important role in the improvement of skin wrinkles such as retinol. Moreover, the retinyl retinoate was found to be a safe cosmetic product that did not lead to allergic or irritant contact dermatitis. All these results combined with the stability of the retinyl retinoate indicated that retinyl retinoate, which was synthesized via a condensing reaction between retinol (hydroxyl end group) and retinoic acid (carboxyl end group), removed the disadvantage of retinol and retinoic acid for application in the cosmetics industry (16, 17). That is, the side effects of retinoic acid and the instability of retinol were overcome by condensing retinol and retinoic acid.

In summary, retinyl retinoate was found to be a new novel hybrid vitamin derivative – retinyl retinoate, which had a higher improvement of fine wrinkles, excellent stability, and fewer side effects compared with retinol, which is currently used in the cosmetics industry as an antiwrinkle agent (17). Thus, retinyl retinoate can be conveniently used as an additive for cosmetics in the prevention and improvement of skin aging and medicines for the treatment of skin problems.

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