

ORIGINAL ARTICLE

# Evaluation of anti-wrinkle effects of a novel cosmetic containing retinol using the guideline of the Japan Cosmetic Industry Association

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## ABSTRACT

Retinol is known to be effective in the treatment of sallowness, wrinkling, red blotchiness and hyperpigmented spots in aging skin. In this study, we have evaluated the anti-wrinkle effects of a new cosmetic containing retinol. An open study was performed in 30 healthy Japanese women who had wrinkles at the corners of the eyes. The tested lotion, Retin-OX+ (RoC SA, Colombes, France), was applied on wrinkles of one side of the face for 8 weeks, and not on the other site as a control. Anti-wrinkle effects were evaluated by two methods: (i) doctors' observation and photos based on the guideline of the Japan Cosmetic Industry Association; and (ii) the Robo Skin Analyzer. This lotion showed marked and moderate improvement in 34% of the subjects with a significant difference as compared with the control sites ( $P < 0.05$ ). Moreover, the length and area decreased in the applied site more than the control site with a significant difference ( $P < 0.01$ ). All the patients completed the study without significant adverse reactions. The tested lotion was well tolerated and may be an optional preparation for the treatment of wrinkles at the corner of the eyes.

**Key words:** anti-wrinkle effects, cosmeceutical, photoaging, retinol.

## INTRODUCTION

Recently, numerous cosmeceutical products have been focused on various clinical signs on photoaging skin including dyspigmentation, fine wrinkles and roughness. Retinoids, vitamin C,  $\alpha$ -hydroxy acids, niacinamide and topical growth factors have been added to cosmeceuticals that could stimulate repair mechanisms resulting in improvements in photodamaged skin.<sup>1,2</sup> In retinoids, tretinoin and isotretinoin are used as therapeutic preparations, while retinol (vitamin A), retinaldehyde and retinyl esters are used as cosmeceuticals. These retinoids are expected to be helpful in: (i) renewing epidermal cells; (ii) acting as ultraviolet (UV) filters; (iii) preventing oxidative

stress; (iv) controlling cutaneous bacterial flora; and (v) improving skin aging and photoaging.<sup>3</sup>

In this study, we investigated anti-wrinkle effects of a novel cosmetic containing retinol in healthy Japanese women according to the guideline of the Japan Cosmetic Industry Association (JCIA).<sup>4</sup>

## METHODS

### Subjects

Thirty Japanese female subjects aged 35–59 years (mean, 50.4) participated in a split face study with left–right randomization that was carried out between April and July 2006. Subjects having fine wrinkles in the eye area were investigated. Recreational sun

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exposure was prohibited. Daily cosmetics that had continued before the test were permitted, while anti-wrinkle cosmetics, pharmaceuticals or aesthetic modalities were prohibited. Informed consent was obtained. There were no conflicts of interest.

### Testing substance

A novel cosmetic, Retin-OX+ (Roc SA, Colombes, France), was evaluated compared with a control without application. Retin-OX+ is an anti-wrinkle cream that contains retinol as a main constituent and "Collagen Ox". Collagen Ox is a mixture of magnesium asparaginate, zinc gluconate and copper gluconate. A dose of 0.3 g Retin-OX+ was applied for a half of the face twice a day after washing the face for 8 weeks.

### Evaluation of wrinkles in the eye area

First, evaluation was conducted by the combination of observation and photographs by two investigators (A. K. and N. K.) based on the guideline of the JCIA.<sup>4</sup> The evaluation was conducted based on the condition of the eyes when opened and closed. This guideline classifies conditions of wrinkles as 8 grades, 0–7. The physicians' overall assessments of the extent of improvement/worsening were graded as "worsened", "no change", "slight improvement", "moderate" and "marked" (Table 1).

For the second, the Robo Skin Analyzer CS50 (Inforward, Tokyo, Japan)<sup>5,6</sup> was used for the evaluation when the eyes were opened. The skin images were taken with a 3CCD digital video camera. This device was connected to a Windows-based personal computer system through a digital video unit and an image acquisition PC card. The software analysis in CS50 calculates the area of wrinkles and pigmentary lesions and measures brightness. Briefly described, blue signals are extracted from a digital image that is composed of red, green and blue signals. An

adaptive binarization process was performed to reveal pixels with weaker intensity. Wrinkles were defined as lines or lesions of which pixels varied to some degrees from surrounding pixels of wrinkle-free lesions in a captured image. For wrinkles, total area of wrinkles on the eye area ( $30 \times 30 \text{ mm}^2$ ) and maximum length of wrinkles from the corner of the eye were calculated.

## RESULTS

The combination of observations and photographs indicated clinical improvement of wrinkles of the tested substance Retin-OX+. Thirty-four percent of subjects showed marked or moderate improvement at 8 weeks in the tested site, whereas 16% did in the unapplied control site (Table 1). These effects were statistically significant ( $P < 0.005$ ). A representative case with marked response is shown in Figure 1.

Reduction rates of total area of wrinkles in CS50 at 8 weeks were 13% on the tested site and 6% in the control site with a significant difference ( $P < 0.01$ ). There was a significant difference ( $P < 0.01$ ) of reduction of maximum length of the wrinkles between the tested (12%) and control site (7%). A representative case with marked response in the digital image is shown in Figure 2.

All the subjects completed the 8-week study. Seven subjects complained of a burning sensation with faint erythema on the applied site, that disappeared in a few weeks without any medication.

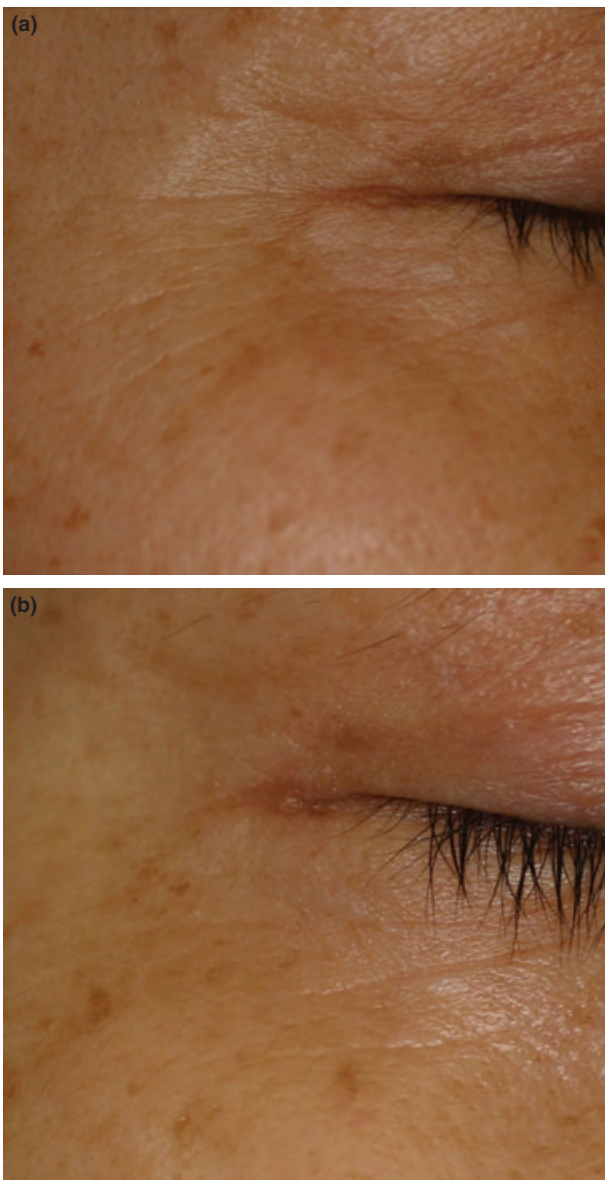
## DISCUSSION

This study revealed the efficacy for wrinkles and tolerability of a cosmetic containing retinol, Retin-OX+, in Japanese women. Overall, 34% of subjects showed marked or moderate improvement with the combination of observation and photographs. Retin-OX+ also showed reduction in both the total area and maximum length of wrinkles using computer analysis of the video-captured image. Significant improvements in the combination of observation and photographs and analysis of digital image based on the JCIA's guideline imply that Retin-OX+ has anti-wrinkle effects as a cosmetic. Retin-OX+ contains retinol as a main constituent and a mixture of magnesium asparaginate, zinc gluconate and copper gluconate. All of

**Table 1.** Overall ratings for the response of wrinkles

Rating	Tested site (%)	Control site (%)
Worsened (0 > change in grades)	0 (0)	0 (0)
No change (no change in grades)	17 (56)	22 (74)
Slight (0.5 > change in grades $\geq$ 0)	3 (10)	3 (10)
Moderate (1.5 > change in grades $\geq$ 0.5)	5 (17)	4 (13)
Marked (change in grades $\geq$ 1.5)	5 (17)	1 (3)

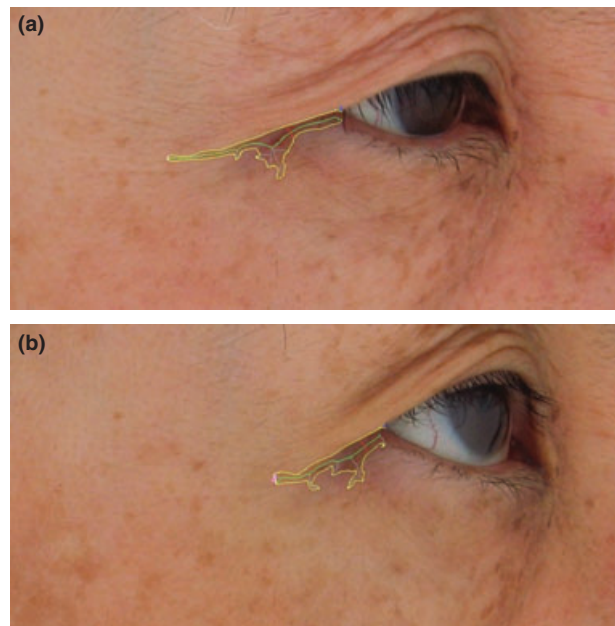
$P < 0.005$  compared with the control site in Wilcoxon's rank sum test.



**Figure 1.** A 52-year-old woman (a) before and (b) 8 weeks after the treatment. Marked improvement was seen.

these constituents may have contributed to the improvement. The combination of observations, photographs and CS50 analysis showed some improvements in the unapplied control site. Protection against UV may affect this improvement because recreational sun exposure was prohibited in this study.

Although retinol is contained in many cosmetics, few studies have demonstrated clinical improvement for aging and photoaging skin. Topical retinol has



**Figure 2.** A 58-year-old woman (a) before and (b) 8 weeks after the treatment. Total area of wrinkles in the eye area and maximum length of wrinkles from the corner of the eye reduced.

been reported to improve fine wrinkles associated with photoaging or natural aging for the skin of white subjects.<sup>7</sup> Our study demonstrated reduction of wrinkles with a cosmetic containing retinol in Japanese female skin. Thus, topical retinol may have anti-wrinkle effects both on white and Mongoloid skin. Retinoic acid (tretinoin), widely used for topical therapy of several skin diseases, improves aging skin with significant biological activity and induces irritation of skin.<sup>3</sup> Tretinoin causes severe irritation of Japanese photodamaged skin and is not officially approved in Japan. Therefore, topical retinol with low irritative potential may be more appropriate than tretinoin for Japanese skin.

In human keratinocytes and mouse skin, retinoic acid is thought to be formed enzymatically from all-*trans*-retinol by a two-step oxidation process in which retinal is the intermediate metabolite.<sup>8-10</sup> When applied to human skin, retinol penetrates and is sequentially oxidized to retinoic acid, enhancing expression of cellular retinoid binding protein mRNA and causing some effects of retinoic acid.<sup>11</sup> The ranking order of retinoid-like activity following topical application is as follows: retinoic acid > retinaldehyde > retinol >> retinyl esters.<sup>3</sup> Similar

mechanisms to retinoic acid<sup>12</sup> may be involved in the improvement of wrinkles with topical retinol. First, increase in collagen production with retinol may cause wrinkle reduction. Topical retinol increases fibroblast growth and collagen synthesis, and concomitantly reduces the levels of collagen-degrading matrix metalloproteinases in naturally aged human skin.<sup>13</sup> Topical retinol also increases protein expression of type I procollagen in chronologically aged skin.<sup>7</sup> These findings suggest that retinol could stimulate fibroblast growth, collagen production and reduce degradation of collagen, leading to the improvement of wrinkles. Second, retinol induces epidermal hyperplasia<sup>11,14</sup> and increase in epidermal glycosaminoglycan skin<sup>7</sup> that has the capacity to bind water. We hypothesized that epidermal thickening and increase of glycosaminoglycan may lead to the improvement of roughness and fine lines.

In conclusion, our study indicated that the topical retinol-containing cosmetic reduced wrinkles in the eye area of Japanese women and was well tolerated. Further study is needed to reveal the precise mechanism of anti-wrinkle effects of retinol.

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