

**CLINICAL IMPROVEMENT OF SKIN AGING BY RETINOL CONTAINING
PRODUCTS : WITH NON-INVASIVE METHODS**

Sun B. K., Lee H. K., Cho J. C., Kim J. I.
Pacific R&D Center, Kyounggi-do, Korea

1. SUMMARY

Retinol as well as RA (retinoic acid) is well known to have many beneficial effects on (photo)aged skin. But the skin irritation potential and unstable condition of the products containing them have been some problems in their cosmetic uses. So, retinol containing gel product (MDC gel) was developed for less skin irritancy and more stability in cosmetic products.

To examine the clinical effects of retinol containing product, we used clinical non-invasive assessment techniques on 40 volunteers for 6 months maintaining double-blind test conditions.

According to our results, the use of retinol containing product improved skin color and hydration level slightly. But there was no statistical differences. There was no erythema reaction compared to the use of RA. Especially, the skin elasticity increased above 20% and skin wrinkles of crows' feet region decreased more than 10%. Besides the instrumental analysis, a large majority of volunteers felt that their skin were improved in the case of wrinkles, elasticity, hydration and color.

From these study, we suggest retinol for a new anti-aging material with safety and real effects.

2. INTRODUCTION

For many years, several well performed studies have been done. They reported that treatment with different concentrations of RA (retinoic acid) have significant effects on the aging symptoms of the skin, especially on the photoaging changes.

However, a main problem of RA treatment is the side-effects such as erythema after prolonged use. The skin tolerability of retinol, on the other side, is excellent and the use of retinol shows the same effects on aged skin such as CRABP II induction, increase of collagen synthesis and epidermal thickness.

Because retinol is unstable under the condition of cosmetic products, we stabilized retinol by use of MDC (matrix double capsule) gel.

In this study, we examined the potency of retinol on skin parameters such as skin hydration, color, elasticity and wrinkles by using non-invasive methods to know if retinol can be an excellent cosmetic active ingredient to improve aged skin with safety.

3. Materials and Methods

The aim of the study was to investigate a mid-time treatment(6 months) with retinol had beneficial effect on skin parameters such as skin elasticity, skin wrinkle and visual skin quality.

To examine the effect of our retinol containing product, we used 2 test products : A is the base lotion as vehicle and B is the base lotion with MDC gel containing 2500 IU/g retinol.

Older women above 40 years with moderate or severe wrinkles on the face were selected as volunteers. None of 40 volunteers had a dermatological problem or used any topical ointment within the past 2 months.

Before using the test products, the initial condition of volunteers were measured as the control of our study. All the measurements of our study were performed in a environmentally controlled room temperature (22-26 centigrade) and relative humidity(35-45%).

The measuring sites are crows' feet region (eye region) and 5cm perpendicular point from crow's feet region on cheek (cheek region). A computer-generated random number list was used to assign volunteers to one of two treatment groups. After assigning, volunteers used their test product once a day at every night before sleeping for 6 months. During 6 months application, we measured the volunteer's skin condition on 1,2,4,6 months after starting application at the same controlled conditions.

The improvements of skin condition were evaluated for 4 aspects: skin hydration, skin color, skin biomechanical properties and wrinkles. Used instruments are Comeometer CM 820(Courage + Khazaka (C+K), Germany) for hydration, Cutometer SEM 474 (C+K, Germany) for skin biomechanical properties, Chromameter CM1000(Minolta, Japan) for skin color and Skin Visiometer SV 400 (C+K, Germany) for wrinkles. All the measured data were processed as % change ((value at measuring point - value at initial point) / value at initial point x 100 (%)) and the statistical significancy of the two test groups was verified by paired t-test as the significant level $p < 0.05$.

4. RESULTS

4.1. Skin hydration

In skin hydration, all measurements were done in triplicate and the average values were used for statistical evaluation. Both in cheek region and eye region, there was no significant difference between group A(base lotion) and group B(base lotion + retinol) on all the measuring points.

Table 1. % changes of skin hydration on eye region(mean±SD)

	1 month	2 month	4 month	6 month
A group	4.7±12.4	5.4±11.1	7.3±10.7	1.7±8.8
B group	6.2±8.9	2.5±7.6	3.6±9.7	3.2±11.1

4.2. Skin color

In skin color, we investigated 3 parameters (L, a, b) and all measurement were done in triplicate and the average values were done in triplicate and the average values were used for statistical evaluation. Both in cheek and eye region, there was no significant difference between two groups for all 3 parameters on each measuring points. Especially, because there was no significant change on a value, our retinol containing test product didn't induce erythema as a side effect.

Table 2. % changes of skin color(a) on eye region(mean±SD)

	1 month	2 month	4 month	6 month
A group	-5.5±7.6	-4.3±7.1	-3.1±6.6	-3.4±9.6
B group	-4.7±4.5	1.7±9.3	-2.0±9.5	-14.1±31.3

4.3. Skin biomechanical properties

The instrumental operating conditions were as follows :

- 1) suction pressure : 500 mbars
- 2) on time : 2 sec, off time : 2 sec
- 3) 3 times repetition

From the cutometric data analysis, we investigated elastic ratio(Ur/Ue) and elastic recovery rate(Ur/Uf) in priority. Our results showed that elastic ratio and elastic recovery rate value of B group increased statistically against A group on both cheek and eye region.

The increase came out within 2 months after use of test product and this effect was maintained to the end point of our study.

Table 3. % changes of elastic ratio(Ur/Ue)

Site	Group	1 month	2 month	4 month	6 month
Cheek	A group	3.0±16.6	14.8±13.3*	6.2±18.7*	-6.9±14.8*
	B group	28.1±20.9	29.3±29.3*	34.4±20.2*	35.8±29.3*
Eye	A group	5.9±14.7*	4.2±12.4*	17.3±13.9*	8.8±16.1*
	B group	59.6±24.9*	46.4±25.9*	61.4±30.7*	48.8±50.6*

* : Significant difference with paired t-test(p<0.05)

Table 4. % changes of elastic recovery(Ur/Uf)

Site	Group	1 month	2 month	4 month	6 month
Cheek	A group	0.7±11.5	9.8±13.8*	3.6±16.9	-1.9±12.1
	B group	22.9±23.6	46.6±24.5*	25.4±18.6	27.2±24.1
Eye	A group	6.4±12.2*	8.8±10.5*	19.7±13.7*	15.6±17.0
	B group	48.5±24.8*	36.4±20.9*	51.7±26.5*	41.1±23.4

* : Significant difference with paired t-test(p<0.05)

4.4. Skin wrinkle

In skin wrinkle measurement, only the replicas of volunteers' crows' feet region(eye region) were made. From the Visiometer SV 400 image analysis program, many skin wrinkle parameters were calculated, and among these, mean peak to valley height value(Rz) of group B was decreased significantly against group A. Although maximum peak to valley height(Rt) and arithmetic mean roughness value(Ra) were not statistically different, these values of group B showed the tendency of decrease as compared with group A.

Table 5. % changes of mean peak to valley height value(Rz) on eye region

	1 month	2 month	4 month	6 month
A group	-1.4*8.3	-2.2*8.7	-1.6*6.8*	-7.1*9.3
B group	-6.1*5.6	-9.9*5.7	-14.4*6.6*	-10.6*7.7

	1 month	2 month	4 month	6 month
A group	-1.4±8.3	-2.2±8.7	-1.6±6.8*	-7.1±9.3
B group	-6.1±5.6	-9.9±5.7	-14.4±6.6*	-10.6±7.7

* : Significant difference with paired t-test(p<0.05)

5. Discussion

The result from the present study shows that some of the significant changes obtained by a 6 month treatment with an active(MDC capsulated) retinol containing product, in skin elasticity and wrinkles. The skin elasticity increased above 20% and skin wrinkles of crows' feet region decreased more than 10%. We were not able to see erythema reaction or any other side effect and all the volunteers were well tolerated during test period.

We thought that this well-tolerance might be important advantage to RA because the side effect of

RA such as erythema was the problem to use this material in cosmetic fields.

Besides the instrumental analysis results, a large majority of volunteers felt that their skin was softer and wrinkles (especially around eye region) were reduced (data were not shown in this paper).

From the above results and other reports, we propose that the effects of retinol such as the increase of elasticity and the decrease of skin wrinkle should be due to the increase of new collagen synthesis mainly.

After obtaining the effect of retinol during a 6 months treatments, we anticipate from these data that further studies should be performed showing the optimum treatment time for reaching the maximum effect on skin wrinkle. Also, we already started another study that how long the anti-aging effect of retinol would be continued after stop of use.

In conclusion, we suggest that the use of retinol as an active ingredients for anti-aging may be sufficient to satisfy the needs of customers and cosmetic scientists.

REFERENCES

1. Retinoid induction of CRABP II mRNA in human dermal fibroblasts : Use as a retinoid bioassay. James T. Elder et al., *J Invest Dermatol.*, 106 : pp 517-521, 1996
2. Retinoic acid isomers applied to human skin in vivo each induce a 4-hydroxylase that inactivates only trans retinoic acid. Elizabeth A. Duell et al., *J Invest Dermatol.*, 106 : pp 316-320, 1996
3. Stability of all-trans-retinol in cream. T. Tsunoda et al., *J. Soc. Cosmet. Chem.*, 46, pp 191-198, 1995
4. All-trans retinoic acid induces cellular retinol-binding protein in human skin in vivo. Gary J. Fisher et al., *J Invest Dermatol.*, 105 : pp 80-86, 1995
5. Long-term effects after topical application of active retinyl palmitate. Erling Thom, *J. Appl. Cosmetol.*, 12, pp 25-30, 1994
6. Retinoids and photodamage. B. A. Gilchrest, *British Journal of Dermatology*, 127, pp 14-20, 1992
7. Effects of all-trans retinoic acid on UVB-irradiated and non-irradiated hairless mouse skin. Simon Chen et al., *J Invest Dermatol.*, 98 : pp 248-254, 1992
8. Clinical panel assessment of photodamaged skin treated with isotretinoin using photographs. Robert B. Armstrong et al., *Arch Dermatol.*, 128, pp 352-356, 1992
9. Liazole inhibits human epidermal retinoic acid 4-hydroxylase activity and differentially augments human skin responses to retinoic acid and retinol in vivo. Sewon Kang et al., *J Invest Dermatol.*, 107 : pp 183-187, 1996
10. CD 23-mediated nitric oxide synthase pathway induction in human keratinocytes is inhibited by retinoic acid derivatives. Pierre-Andre Becherel et al., *J Invest Dermatol.*, 106 : pp 1182-1186, 1996
11. Retinoids can be classified according to their effects on vitamin A metabolism in HeLa cells. Eva Stenstrom et al., *Skin Pharmacology*, 9 : pp 27-34, 1996
12. Hyaluronic acid and dermatan sulfate are selectively stimulated by retinoic acid in irradiated and nonirradiated hairless mouse skin. Dominique Margelin et al., *J Invest Dermatol.*, 106 : pp 505-509, 1996
13. Biological activity of all-trans retinol requires metabolic conversion to all-trans retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. Sara B. Kurlandsky et al., *J Biol. Chem.*, 269, 52, pp 32821-33827, 1994
14. An in vivo experimental model for effects of topical retinoic acid in human skin. C. E. M. Griffiths et al., *Br. J. Dermatol.*, 129 : pp 389-394, 1993

15. Mechanisms of action of retinoic acid in skin repair. C. E. M. Griffiths et al., *Br. J. Dermatol.*, 127 : pp 21-24, 1992
16. Topical all-trans retinoic acid stimulates collagen synthesis in vivo. Elaine Schwartz et al., *J Invest Dermatol.*, 96 : pp 975-978, 1991
17. Changes of photo-aged human skin following topical application of all-trans retinoic acid. Dean S. Rosenthal et al., *J Invest Dermatol.*, 95 : pp 510-515, 1990

Skin Hydration

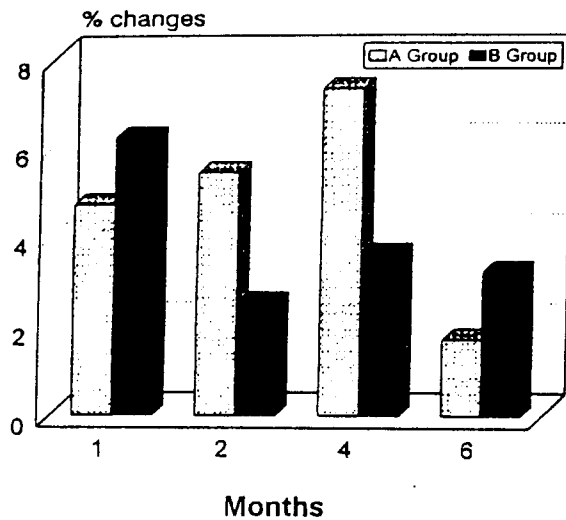


Fig 1. % changes of skin hydration on eye region

Skin Color (a)

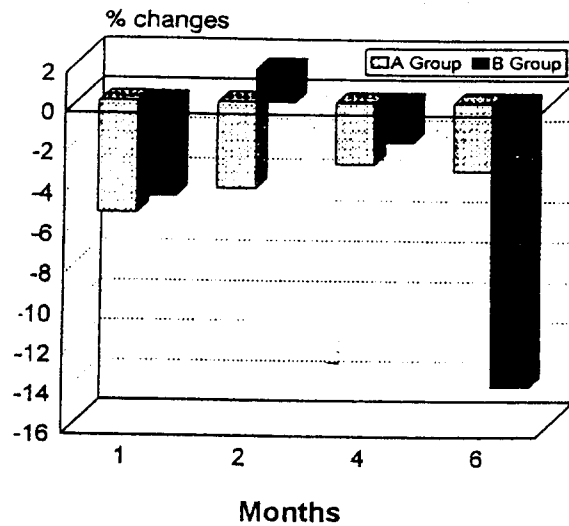


Fig 2. % changes of skin color (a) on eye region

Skin elastic ratio (Ur/Ue)

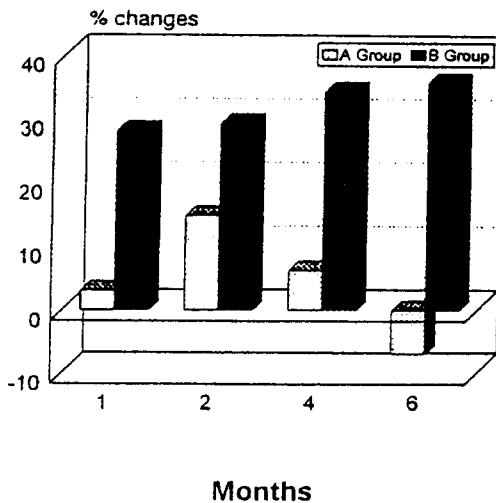


Fig 3. % changes of skin elastic ratio on cheek region

Skin elastic ratio (Ur/Ue)

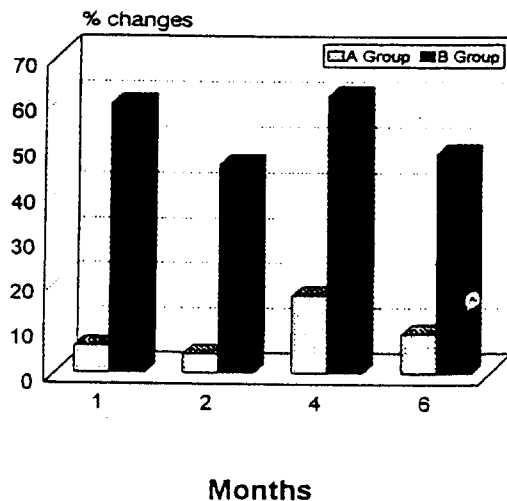
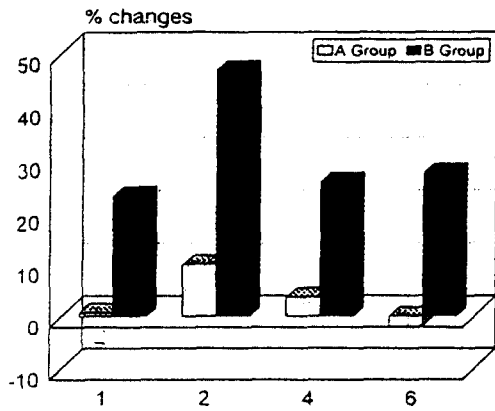


Fig 4. % changes of skin elastic ratio on eye region

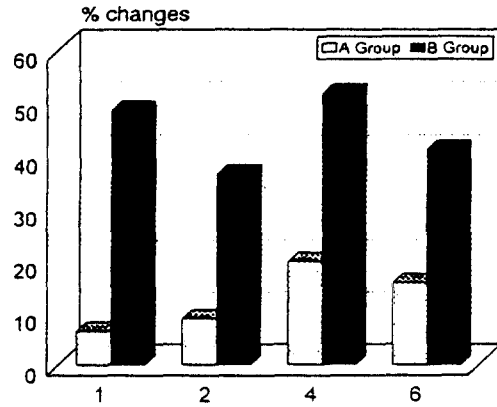
Skin elastic recovery (Ur/Uf)



Months

Fig 5. % changes of skinelastic recovery on cheek region

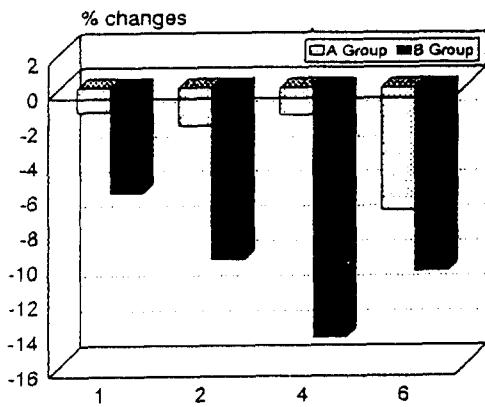
Skin elastic recovery (Ur/Uf)



Months

Fig 6. % changes of skinelastic recovery on eye region

Skin wrinkle (Rz)



Months

Fig 7. % changes of mean peak to valley height value (Rz) on eye region