The effects of succinylated atelocollagen and adenosine on periorbital wrinkles

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The effects of succinylated atelocollagen and adenosine on periorbital wrinkles

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ABSTRACT

The effects of succinylated atelocollagen and adenosine on periorbital wrinkles

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Degradation of structural collagen and reduced collagen synthesis contribute to the characteristic appearance of wrinkles. Many ingredients have been studied about the effects on periorbital wrinkles, but the potential anti-wrinkle effects of topical applications of collagen have not been investigated. In this study, we evaluated the effects of topical application of succinylated atelocollagen on periorbital wrinkles and compared the results to the results of treatment with adenosine, which is an approved anti-wrinkle ingredient. In all participants, right or left periorbital area of each their face was applied with either a

solution containing succinylated atelocollagen and adenosine, or a solution containing only succinylated atelocollagen for two months. A placebo solution was applied to the other side of periorbital area of each patient's face for two months. Clinical improvement was evaluated by two dermatologists and also by subjects themselves using a five-point grading scale. Silicone casts of periorbital wrinkles were used to assess changes of periorbital wrinkles. Skin biopsies were done in eight volunteers before treatment and after two months of treatment. Based on objective and subjective measurements of clinical improvement, the assessment scores of treated sites were statistically significant higher than those of placebo sites after two months of treatment. Analysis of silicone casts of periorbital wrinkles showed partial effects of succinylated atelocollagen on periorbital wrinkles. However, we did not observe any effects of adenosine on periorbital wrinkles. In conclusion, succinylated atelocollagen may be an effective treatment option for periorbital wrinkles, but further study including a longer treatment period and larger subject group are needed to verify these results.

Key words: adenosine, succinylated atelocollagen, periorbital wrinkles

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I. INTRODUCTION

Skin aging is expressed by symptoms such as changes in skin thickness, color, elasticity, vascular dilatation and wrinkles. Epidermal atrophy and the degeneration of dermal collagen and elastic fibers lead to the formation of wrinkles and fine lines. Due to the movements of mimetic muscles and remodeling of bony tissues, deep wrinkles occur and become more prominent with the aging process[1]. Sunlight, smoking and pollutants increase the production of collagenase, an enzyme that breaks down collagen through a signal transduction cascade, resulting in the acceleration of wrinkle formation[2].

In the field of dermatology, there is an ever-increasing demand for medications and cosmetics that prevent or treat wrinkles and that are effective, convenient, and have few side effects.

There are ongoing studies of agents that may be effective for treating and preventing wrinkles. Retinoids, vitamin C and adenosine are considered especially effective[1]. Several anti-oxidants, including vitamin E, ferulic acid, co-enzyme Q10, idebenone, green tea, silymarin and pycnogenol, are incorporated into some skin care products[1]. Asiaticoside, phosphatidylserine, ginseng saponins isolated from red ginseng, and K6PC-5, a novel sphingosine kinase activator, are under survey, having demonstrated antioxidative effects and wrinkle improvement[3-5]. However, the potential anti-wrinkle effects of topical applications of collagen, a component of the dermis, have not been investigated.

Collagen is a fundamental component of the dermis, and type I collagen makes up 80% of the collagen in the skin[6]. As aging progresses, fragmented type I collagen fibrils become prominent and impair the mechanical properties of the skin, leading to wrinkle formation[7]. Therefore, supplying collagen, the major component of the skin's extracellular substrate, may constitute an effective fundamental treatment for wrinkles. However, collagen is insoluble in neutral solution and has a large molecular weight (300kDa) so it is not sure

that collagen penetrates into the skin. In addition, when collagen is kept at room temperature, it becomes denatured and loses its triple helix structure. Because of these limitations to the topical application of collagen, direct injections of small collagen particles dispersed in distilled water into the skin are often used by dermatologists.

Among the succinylated atelocollagen used in this research, the telopeptides on both ends, which causes immune reactions, was eliminated to minimize the immune reactions in human body. Furthermore, it was treated with succinic anhydride to modify the surface form as electrically anionic status, to improve the solubility in water or neutral solution. We performed a double blind, randomized, prospective, split-face clinical study on Korean participants with periorbital wrinkles to evaluate the effects of topical application of succinylated atelocollagen on periorbital wrinkles and compared the results to the results of treatment with adenosine, which is an approved anti-wrinkle ingredient.

II. PATIENTS AND METHODS

1. Participants

Thirty-two female participants (age range 32-59 years) with periorbital wrinkles were enrolled in a double-blind, randomized, prospective, split-face clinical study. The participants were stratified based on a baseline facial grading scale, ranging from 0 to 4[8]. Exclusion criteria included history of keloid scarring or other skin diseases, any laser procedures or isotretinoin use within six months of study initiation, pregnancy, or systemic diseases that are known to affect metabolism. Patients graded as 0 or 1 on the facial grading scale were also excluded. The study protocol was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea and informed consent was obtained from each participant.

2. Treatments

In all subjects, one side of each participant's face (the right or left periorbital area) was randomly assigned to be treated with either a solution containing succinylated atelocollagen and adenosine (sodium hyaluronate, L-lysine, succinylated atelocollagen, adenosine) (Group A), or a solution containing

only succinylated atelocollagen (sodium hyaluronate, L-lysine, succinylated atelocollagen) (Group B). A placebo (sodium hyaluronate, L-lysine) solution (Group P) was applied to the other side of each patient's face (Table 1).

Ingredients (gm/30ml)	Group A (Collagen + adenosine)	Group B (Collagen)	Group P (Placebo)
Sodium hyaluronate	0.12	0.12	0.12
L-lysine	0.00006	0.00006	0.00006
Succinylated atelocollagen	0.12	0.12	-
Adenosine	0.012	-	-

Table 1. Ingredients of the test and placebo solutions.

All subjects were instructed to apply the treatment and placebo solutions twice a day for two months on each side of the face in the periorbital area. They were also instructed not to apply any other topical creams or solutions during the study period. Each subject was assessed once every month during the duration of the study. Skin biopsies were obtained from eight subjects, four from each group, for histologic analyses of both sides of the periorbital area before and after the two-month-treatment period.

3. Assessment of clinical effects

Before treatment, and after one and two months of treatment, digital

photographs were taken of each patient using identical camera settings and lighting conditions. Two dermatologists evaluated the subjects' clinical improvements by observing these photographs in a blind fashion, and the participants themselves subjectively evaluated their clinical improvements at the same time points. They followed a five-point grading scale: 0 = no improvement, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, 4 = 76%-100% improvement.

We used silicone casts (Replica^{TM}, Cuderm Inc., Dallas, TX, USA) of the participants' periorbital wrinkles to assess skin texture before and after the two-month treatment period. An adhesive ring locator was placed against each periorbital area and filled with pre-mixed resin so that the resin overflowed 2-3mm onto the cardboard surface. After the resin set and the cast was peeled from the skin surface, the foam adhesive spacer layer was separated from the cardboard frame and discarded. The casts were labeled with the date, initials and group and stored in a paper envelope until analysis. For analysis, a collimated light source was directed at a 25˚ angle from the plane of the cast surface. Each cast was placed in a holder that fixed the direction of the tab position of the cast (Figure 1).

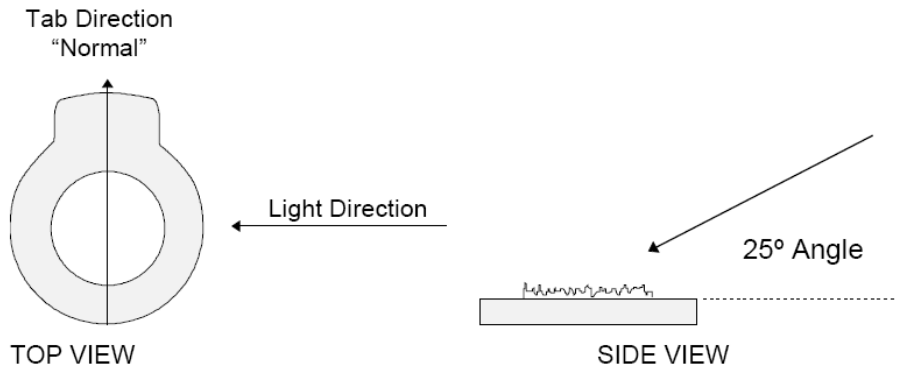

Figure 1. Top and side views of silicone cast used to assess skin texture.

The textures of shadows produced by oblique lighting of the negative casts were analyzed via two assay methods:

A. Measuring the luminance along a set of 10 equal-length parallel lines (passes) running across the cast parallel to the lighting direction. The variations in luminance were treated as indicators of surface roughness.

(A) Rz - the average maximum difference in the luminance value for five equal-length segments in each of the 10 lines traversing the sample.

(B) Ra - the average deviation of the luminance curve about the mean luminance for the same 10 lines.

The "R" parameters are reported in units of brightness (Gray levels) ranging from 0 to 255 and increase with increasing roughness.

(C) FNum - number markers indicative of fine and coarse lines per mm.

(D) IDL - the integrated developed length of the luminance traces of the 10 scan lines. The IDL is the total length of the luminance lines as a proportion of

the straight-line distance and it increases with the roughness of the surface.

B. The cast image area was divided into 10 equal width bands or sub-areas. Shadow-like features were detected in each of these bands based on their luminance values being less than the detection threshold. Four parameters were determined from the detected features.

(A) Spacing - the mean distance in millimeters between adjacent detected features. This parameter increases with disappearance of wrinkles.

(B) Breadth - the average breadth of the detected features in millimeters. This parameter is proportional to the depth of the wrinkle producing the shadow and decreases as wrinkles become shallower.

(C) Shadows - percent of the sampled replica area with luminance values less than the detection threshold. This is the relative area of shadow cast by the wrinkles in the cast, and shadows decrease with smoothing of the skin.

(D) NumWr - the total number of shadowy features detected in the 10 bands or sub-areas. This parameter decreases with smoothing of the skin.

4. Assessment of histologic changes

Skin biopsies were taken from eight volunteers before treatment and after the two-month-treatment period using a 3 mm biopsy punch. At the first visit, specimens were obtained from crow's feet at a 1.5cm distance from the lateral

margin of both eyes. The biopsies performed after the two-month-treatment period was taken right next to the previous biopsy sites. The specimens were fixed in formalin and embedded in paraffin. Skin samples were stained with hematoxylin and eosin to measure the epidermal thickness, Masson trichrome to evaluate changes in dermal collagen, monoclonal anti-human procollagen type I antibody (Abcam Inc., Cambridge, MA, USA) to demonstrate changes in collagen formation and monoclonal anti-human Ki-67 antibody (Abcam Inc.) to evaluate changes in cellular proliferation in the skin. Images of each section were taken at a magnification of x200 with a 12.5 megapixel digital camera (DP70, Olympus Optical Co., Tokyo, Japan) connected to a light microscope (BX40, Olympus Optical Co.). The images were analyzed by MetaMorph (Molecular Devices, Sunnyvale, CA, USA).

5. Statistical analysis

Statistical analysis was performed using the Wilcoxon signed-rank and rank-sum tests for evaluation of objective and subjective improvement scores and histological changes. One sample t-test and an independent group t-test were used to evaluate changes in the casts. P-values less than 0.05 were considered statistically significant, and P-values less than 0.10 were considered directionally significant.

III. RESULTS

Twenty eight of thirty two participants completed the 2-month study. All four dropped patients were lost due to personal causes without having any side effects.

1. Degree of clinical improvement

The mean clinical improvement scores measured by dermatologists at the one-month treatment assessment were 1.53±0.64, 1.08±0.86 and 0.57±0.50 in groups A, B and P, respectively. The mean clinical improvement scores at the two-month treatment assessment were 2.27±0.96, 2.31±1.25 and 0.96±0.74 in groups A, B and P, respectively (Figure 2). A statistically significant difference in clinical improvement was observed between groups A and P ($P=0.0005$), but we did not observe a statistically significant difference between groups B and P at the one-month treatment assessment ($P > 0.05$). Statistically significant differences were observed between groups A and P, and between groups B and P at the two-month treatment assessment ($P=0.0005$, $P=0.0039$). There were no statistically significant differences between groups A and B at the one-month or two-month treatment assessments ($P > 0.05$).

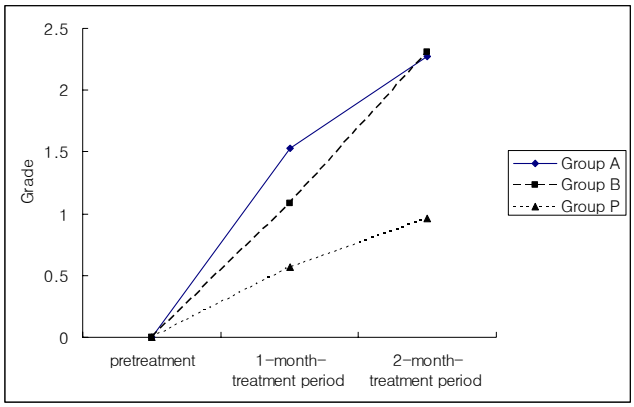

Figure 2. Mean clinical improvement scores assessed by physicians (grading scale: 0 = no improvement, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, 4 = 76%-100% improvement).

The clinical improvement scores assessed by the physicians and by participants were similar (Figure 3). However, at the one-month treatment assessment, no statistically significant differences were observed among the three groups ($P > 0.05$) by patients. At the two-month treatment assessment, participant evaluations were significantly different between groups A and P ($P=0.01$), and between groups B and P ($P=0.001$). There was no significant difference between groups A and B at the two-month treatment assessment ($P > 0.05$). Photographs of representative subjects in the three groups can be seen in Figure 4.

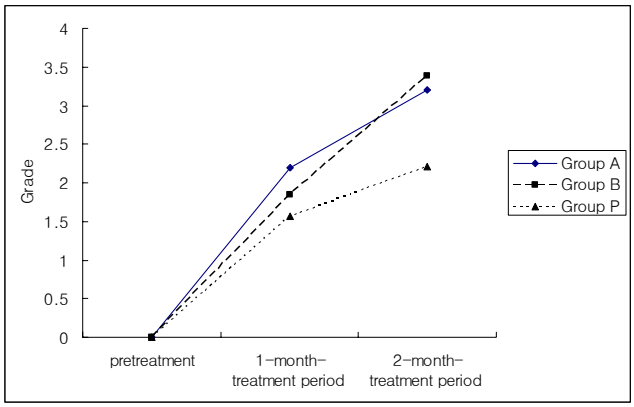

Figure 3. Mean clinical improvement scores assessed by participants (grading scale: 0 = no improvement, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, 4 = 76%-100% improvement).

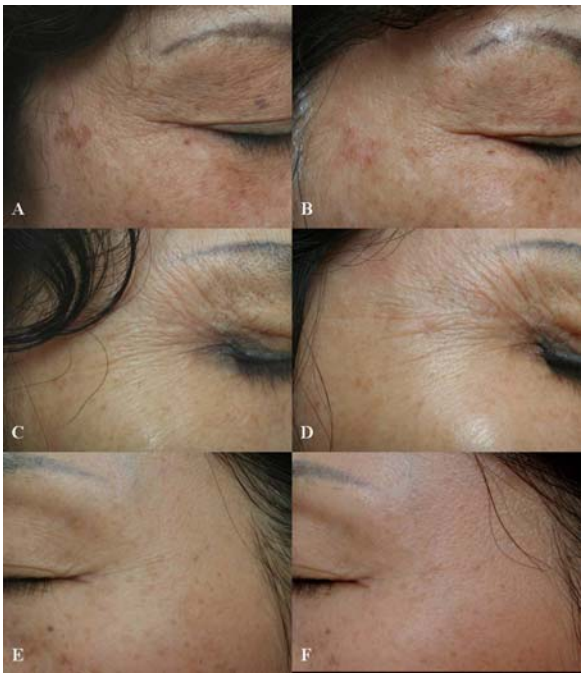

Figure 4. Comparison of clinical improvement of periorbital wrinkles at baseline and after two months of treatment. Before treatment (A) and after two months of treatment (B) in subject group A. Before treatment (C) and after two months of treatment (D) in subject group B. Before treatment (E) and after two months of treatment (F) in subject group P.

2. Cast image analysis

A total of 112 casts were evaluated. The casts represent treated sites for groups A and B, and placebo sites for group P at two visit times: baseline (BL) and after the two-month treatment period (V2). The results are summarized in Table 2.

	Group A		Group B		Group P	
	BL	V2	BL	V2	BL	V2
Rz	128.7	109.6	139.5	113.0	144.7	126.8
Ra	24.5	19.7	26.9	20.5	27.9	24.3
FNum	0.476	0.439	0.399	0.386	0.469	0.469
IDL	6.95	5.48	6.84	5.28	6.93	5.88
Spacing	1.948	1.946	1.840	2.239	1.389	2.005
Breadth	0.188	0.198	0.247	0.206	0.222	0.216
Shadows	4.2	3.1	5.4	2.7	5.8	4.4
NumWr	71.5	57.3	73.7	46.4	83.3	72.1

Table 2. The results of cast image analysis at baseline (BL) and after two months of treatment (V2). (Group A; succinylated atelocollagen+adenosine, group B; succinylated atelocollagen, group P; placebo.)

Changes from baseline were calculated by subtracting each subject's BL values from the appropriate V2 values. Table 3 summarizes the mean changes from baseline for groups A, B and P. Changes from baseline were significant for three measured parameters in Group A and six parameters in Group B. The changes for both groups represented improvements in texture (increased smoothness), with decreases from baseline for Rz, Ra and IDL (both groups A

and B), Breadth, Shadows and NumWr (Group B only). In group P, changes from baseline were significant for five measured parameters. The changes represented improvements in texture: decreases from baseline for Rz, Ra, IDL and Shadows parameters, with an increase in the Spacing parameter. Cast images of representative subjects from the three groups can be seen in Figure 5.

	Group A		Group B		Group P	
	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
Rz	-19.1±40.5	0.0893	-26.5±37.1	0.024	-17.8±27.7	0.0021
Ra	-4.8±8.4	0.0452	-6.4±8.2	0.0164	-3.6±6.5	0.0063
FNum	-0.038±0.096	0.1502	-0.014±0.117	0.685	0.001±0.089	0.9783
IDL	-1.47±2.80	0.0624	-1.56±2.82	0.0682	-1.04±1.59	0.0017
Spacing	-0.002±1.665	0.9960	0.372±1.768	0.4812	0.623±1.193	0.0117
Breadth	0.01±0.056	0.4980	-0.041±0.059	0.0261	-0.006±0.045	0.5047
Shadows	-1.1±4.0	0.2882	-2.7±3.3	0.0124	-1.4±3.2	0.0286
NumWr	-14.1±57.2	0.3547	-27.3±48.2	0.0638	-11.2±42.0	0.1705

Table 3. Mean changes from baseline in groups A, B and P. (Group A; succinylated atelocollagen+adenosine, group B; succinylated atelocollagen, group P; placebo.)

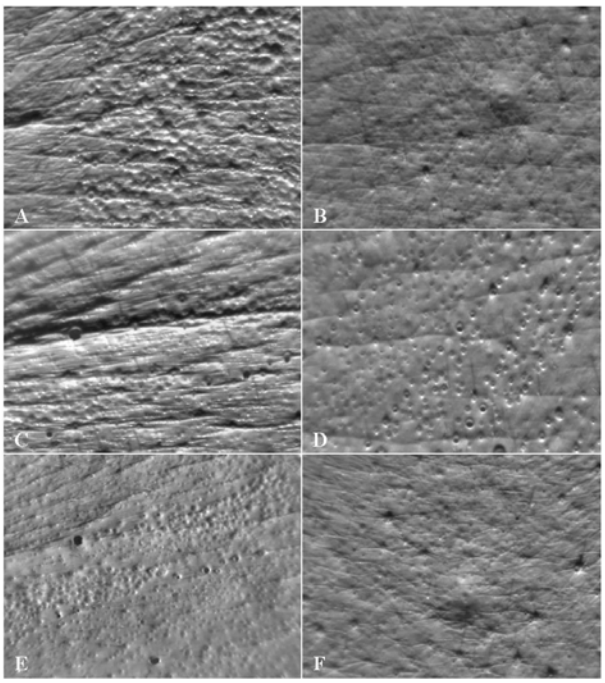

Figure 5. Cast images of representative subjects in groups A, B and P at baseline and after two months of treatment. Before treatment (A), after two months of treatment (B) in subject group A. Before treatment (C), after 2two months of treatment (D) in subject group B. Before treatment (E), after two months of treatment (F) in subject group P.

Similarly, the differences between treated and placebo site pairs (treatment effects) were calculated by subtracting the appropriate baseline corrected values for each subject. These results are tabulated in Table 4. Treatment effects were directionally significant for two parameters for group B only. Significant treatment effects for Rz and Ra were in the direction of active smoother than group P.

	Group A		Group B	
	Mean±SD	P value	Mean±SD	P value
Rz	9.2±51.4	0.5019	-20.8±41.3	0.0950
Ra	1.1±10.5	0.6794	-5.3±9.0	0.0523
FNum	-0.035±0.129	0.3176	-0.018±0.134	0.6410
IDL	0.33±3.11	0.6849	-1.39±2.85	0.1036
Spacing	-0.615±2.188	0.2951	-0.334±1.978	0.5706
Breadth	0.008±0.063	0.6507	-0.026±0.069	0.2037
Shadows	0.3±4.3	0.8169	-1.2±2.8	0.1439
NumWr	-4.1±66.3	0.8157	-14.8±49.4	0.3001

Table 4. Treatment effects calculated by subtracting value of group P from value of group A or B. (Group A; succinylated atelocollagen+adenosine, group B; succinylated atelocollagen.)

Comparisons of groups A and B were also analyzed. A direct comparison of group A and B treatment effects confirmed that the differences in Ra parameters between groups were directionally significant, with Group B being smoother than Group A (P=0.0923).

3. Histologic changes

Changes in histologic features between baseline and after two months of treatment are tabulated in Table 5. Changes from baseline were calculated by subtracting each subject's BL values from the V2 values.

Change	Group A	Group B	Group P
Epidermal thickness (μm)	10.23±7.60	13.15±5.65	6.96±10.15
Collagen (OD)	707.07±1354.46	873.87±331.01	756.72±476.35
Ki-67 (OD)	224.28±102.03	181.49±283.44	-102.47±266.31
Procollagen type I (OD)	346.29±570.80	406.87±665.45	529.29±618.73

Table 5. Quantitative changes in histologic features between baseline and after two months of treatment. (Group A; succinylated atelocollagen+adenosine, group B; succinylated atelocollagen, group P; placebo.) OD : optical density.

Epidermal thickness increased after two months of treatment, compared with values before treatment in all groups (Figure 6). The periorbital areas treated with succinylated atelocollagen and adenosine containing solution, or succinylated atelocollagen containing solution showed greater increases in epidermal thickness. However, there were no statistically significant differences among the three groups ($P > 0.05$).

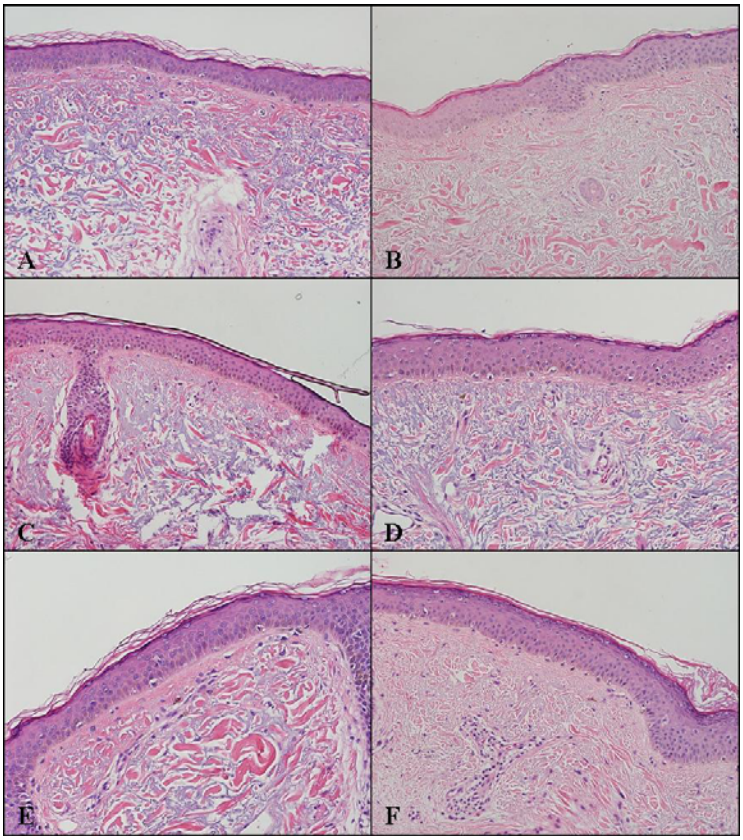

Figure 6. Hematoxylin and eosin stain (x 200). Before treatment (A) and after two months of treatment (B) using succinylated atelocollagen and adenosine-containing solution; before treatment (C) and after two months of treatment (D) using succinylated atelocollagen-containing solution; before treatment (E) and after two months of treatment (F) using placebo solution.

Quantitative image analyses of pre- and post-treatment biopsies revealed that after the two-month treatment period, the quantities of collagen fibers of each group had increased compared with baseline (Table 5 and Figure 7). However, there were no statistically significant differences among the three groups ($P > 0.05$).

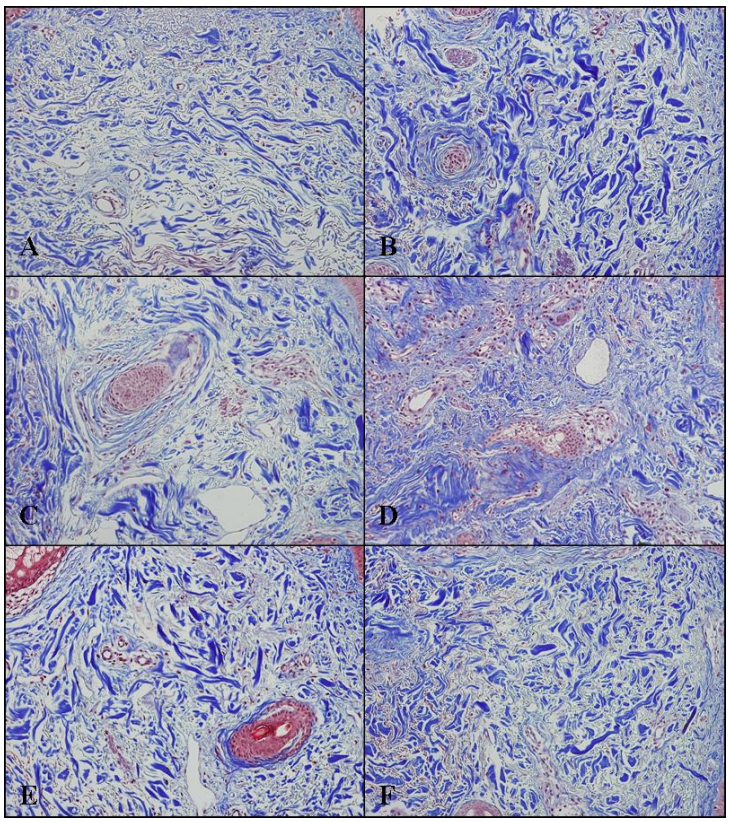

Figure 7. Masson trichrome stain (x 200). Before treatment (A) and after two months of treatment (B) using succinylated atelocollagen and adenosine-containing solution; before treatment (C) and after two months of treatment (D) using succinylated atelocollagen-containing solution; before treatment (E) and after two months of treatment (F) using placebo solution.

Quantitative image analyses of Ki-67 expression on pre- and post-treatment biopsies showed the increase after the two-month treatment period in groups A and B. However, there was a decrease in the optical density of Ki-67 expression in group P (Table 5). There was significant statistical difference between groups A and P (P=0.0455), but there were no statistically significant differences between groups A and B, or between groups B and P (P > 0.05) (Figure 8).

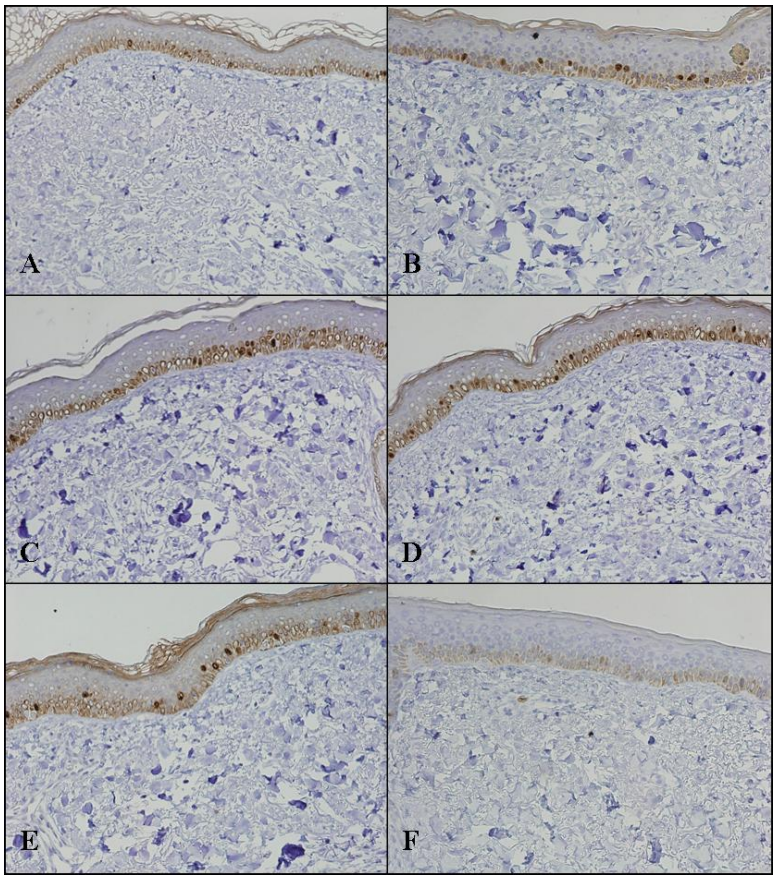

Figure 8. Immunohistochemical staining of Ki-67 expression (x 200). Before treatment (A) and after two months of treatment (B) using succinylated atelocollagen and adenosine-containing solution; before treatment (C) and after two months of treatment (D) using succinylated atelocollagen-containing solution; before treatment (E) and after two months of treatment (F) using placebo solution.

Immunohistochemical staining of procollagen type I expression revealed that procollagen expression was increased in all groups (Figure 9). Quantitative analyses of procollagen type I expression showed that the changes from baseline were 346.3±570.8, 406.9±665.5 and 529.3±618.7 in each group A, B, and P, respectively. However, there were no statistical differences among the three groups ($P > 0.05$).

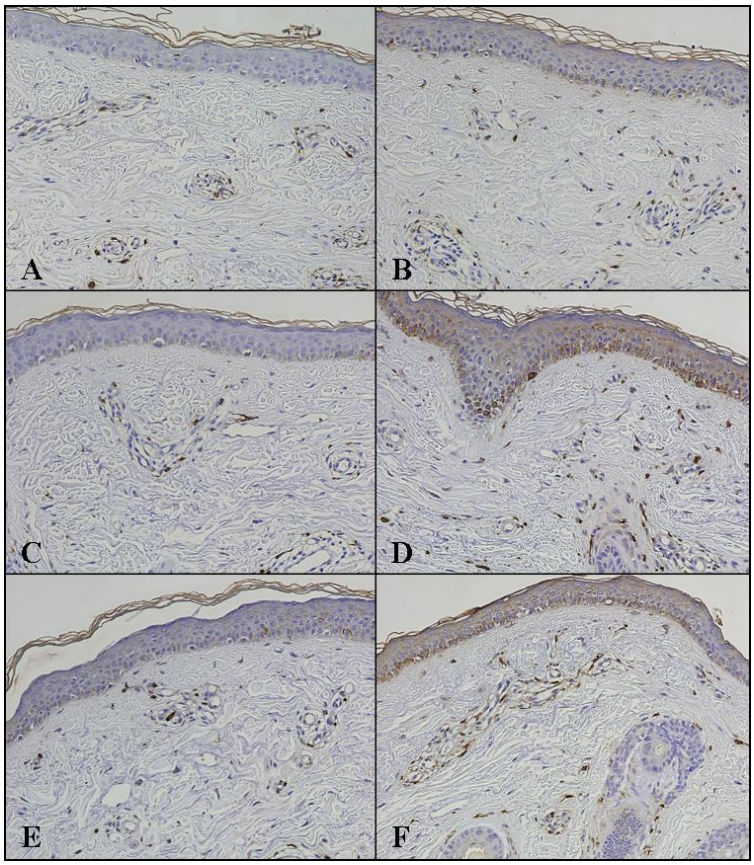

Figure 9. Immunohistochemical staining of procollagen type I expression (x 200). Before treatment (A) and after two months of treatment (B) using succinylated atelocollagen and adenosine-containing solution; before treatment (C) and after two months of treatment (D) using succinylated atelocollagen-containing solution; before treatment (E) and after two months of treatment (F) using placebo solution.

4. Complications

The treatments were well tolerated and there were no reported complications for any of the participants.

IV. DISCUSSION

Wrinkle formation represents both photoaging and chronological aging. In particular, periorbital wrinkles are generated early in the formation of wrinkles. At the molecular level, ultraviolet (UV) irradiation activates cytokine receptors and other growth factors on the surfaces of fibroblasts and keratinocytes. Activated receptors and growth factors stimulate transcription factor AP-1 through a signal transduction cascade, and AP-1 stimulates the transcription of matrix metalloproteinase (MMP) genes. MMPs break down collagen, the main component of the dermal extracellular matrix, and other structural proteins[2]. The activation of MMP-1 and MMP-9 leads to partial degradation of collagen, which regulates procollagen type I synthesis and down-regulates procollagen type I formation[9]. AP-1 stimulated by UV irradiation also interferes with collagen synthesis through down-regulation of procollagen type I gene expression[10]. Collagen provides skin with its tensile strength, so degradation of structural collagen and reduced collagen synthesis are probably major contributors to the characteristic appearance of wrinkles[11]. In chronological aging, similarities among important molecular features of photoaging have been identified[2].

As mentioned above, some ingredients have been proven to repair and

prevent wrinkles. Many controlled studies have demonstrated that topical applications of retinoids reduce the severity of wrinkles[12]. All-trans-retinoic acid, a first-generation retinoid, induces production of procollagen types I and III through induction of TGF-β, a key player of collagen synthesis in fibroblasts[11]. Increases in levels of collagen types I and III provide resiliency and strength to skin. Another constituent, adenosine, has also been shown to be effective in treating wrinkles. Adenosine acts as a regulator of cellular and organ function, and interacts with a family of 4G protein-coupled receptors, A_1, A_{2A}, A_{2B} and A_3. After binding to A_{2A} receptors, collagen production is stimulated by fibroblasts via a MEK-1/MAPK-mediated pathway and collagen degradation is diminished by down-regulation of MMP expression[13].

Increased collagen synthesis and decreased collagen degradation are the keystones of strategies for preventing or improving the appearance of wrinkles. However, there have not been any reported clinical studies that evaluate the effect of applying collagen topically on periorbital wrinkles. Collagen enhances the attachment and proliferation of fibroblasts[14]. Fibroblasts bind to intact collagen fibrils via cell surface integrin receptors, and this binding creates dynamic mechanical tension within fibroblasts and maintains their shape. Mechanical tension and cell shape are critical for its cellular function to synthesize procollagen fibrils[7]. Recently, there has been an

increase in the use of collagen for the treatment of chronic wounds[15].

In this study, we investigated the efficacy of topical application of succinylated atelocollagen-containing solution and compared these effects with the effects of adenosine on periorbital wrinkles. The succinylated atelocollagen-containing solution that we tested was composed of succinylated atelocollagen, L-lysine, sodium hyaluronate and purified water. The size of the succinylated atelocollagen molecule is 300 nm x 2.4 nm. The telopeptide consists of 12-27 amino acids in a collagen molecule, and the sequence of the telopeptide is different between individuals. Complete removal of the telopeptide can prevent immune reactions and reduce the size of the molecule[16]. The succinylated atelocollagen was treated with succinic anhydride to create an electrically anionic surface, in order to improve the solubility in water or neutral solution. L-lysine has been widely used as an adhesion agent of cells and a mediator for drug delivery. Because L-lysine has polycationic sites, it adheres to anionic sites on the cell surface and the succinylated atelocollagen. By combining the succinylated atelocollagen with L-lysine, its ability to penetrate into the skin has been improved. Brown et al [17] showed that hyaluronic acid, which has hydrophilic ability and large molecular weight (360-400 kDa), can be absorbed from the surface of the intact skin by passive diffusion and active transport and be observed in the

deeper layers of epidermis and dermis. Succinylated atelocollagen has increased solubility and similar molecular weight so it may be absorbed through intact human skin. Hyaluronic acid is also a component of the extracellular matrix and has the ability to induce fibroblasts to proliferate and to produce more collagen[18]. In addition, succinylated atelocollagen and hyaluronic acid can hold water within their molecules. Water in their molecules have a bulk effect and may improve periorbital wrinkles.

Based on the objective measurements of clinical improvement, groups A and B showed more significant improvement than group P after a two-month treatment period. We found that the mean improvement scores of group A were 1.53±0.64 after one month of treatment and 2.27±0.96 after two months of treatment. For group B, the mean improvement scores were 1.08±0.86 after one month of treatment and 2.31±1.25 after two months of treatment. The treatment effects of groups A and B increased with time. However, we did not observe any significant effects after two months of adenosine treatment. The subjective assessments of clinical improvement by the participants were similar to the objective assessments of clinical improvement. In the analysis of the casts, the treated sites for both group A and B exhibited consistent smoothing of the periorbital wrinkles. In paired comparisons with group P, group B only showed directionally significant improvement for two

parameters of roughness. Although there was no statistical significance, other parameters were in the direction of active smoother than group P. It was confirmed that only one parameter of roughness (Ra) in group B was lower than in Group A (P=0.0923). Overall, the results suggest that the treatment effect in group B was somewhat superior to that in groups A or P.

Analysis of histologic features revealed increased epidermal thickness, collagen and procollagen type I density with treatment in all groups. There were no statistically significant differences among the three groups. The density of Ki-67 in group A was significantly higher than that of group P. The Ki-67 protein is a cellular marker for proliferation and can be exclusively detected within the cell nucleus during interphase[19]. It is thought that adenosine, which was included in group A's treatment, may affect the proliferation of keratinocytes. However, we did not observe proliferation of dermal fibroblasts with Ki-67 staining. This may be due to the short-term treatment time or low penetration rate of the treatment solution. We were not able to distinguish the effects of succinylated atelocollagen or adenosine on histologic features. The lack of significant differences among the three groups may be due to the small number of subjects (each group, n=4) and relatively short duration of treatment.

V. CONCLUSION

Based on objective and subjective measurements of clinical improvement, succinylated atelocollagen was shown to be effective in the treatment of periorbital wrinkles. Analysis of cast images showed partly significant improvement between the areas treated with succinylated atelocollagen-treated and the areas treated with placebo. However, we did not observe any effects of adenosine on periorbital wrinkles. This may be due to the small number of subjects or to the short treatment period.

The results of our study show that succinylated atelocollagen may be an effective treatment option for periorbital wrinkles, but a further study with a longer treatment period and larger subject group are needed to verify these results.

REFERENCES

1. Baumann L. Skin aging and its treatment. J Pathol 2007;211:241-51.

2. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. Arch Dermatol. 2002;138:1462-70.

3. Chiu A, Kimball AB. Topical vitamins, minerals, and botanical ingredients as modulators of environmental and chronological skin damage. Br J Dermatol 2003;149:681-91.

4. Park HY, Youm JK, Kwon MJ, Park BD, Lee SH, Choi EH. K6PC-5, a novel sphingosine kinase activator, improves long-term ultraviolet light-exposed aged murine skin. Exp Dermatol 2008;17:829-36.

5. Kim YG, Sumiyoshi M, Sakanaka M, Kimura Y. Effects of ginseng saponins isolated from red ginseng on ultraviolet B-induced skin aging in hairless mice. Eur J Pharmacol 2009;602:148-56.

6. Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ et al. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 2006;168:1861–8.

7. Fisher GJ, Quan T, Purohit T, Shao Y, Cho MK, He T et al. Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. Am J Pathol

2009;174:101-14.

8. Bissett DL, Oblong JE, Berge CA. Niacinamide: A B vitamin that improves aging facial skin appearance. Dermatol Surg 2005;31:860-5.

9. Varani J, Spearman D, Perone P, Fligiel SE, Datta SC, Wang ZQ, et al. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am J Pathol 2001;158:931-42.

10. Fisher GJ, Datta S, Wang Z, Li XY, Quan T, Chung JH, et al. c-Jun-dependent inhibition of cutaneous procollagen transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. J Clin Invest 2000;106:663-70.

11. Singh M, Griffiths CE. The use of retinoids in the treatment of photoaging. Dermatol Ther 2006;19:297-305.

12. Samuel M, Brooke RC, Hollis S, Griffiths CEM. Interventions for photodamaged skin. Cochrane Database Syst Rev 2005;25: CD001782.

13. Chan ES, Fernandez P, Merchant AA, Montesinos MC, Trzaska S, Desai A, et al. Adenosine A_{2A} receptors in diffuse dermal fibrosis: pathogenic role in human dermal fibroblasts and in a murine model of scleroderma. Arthritis Rheum 2006;54:2632-42.

14. Srivastava S, Gorham SD, Courtney JM. The attachment and growth of an

established cell line on collagen, chemically modified collagen, and collagen composite surfaces. Biomaterials 1990;11:162-8.

15. Shin JU, Choi YJ, Roh MR, Chung KY, Suh H. Diabetic ulcers treated with bi-layered collagen membrane. Korean J Dermatol 2009;47:831-4.

16. Shin JU. The effect of succinylated atelocollagen and ablative fractional resurfacing laser on striae distensae[dissertation]. Seoul: Yonsei Univ.; 2009.

17. Brown TJ, Alcorn D, Fraser JR. Absorption of hyaluronan applied to the surface of intact skin. J Invest Dermatol 1999;113:740-6.

18. Mariggiò MA, Cassano A, Vinella A, Vincenti A, Fumarulo R, Lo Muzio L. Enhancement of fibroblast proliferation, collagen biosynthesis and production of growth factors as a result of combining sodium hyaluronate and aminoacids. Int J Immunopathol Pharmacol 2009;22:485-92.

19. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. Cell Physiol 2000;182:311–22.

ABSTRACTS (IN KOREAN)

눈가 주름에 대한 숙신화 아텔로콜라겐과 아데노신의 효과

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류동진

주름 형성은 광노화와 내인성 노화 모두에서 가장 대표적으로 관찰되는 증상이다. 주름의 특징적인 양상은 진피의 대부분을 구성하는 콜라겐의 분해와 콜라겐 합성의 감소로 인해 주로 발생한다. 눈가 주름에 대한 효과를 관찰하기 위해 많은 성분들이 연구되고 있으나 지금까지 콜라겐을 국소적으로 도포하여 그 항 주름 효과를 비교한 연구는 시행되지 않았다. 본 연구에서는 숙신화 아텔로콜라겐을 눈가 주름에 국소 도포하여 그 효과를 보고자 하였고 항 주름 성분으로 증명된 아데노신의 효과와 비교해보고자 하였다. 모든 환자들은 오른쪽 혹은 왼쪽 눈가 주름에, 숙신화 아텔로콜라겐과 아데노신을 포함한 제제나 숙신화 아텔로콜라겐만 포함되어 있는 제제 중 하나를 두 달 동안 도포하도록 하였고 나머지 반대쪽 눈가 주름에는 위약 제제를 두 달 동안 바르도록 하였다. 두 명의 피부과 의사와 환자가 5-point grading scale (0 = no improvement, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, 4 = 76%-100% improvement)을 이용하여 임상적 호전 정도를 평가하였고 눈가 주름을 실리콘으로 본을 떠서 눈가 주름의 변화를 측정하였다. 또한 8명의 환자는 연구시작전과 2달간의 치료 후에 눈가 주름에서 조직

검사를 시행하였다. 의사에 의한 객관적 평가 및 환자에 의한 주관적 평가에서 숙신화 아텔로콜라겐과 아데노신, 혹은 숙신화 아텔로 콜라겐을 도포한 부위는 플라시보를 도포한 부위에 비해 통계적으로 유의하게 호전된 결과를 보였다. 눈가 주름의 실리콘 모형에 대한 평가에서는 숙신화 아텔로콜라겐를 도포한 부위가 플라시보를 도포한 부위보다 부분적으로 더욱 눈가 주름이 호전된 양상을 보였다. 그러나, 본 연구에서는 아데노신의 눈가 주름에 대한 효과를 관찰할 수는 없었다. 결론적으로, 숙신화 아텔로콜라겐이 눈가 주름에 대한 효과적인 치료 방법이 될 수 있음을 알 수 있었으나 이러한 결과를 확증하기 위해서는 대규모 연구 및 장기간의 연구가 추가로 필요함을 알 수 있었다.

핵심되는 말 : 아데노신, 숙신화 아텔로콜라겐, 눈가 주름