BJD
British Journal of Dermatology

Improvement in skin wrinkles from the use of photostable retinyl retinoate: a randomized controlled trial

H. Kim,*† N. Kim,‡ S. Jung,‡ J. Mun,‡ J. Kim,† B. Kim,† J. Lee,† H. Ryoo† and H. Jung*

*Department of Biotechnology, Yonsei University, 134 Shinchon-dong, Seoul, 120-749, Korea
†Department of Skin Science and Cosmetics, ENPRANI Co. Ltd, Shinhung-dong 3 Ga, Chung-Gu, Inchon, 400–103, Korea Department of Biotechnology, Yonsei University, 134 Shinchon-dong, Seodaemun-Gu, Seoul, 120–749, Korea
‡Department of Clinical Evaluation, Ellead Skin Evaluation Center, Seohyun-dong, Seongnam-si, Kyunggi-do, 463-824, Korea

Correspondence
Hyungil Jung.
E-mail: hijung@yonsei.ac.kr

Accepted for publication
20 August 2009

Key words
anti-ageing, photoage, retinoid, retinyl retinoate, wrinkle

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2009.09483.x

Summary

Background Photoaged skin can be treated with retinoids, which are natural and synthetic vitamin A derivatives. However, these are photounstable and can cause skin irritation, which is a major limitation in their use in general cosmetics. Retinyl retinoate, which is an ester of all-*trans* retinoic acid (RA) and all-*trans* retinol, has reduced toxicity due to blocking of the carboxyl end group of RA and higher skin regeneration activity than retinol.

Objectives To assess the efficacy of a photostable retinyl retinoate in treating women over 30 years old with periorbital wrinkles.

Methods We conducted two clinical studies with a total of 46 Korean women with periorbital wrinkles, who were not pregnant, nursing or undergoing any concurrent therapy. In the first clinical study, the efficacy of retinyl retinoate was compared with placebo. Twenty-four patients completed a 12-week trial of 0·06% retinyl retinoate applied twice daily to one side of the face and a placebo applied to the other side. In the second clinical study, the efficacy of retinyl retinoate was compared with retinol. Twenty-two patients completed an 8-week trial of 0·06% retinyl retinoate applied twice daily to one side of the face and 0·075% retinol applied to the other side. Efficacy was based on a global photodamage score, photographs, and image analysis using replicas and visiometer analysis (Skin-Visiometer SV 600; Courage & Khazaka, Cologne, Germany) every 4 weeks. The standard wrinkle and roughness parameters used in assessing skin by visiometer were calculated and statistically analysed.

Results The retinyl retinoate-treated wrinkles improved compared with wrinkles treated with placebo or retinol, as assessed by both the investigators and the subjects. Also, skin replica analysis indicated significant improvements in retinyl retinoate-treated skin in both studies, especially in average roughness.

Conclusions Retinyl retinoate applied twice daily was significantly more effective than a placebo or retinol in treating periorbital wrinkles. Importantly, no severe side-effects were observed.

Photoaged skin is largely the consequence of chronic sun exposure and is histopathologically characterized by loss of epidermal polarity, a basket weave appearance of the epidermis, keratinocyte atypia, and reduction and alteration in collagen.[1,2] Photoageing manifests clinically with fine and coarse wrinkling, thickening, inelasticity, dryness, roughness, shallowness and pigmentary mottling.[3,4] Kligman and Kligman first reported that all-*trans* retinoic acid (RA), which modulates genes involved in cellular differentiation and proliferation, is a good candidate for both treating and preventing the photoageing process, leading to the effacement of wrinkes.[5] A number of investigators have also revealed that RA improves photoaged skin.[6–9] However, the side-effects of RA, such as skin dryness, wounds, and scraping during the latent period[10,11] limits its application as a component of medicines and cosmetics.[12,13] Although some retinoid derivatives, such as tretinoin, isotretinoin, etretinate, tazarotene and adapalene, were developed to overcome these problems,[14] local cutaneous irritation including burning, itching, erythema, peeling or dryness, is still observed in approximately one-quarter of

patients.[15] Retinol has received a great deal of attention as an alternative anti-ageing agent for long-term treatment because the carboxyl end group of RA, which is responsible for the adverse side-effects, was replaced with a hydroxyl group.[16–19] However, the instability of retinol in light, oxygen, heat, lipid-peroxidation or water is a significant drawback to the use of these derivatives in general cosmetics.[20,21]

Retinyl retinoate (Fig. 1) is a new synthetic retinoid in which an ester bond was formed between retinol (hydroxyl end group) and retinoic acid (carboxyl end group) via a condensing reaction.[22] This novel hybrid retinoid derivative has enhanced thermal stability and decreased photosensitivity, and caused less cell toxicity compared with retinol in *in vitro* studies.[22] In this present study, we compared retinyl retinoate against a placebo or retinol in improving skin wrinkles in aged skin.

Patients and methods

Patients

Forty-six generally healthy Korean women between 34 and 53 years of age were selected from volunteers. All patients satisfied the inclusive criteria with periorbital wrinkles (global photodamage score 1~6),[23] confirmed by a dermatologist's medical interview and physical examination. Twenty-three patients had mild photodamage (grades 2–3 on a 0–7 scale), and 23 had moderate photodamage (grades 4–6 on a 0–7 scale). None of the women had used topical retinoids to treat their photodamaged areas within 3 months prior to this study. None of the women had undergone wrinkle removal or peeling procedures within 6 months prior to the study. None of the women were pregnant or breastfeeding or had atopic dermatitis, allergic diathesis or hypersensitive skin. All the patients were informed by the investigators about the study objectives, outlines, test methods and possible adverse effects. Patients completed their profile, case report form and questionnaire, and signed the informed consent statement.

The study was conducted in accordance with the Guideline for Functional Cosmetics [KFDA 11-1470000-000863-01; http://www.kfda.go.kr/index3.html (last accessed 9 September 2009)], which require at least 20 patients for the statistical analysis. Forty-eight patients were divided into two studies satisfying the guideline. In the first clinical study, retinyl retinoate lotion was compared with placebo in 24 women (age range 34–50 years). In the second clinical study, retinyl retinoate lotion was compared with retinol lotion in 22 women (age range 39–53 years) excluding two women who abandoned study procedures.

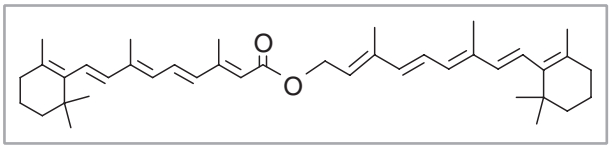

Fig 1. Retinyl retinoate.

Methods

Treatment regimen

The two studies were prospective, double-blinded, randomized and controlled, in which the investigator and patients did not know the cream and area being tested. Independent researchers dispensed either active or placebo inhalers according to a computer-generated randomization list. Women had an equal probability of assignment to the groups. The randomization code was developed using a computer random number generator to select random permuted blocks. The block lengths were 4, 8 and 12 and varied randomly. In the first, independent researchers listed 24 subjects in order of precedence in the randomization list and subjects were randomly divided into groups A and B. The first 12 listed subjects were allocated to group A and the left periorbital (eye) area was treated with 0·06% retinyl retinoate (lotion A; Enprani, Inchon, Korea), and the right periorbital area was treated with placebo (lotion P; Enprani). In group B, the 13th to 24th listed subjects were allocated and the retinyl retinoate and placebo were applied to the opposite sides of the face, compared with group A. Subjects were instructed to apply these to their periorbital areas in the morning and at night for 12 weeks. In the second clinical study, independent researchers listed 22 subjects in order of precedence in the randomization list and subjects were divided into groups P and Q. In group P, the first 11 listed subjects were allocated and the left periorbital area was treated with 0·06% retinyl retinoate, and the right periorbital area was treated with 0·075% retinol (lotion B; Enprani). In group Q, the 12th to 22nd listed subjects were allocated and each substance was applied to the opposite side, compared with group P. Subjects were instructed to apply the treatments in the morning and at night for 8 weeks.

Clinical evaluations were made at weeks 0 (baseline), 4, 8 and 12. Dermatologist's visual assessment, baseline photographs obtained from each subject, and image analysis of replicas using a Visiometer (Skin-Visiometer SV 600; Courage & Khazaka, Cologne, Germany) were used to analyse changes in skin wrinkles. Replicas of wrinkles in the left and right periorbital areas were acquired.

Self-assessment questionnaire

Subject self-assessments via questionnaire were made at weeks 0 (baseline), 4, 8 and 12. Subjects scored changes as 0, no change; 1, mild; 2, good; or 3, excellent.

Investigator's assessment

Subject's periorbital wrinkles were evaluated with a double-blind test by two dermatologists. The dermatologists evaluated subjects' periorbital wrinkles based on a global photodamage score (0, none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe;

7, very severe) at weeks 0 (baseline), 4, 8 and 12.[23] If the dermatologists' evaluations differed, low-grade efficacy and high-grade adverse effect were selected. Subjects' periorbital wrinkles were classified into eight grades. Adverse effects such as erythema, oedema, scaling, itching, stinging, burning, tightness and prickling were recorded by the investigator.

Image analysis using replicas and visiometers

Wrinkle improvement was evaluated by measuring skin roughness and wrinkles using the Skin-Visiometer SV 600.[24] Replicas of right and left periorbital areas were taken at weeks 0 (baseline), 4, 8 and 12. Skin replicas of crow's feet were obtained according to the technique reported by Grove *et al.*[25] and analysed with visiometer software. Light intensity was analysed with the Lambert–Beer law, and the degree of skin wrinkle improvement was calculated ($I_{ex} = I_{in}\ e^{-kd}$), where I_{ex} is the transmitted light intensity, I_{in} is the unattenuated light intensity, k is the Napierian absorption coefficient of the medium, d is the thickness of the medium. Evaluations were performed in the same location with the same lighting at each visit. Parameters used in the assessment of skin with the Visiometer SV 600 were as follows: R1, skin roughness; R2, maximum roughness; R3, average roughness; R4, smoothness depth; and R5, arithmetic average roughness.

Statistical data analysis

The changes from the baseline of wrinkle and roughness parameters (R1, R2, R3, R4 and R5) were evaluated. A statistically significant difference in efficacy was achieved (P-value $< 0{\cdot}05$) by the paired t-test. The statistical data analysis was performed using SPSS software version 10.0 (SPSS Inc., Chicago, IL, U.S.A.).

Adverse effects

We assayed for erythema, oedema, scaling, itching, stinging, burning, tightness and prickling.

Results

Subject self-assessment

Subjects performed self-assessments in a blinded evaluation. In the first clinical study of retinyl retinoate vs. placebo, 21 of 24 (87·5%) subjects reported slight or significant improvement in wrinkles, and three subjects (12·5%) reported no improvement. In the second clinical study of retinyl retinoate vs. retinol, 20 (90·9%) of 22 subjects reported slight or significant improvement in their wrinkles, and two subjects (9·1%) reported no improvement. Placebo, 0·075% retinol and 0·06% retinyl retinoate were all safe products that did not lead to allergic contact dermatitis or irritant contact dermatitis.

Investigator's assessment

Periorbital wrinkles were evaluated in a double-blind test by two dermatologists at weeks 0, 4, 8 (clinical study 2) and 12 (clinical study 1) based on a global photodamage score of 0, none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe; and 7, very severe. After weeks 12 and 8 in clinical study 1 and 2, respectively, the average photodamage score of all patients significantly improved on the retinyl retinoate-treated side compared with the placebo-treated side ($-0{\cdot}42 \pm 0{\cdot}50$ vs. $-0{\cdot}17 \pm 0{\cdot}38$, $P = 0{\cdot}031$) and with retinol treatment ($-1{\cdot}14 \pm 0{\cdot}71$ vs. $-0{\cdot}77 \pm 0{\cdot}75$, $P = 0{\cdot}042$), respectively (Table 1). In particular, 86% of the patients (19 of 22) had decreased photodamage scores with retinyl retinoate treatment, in contrast to 59% (13 of 22) of those using retinol. Figures 2 and 3 illustrate remarkable

Table 1 Changes in the global photodamage score in patients treated with placebo and 0·06% retinyl retinoate in clinical study 1, and in patients treated with 0·075% retinol and 0·06% retinyl retinoate in clinical study 2

Clinical study 1					
Week	Placebo (lotion P)	△	Retinyl retinoate (lotion A)	△	P-value (A : P)
0	3·75 ± 0·79		3·83 ± 0·82		
4	3·71 ± 0·81	−0·04 ± 0·20	3·79 ± 0·83	−0·04 ± 0·20	1·000
8	3·71 ± 0·81	−0·04 ± 0·20	3·71 ± 0·86	−0·13 ± 0·34	0·328
12	3·58 ± 0·65	−0·17 ± 0·38	3·42 ± 0·78*	−0·42 ± 0·50	0·031*
Clinical study 2					
Week	Retinol (lotion B)	△	Retinyl retinoate (lotion A)	△	P-value (A : B)
0	3·45 ± 0·96		3·45 ± 0·74		
4	3·18 ± 0·73	−0·27 ± 0·63	2·86 ± 0·71*	−0·59 ± 0·67	0·069
8	2·68 ± 0·89*	−0·77 ± 0·75	2·32 ± 0·57*	−1·14 ± 0·71	0·042*

*$P < 0{\cdot}05$.

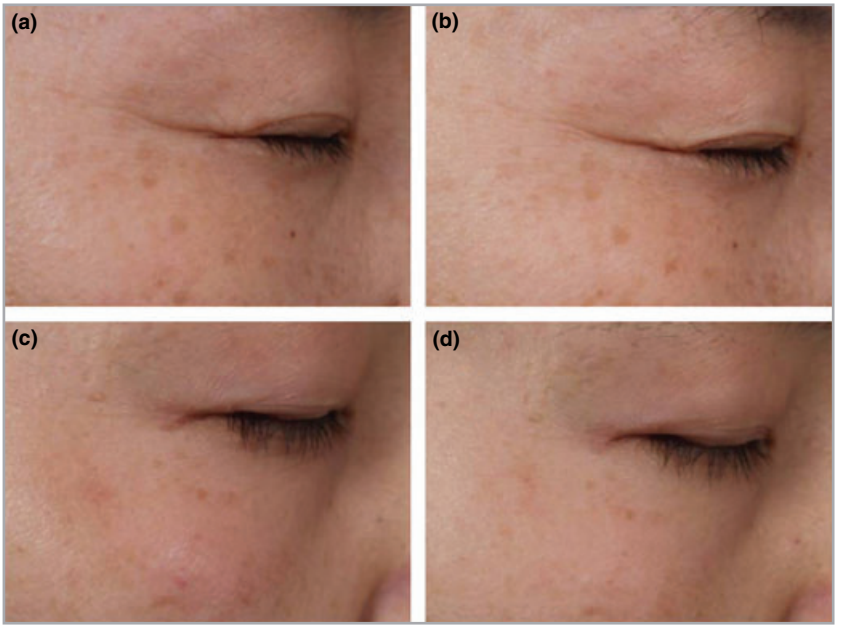

Fig 2. A patient with wrinkles before (a,c) and 12 weeks after (b,d) treatment with placebo (a,b) or retinyl retinoate (c,d).

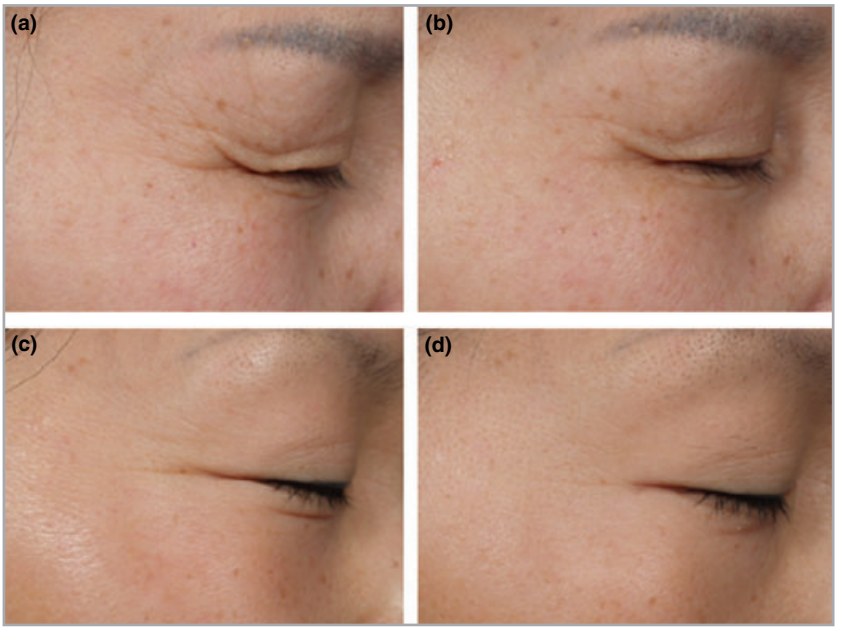

Fig 3. A patient with wrinkles before (a,c) and 8 weeks after (b,d) retinol (a,b) or retinyl retinoate (c,d) treatment.

improvements in wrinkles after 12 weeks (clinical study 1) and 8 weeks (clinical study 2) of retinyl retinoate treatment.

Image analysis using replicas and visiometers

A replica from the right and left periorbital areas was taken at weeks 0 (baseline), 4, 8 (clinical study 2), and 12 (clinical study 1) and analysed based on five parameters using the Visiometer and dedicated software. Figure 4 compares parameters at weeks 4 and 8 for retinol and retinyl retinoate in clinical study 2. Visiometer R-values R1 through R5 decrease as wrinkles diminish. At week 8, a statistically significant difference in the average roughness (R3), which is the average roughness in wrinkle formation, was observed in retinol-treated subjects ($P = 0{\cdot}005$). This significant difference in R3, however, was observed after 4 weeks with retinyl retinoate ($P = 0{\cdot}025$). This result, combined with the significant difference in the maximum roughness (R2) in retinyl retinoate after 8 weeks, indicated the faster and greater effect of retinyl retinoate on wrinkle improvement.

Discussion

Exposure of the human epidermis to ultraviolet (UV) irradiation leads to changes in the physiological and biochemical features of the skin. Photoaged skin is clinically characterized by

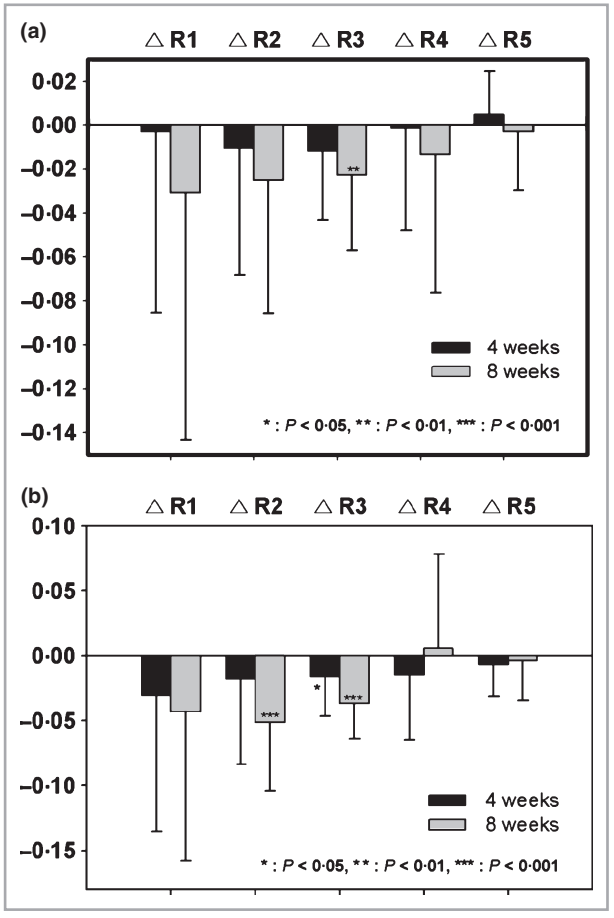

Fig 4. Changes in wrinkles were analysed by the Skin Visiometer SV 600 after 4 and 8 weeks of 0·075% retinol (a) and 0·06% retinyl retinoate (b) treatment. R1, skin roughness; R2, maximum roughness; R3, average roughness; R4, smoothness depth; and R5, arithmetic average roughness.

wrinkled, dry, inelastic and irregularly pigmented skin. These phenomena usually result from the increase of epidermal thickness, reduction in collagen in the dermis, and increased numbers of keratinizing cysts in the lower dermis. Topical retinoid derivatives can repair UV-damaged skin *in vivo*, leading to the effacement of wrinkles.[26]

Retinoic acid is the most bioactive form of the retinoids when topically applied to the skin, causing thinning of the stratum corneum, which leads to a smoother skin texture, and increased collagen content in photodamaged skin, which reduces fine wrinkles and increases skin tensile strength.[27] However, topical retinoic acids have a limited effect in the long-term treatment of ageing and photoageing due to side-effects such as irritation of the skin.[28] Although retinol was developed to reduce such side-effects, it is photounstable and has a lower efficacy than other retinoids. This photoinstability and skin irritation potential is a major drawback for its use in cosmetics. Retinyl retinoate was synthesized with a condensing reaction between retinol and retinoic acid to improve the photostability and biological activity. It has enhanced thermal stability and decreased photosensitivity, and exhibits decreased cell toxicity compared with retinol.[22]

The present study demonstrated a significant improvement in the treatment of fine photoageing-induced wrinkles using topical application of retinyl retinoate in women with facial photodamage. Clinical data based on subject and investigator assessment revealed a greater improvement in fine wrinkles using retinyl retinoate than with a placebo or retinol. In these clinical studies, the overall average photodamage scores significantly improved with retinyl retinoate treatment compared with treatment with placebo or retinol. These results indicated that the decrease of the photodamage scores with retinyl retinoate treatment was not caused by the individual variation, because retinyl retinoate was applied to one side of the face and a placebo or retinol was applied to the other side.

The main aim of this study was to evaluate the clinical effects of retinyl retinoate. We analysed images using replicas and visiometers, an objective technique to reproduce changes in photodamaged skin. The image analysis using replicas was performed at week 12 (clinical study 1) and week 8 (clinical study 2). The R2 and R3 parameters in the visometer image analysis measured maximum and average roughness in wrinkle formation, respectively. In clinical study 2, the statistically significant difference in R2 and R3 that was observed in retinyl retinoate indicated that the application of retinyl retinoate significantly improved the wrinkle. This analysis correlated well with the clinical findings. Moreover, the significant difference in R3 was observed after 4 weeks with retinyl retinoate, compared with the 8-week observation of retinol; this result indicated the faster effect of retinyl retinoate on wrinkle improvement.

In our previous *in vitro* biological activity study, the biological activity of retinyl retinoate was in between the properties of retinol and retinoic acid. Retinyl retinoate showed higher biological activity than that of retinol, such as RAR activity and collagen synthesis. Also, retinyl retinoate showed a similar side-effect to that of retinol, and not retinoic acid.[22] This suggested that the improvement of retinyl retinoate-treated wrinkles may be in between compared with improvement of retinol- and retinoic acid-treated wrinkles.

Moreover, retinyl retinoate is photostable, overcoming the main drawback of retinol. These findings imply that the hybrid retinoid, retinyl retinoate, has advantages of both retinol and retinoic acid, and has the potential to satisfy a new generation of topical retinoids. In this study, we showed that retinyl retinoate plays a more important role in the improvement of skin wrinkles than retinol, which is used in the cosmetics industry as an antiwrinkle agent. Thus, due to its excellent stability under severe and UV-irradiated conditions, retinyl retinoate may be conveniently used as an additive in cosmetics and medications to prevent and improve skin ageing and for the treatment of skin ailments such as acne and psoriasis.

Acknowledgments

This work was supported by a grant from Korea Health and Medical Technology R&D Project, Ministry of Health & Welfare (0405-CS00-0501-0010).

References

1 Green LJ, McCormick A, Weinstein GD. Photoaging and the skin: the effects of tretinoin. *Dermatol Clin* 1993; **11**:97–105.
2 Gilchrest BA. A review on skin ageing and its medical therapy. *Br J Dermatol* 1996; **135**:867–75.
3 Kang S, Fisher GJ, Voorhees JJ. Photoaging: pathogenesis, prevention, and treatment. *Clin Geriatr Med* 2001; **17**:643–59.
4 Fisher GJ, Wang ZQ, Datta SC *et al.* Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997; **337**:1419–29.
5 Kligman LH, Kligman AM. The nature of photoaging: its prevention and repair. *Photodermatol* 1986; **3**:215–17.
6 Weiss JS, Ellis CN, Headington JT, Voorhees JJ. Topical tretinoin in the treatment of aging skin. *J Am Acad Dermatol* 1988; **19** (1 Part 2):169–75.
7 Jessica HR, Adam JM, Patrick JS *et al.* Photoaging: mechanisms and repair. *J Am Acad Dermatol* 2006; **55**:1–19.
8 Chen S, Kiss I, Tramposch KM. Effects of all-*trans* retinoic acid on UVB-irradiated and non-irradiated hairless mouse skin. *J Invest Dermatol* 1992; **98**:248–54.
9 Bryce GF, Bogdan NJ, Brown CC. Retinoic acids promote the repair of the dermal damage and the effacement of wrinkles in the UVB irradiated hairless mouse. *J Invest Dermatol* 1988; **91**:175–80.
10 Kim BH, Lee YS, Kang KS. The mechanism of retinol-induced irritation and its application to anti-irritant development. *Toxicol Lett* 2003; **146**:65–73.
11 Kang S, Duell EA, Fisher GJ *et al.* Application of retinol to human skin *in vivo* induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. *J Invest Dermatol* 1995; **105**:549–56.
12 Nau H. Embryotoxicity and teratogenicity of topical retinoic acid. *Skin Pharmacol* 1993; **6** (Suppl. 1):35–44.
13 Mills OH Jr, Berger RS. Irritation potential of a new topical tretinoin formulation and a commercially-available tretinoin formulation as measured by patch testing in human subjects. *J Am Acad Dermatol* 1998; **38**:S11–16.
14 Chandraratna RA. Rational design of receptor-selective retinoids. *J Am Acad Dermatol* 1998; **39**:S124–8.
15 Guenther LC. Topical tazarotene therapy for psoriasis, acne vulgaris, and photoaging. *Skin Therapy Lett* 2002; **7**:1–4.
16 Dawson MI, Hobbs PD. The synthetic chemistry of retinoids. In: *The Retinoids: Biology, Chemistry, and Medicine* (Sporn MB, Roberts AB, Goodman DS, eds), 3rd edn. New York: Raven Press, 1994; 5–178.
17 Bernard BA. Adapalene, a new chemical entity with retinoid activity. *Skin Pharmacol* 1993; **6** (Suppl. 1):61–9.
18 Weinstein GD. Safety, efficacy and duration of therapeutic effect of tazarotene used in the treatment of plaque psoriasis. *Br J Dermatol* 1996; **135**:32–6.
19 Varani J, Warner RL, Gharaee-Kermani M *et al.* Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. *J Invest Dermatol* 2000; **114**:480–6.
20 Brisaert M, Plaizier-Vercammen J. Investigation on the photostability of a tretinoin lotion and stabilization with additives. *Int J Pharm* 2000; **199**:49–57.
21 Han HS, Kwon YJ, Park MS *et al.* Efficacy validation of synthesized retinol derivatives *in vitro*: stability, toxicity, and activity. *Bioorg Med Chem* 2003; **11**:3839–45.
22 Kim H, Kim B, Kim H *et al.* Synthesis and *in vitro* biological activity of retinyl retinoate, a novel hybrid retinoid derivative. *Bioorg Med Chem* 2008; **16**:6387–93.
23 Chung JC, Lee SH, Youn CS *et al.* Cutaneous photodamage in Koreans; influence of sex, sun exposure, smoking, and skin color. *Arch Dermatol* 2001; **137**:1043–51.
24 Paepe KDP, Lagarde JM, Gall Y *et al.* Microrelief of the skin using a light transmission method. *Arch Dermatol Res* 2000; **292**:500–10.
25 Grove GL, Grove MJ, Leyden JJ *et al.* Skin replica analysis of photodamaged skin after therapy with tretinoin emollient cream. *J Am Acad Dermatol* 1991; **25**:231–7.
26 Kambayashi H, Odake Y, Takada K *et al.* N-retinoyl-D-glucosamine, a new retinoic acid agonist, mediates topical retinoid efficacy with no irritation on photoaged skin. *Br J Dermatol* 2005; **153** (Suppl. 2):30–6.
27 Boswell CB. Skincare science: update on topical retinoids. *Aesthet Surg J* 2006; **26**:233–9.
28 Nau H. Embryotoxicity and tetrato-, genicity of topical retinoic acid. *Skin Pharmacol* 1993; **6** (Suppl. 1):35–44.