Effect of Glycolic Acid on Collagen Gene Expression in Cultured Human Skin Fibroblasts

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Background **: Glycolic acid is currently reported to have beneficial effects on the photoaged skin. These effects may be through the modulatory action of glycolic acid in the production of dermal extracellular matrix proteins of fibroblast.**

Objective : The aim of this study was to investigate the specific effect of glycolic acid on the collagen and elastic fiber gene expression *in vitro*.

Methods **: Human skin fibroblasts were cultured from normal skins of excised specimens and glycolic acid (75μg/ml) was treated. After 24 hours of treatment, six each cell lines were processed for extraction of RNA and subjected to Northern analysis.**

Results **: Type I collagen mRNA expression was significantly increased in the glycolic acid-treated fibroblasts. Elastin mRNA expression did not show any significant change.**

Conclusion **: These results suggested that glycolic acid induced the increase of type I collagen mRNA expression and had the specific biologic effect on fibroblast. (Ann Dermatol 13(2) 92~95, 2001).**

Key Words : Glycolic acid, Fibroblast, Collagen

Glycolic acid is one of alpha hydroxy acids and new superficial chemical peeling agent[1]. Formerly, it was used to treat the disorders of keratinization[2]. Since the beneficial effect on photoaged skin was reported, this substance is now widely used to manage the photoaged skin[3-7]. It has been reported that the underlying events in clinical improvements by glycolic acids were histologically associated with dermal deposition of extracellular matrix[6,7]. However, the mechanism by which glycolic acid act is still not clear. This study was perfomed in order to investigate the specific effect of glycolic acid on the collagen and elastic fiber gene expression in cultured human dermal fibroblasts.

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MATERIALS AND METHODS

Materials

[^{32}P]dCTP (250μCi/mmol), multiprime DNA labeling kit, and nylon membrane were purchased from Amersharm (Buckinghamshire, England). Dulbecco' s modified Eagle' s medium (DMEM) and fetal calf serum (FCS) were from Gibco BRL (NY, USA). Glycolic acid was purchased from Sigma, USA.

Fibroblast culture

Normal skins from six different surgical excisions or circumcisions were used to isolate fibroblasts by collagenase digestion (Table 1). The fibroblasts were cultured in DMEM containing 10% FCS, 2 mM glutamine, and antibiotics at 37°C in a humidified atmosphere of 5% carbon dioxide. Cells in fourth to fifth passages were used.

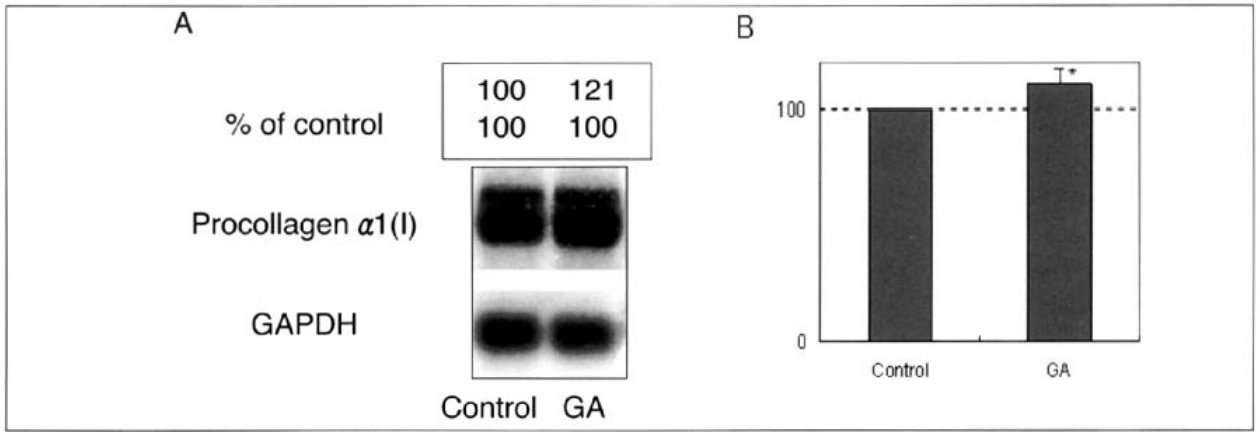

Fig. 1. A: A representative of autoradiography. B: Mean of relative abundance of procollagen α1(I) mRNA (mean ± SD, n = 6). * Glycolic acid(GA) treated fibroblast showes significant increase of procollagen α1(I) mRNA expression ($p<0.05$). Northern analysis reveals increase of procollagen α1(I) mRNA level in glycolic acid (GA) treated fibroblasts.

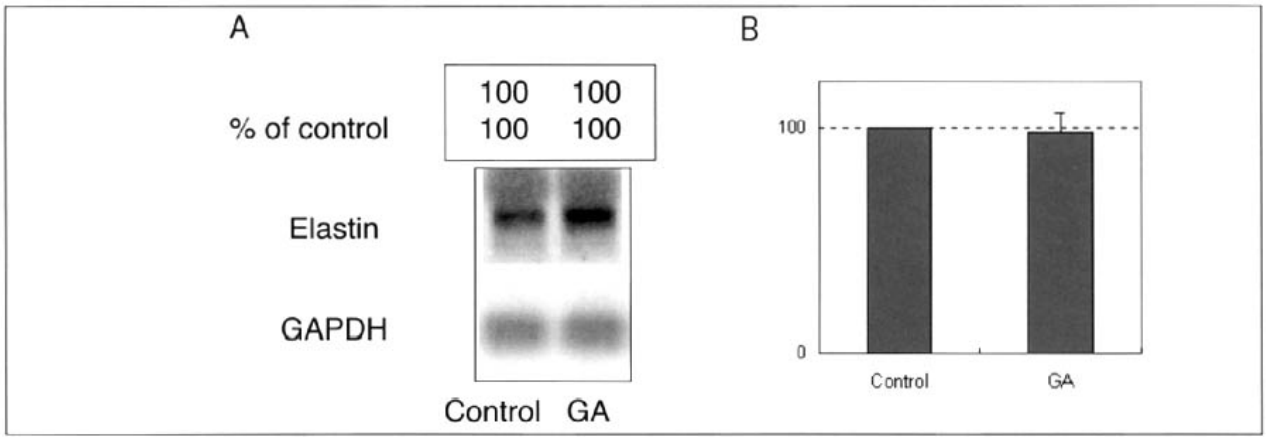

Fig. 2. Elastin mRNA expression. A: A representative of autoradiography. B: Mean of relative abundance of elastin mRNA (mean ± SD, n = 6). The difference is not significant ($p>0.05$). Northern analysis reveals no change of elastin mRNA level in glycolic acid (GA) treated fibroblasts.

Glycolic acid treatment

At the third or fourth passage, each cell line was divided into two dishes. One is the control dish and the other is the experiment dish. When cells reached about 70% to 80% of confluence, medium was removed and replaced with DMEM with 10% FCS containing 75 μg /ml glycolic acid. Glycolic acid was diluted in distilled water. After 24 hours of incubation, total RNA was extracted. Control was treated with same amount of distilled water instead of glycolic acid.

Northern blot analysis

Total RNA was isolated from cells using a previously described procedure[8]. An aliquot of total RNAs (10μg per lane) was size-fractionated by 1 % agarose gel electrophoresis and transferred to nylon membrane. The transferred RNA was hybridized to ^{32}P-labeled probes under stringent conditions at 42°C for 12 hours. The following cDNA probes radioactively labeled to the specific activity of 10^8cpm/μg DNA by random priming were used: procollagen α1(I) and elastin. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as a constitutive expression control. The membranes were washed twice with 2 × saline sodium citrate (SSC), 0.1% sodium dodecyl sulphate (SDS) for 5 min at room temperature and twice with 0.1 × SSC, 0.1% SDS at 50°C for 5 min and then exposed to X-ray film (Kodak, NY, USA) at -

Table 1. Clinical characteristics of patients whose fibroblasts were established

Patient number	Age/Sex	Site
1	27/F	back
2	36/F	back
3	18//F	buttock
4	13/M	foreskin
5	29/M	foreskin
6	21/M	foreskin

70°C. The autodiograms were analyzed with image analyzer (Bio-profil®, Vilber Lourmat, France). The mRNA levels were standardized to GAPDH mRNA levels in the same RNA sample. The abundance of mRNA was expressed as a percentage of control.

Cell viability assay

Cell viability was determined by the MTT assay[9]. After glycolic acid treatment, the cells were cultured for 24 hours; 20μl of MTT (5mg/ml) was added to each well, and the cells were incubated for 4 hours at 37°C. The supernatant was removed and 200 μl of dimethylsulfoxide was added to each well to dissolve formazan products. The absorbance was determined spectrophotometrically at 570nm with an ELISA reader. The values were statistically compared with those of control that was not treated with glycolic acid.

Statistical analysis

The difference of mRNA level between glycolic acid-treated and -untreated fibroblasts were compared by a Wilcoxon rank sum test and considered to be statistically significant if $p<0.05$.

RESULTS

1. Effects of glycolic acid on the mRNA level of collagen (Fig. 1)

The mean of relative abundance of procollagen α1(I) mRNA expression in glycolic acid treated fibroblast was 111 $\pm$ 6.7 (n=6). This increase was statistically significant ($p<0.05$), compared with control. The treatment of glycolic acid resulted in an increase of level of procollagen α1(I) mRNA.

2. Effects of glycolic acid on the mRNA level of elastin (Fig. 2)

The mean of relative abundance of elastin mRNA was 97.9 $\pm$ 8.9 (n=6). This decrease was not statistically significant. Glycolic acid did not induce the increase of elastin mRNA expression.

3. Cell viability

The viability of cultured fibroblasts measured by the MTT assay after glycolic acid treatment (75ug/ml) did not show a significant decrease (data not shown).

DISCUSSION

We investigated the *in vitro* effect of glycolic acid on the collagen and elastic fiber gene expression by cultured human dermal fibroblasts. Our findings showed that glycolic acid significantly stimulated type I collagen gene expression and did not induce the increase of elastin gene expression. This observation suggested that glycolic acid could have the specific biologic influence on fibroblasts.

Several clinical works reported that glycolic acid could clinically improve wrinkles and photoaging lesions. Those beneficial clinical effects were associated with histologic changes such as increased synthesis of glycosaminoglycans and collagen or the increased dermal repair zone[1,3-7,10]. However, only a few works have been performed regarding mechanism of action of glycolic acid. Griffin et al.[11] found that glycolic acid treatment leads to mast cell degranulation and increased expression of factor XIIIa transglutaminase by activated dermal dendrocytes and suggested that glycolic acid effect may be somehow related to this process. Besides those indirect and staged effects of glycolic acid on fibroblasts, Moy et al.[12] demonstrated direct influence of glycolic acid on collagen production in cultured human fibroblasts. And Kim et al.[13] showed glycolic acid had the capacity of stimulation of fibroblast proliferation.

Increased synthesis of type I collagen mRNA, though small, could support the earlier report that glycolic acid increased the collagen production. This capacity of glycolic acid may probably be one of action mechanisms.

The elastic fibers after alpha hydroxy acid treatment were to be longer, thicker, and less fragmented, compared with control[7]. From that finding glycolic acid appeared to cause elastic fiber metabolism. We checked the influence of glycolic acid on elastin gene expression. But elastin gene expression showed no significant change. Glycolic acid seemed not to have a stimulatory

effect on elastic fiber production.

In conclusion, glycolic acid treatment can cause a significant increase of collagen type I mRNA expression and this result suggests glycolic acid may have specific biologic effect on fibroblasts.

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