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A New Strategy to Promote Collagen Production and Wound Healing by the Combined Treatment of Ascorbic Acid and Glycinamide in Human Dermal Fibroblasts

Poster ID 582

Ji Eun Lee and Yong Chool Boo

KYUNGPOOK NATIONAL UNIVERSITY

Abstract:

Background: A decrease of dermal collagen is a common feature of natural aging and photoaging of the skin.

Objectives: To present a novel strategy to enhance dermal collagen production.

Methods: To identify amino acid analogs with excellent collagen production enhancing effects, human dermal fibroblasts (HDFs) were treated with 20 kinds of amidated amino acids individually at 1 mM. Cell viability, collagen production, and mRNA expression of procollagen genes (COL1A1, COL3A1) were compared when glycinamide, glycine analogs, ascorbic acid (AA), AA analogs, and transforming growth factor (TGF)-β1 were treated each or in combinations.

Results: Of the 20 different amidated amino acids, glycinamide enhanced collagen production most effectively. Glycine, a free amino acid, enhanced collagen production to a lesser degree but other glycine analogs, such as N-acetyl glycine, N-acetyl glycinamide, glycine methyl ester, glycine ethyl ester, and glycyl glycine, did not show such effect. AA and TGF-β1 increased COL1A1 and COL3A1 mRNA expression and collagen production, whereas glycinamide increased collagen production without changes in the mRNA expression. The combination of AA and glycinamide synergistically enhanced collagen production, cell proliferation, and wound healing in HDFs to a similar level in cells treated with TGF-β1. AA analogs, such as magnesium ascorbyl phosphate, 3-O-ethyl ascorbic acid, ascorbyl glucoside, and ascorbyl tetra-isopalmitate, enhanced collagen production, especially when they were co-treated with glycinamide.

Conclusions: This study provided a new strategy to enhance cell collagen production using glycinamide in combination with AA or its analogs. This strategy may be useful in antiaging cosmetics.

Introduction:

Since ECM proteins, including collagen, have a unique amino acid composition, their production in dermal fibroblasts is affected by the availability of certain amino acids. The purpose of this study is to find an optimized condition enhancing collagen production in cells. This study provided a new strategy to effectively enhance cell collagen production and wound healing at the cell level.

Materials & Methods:

1. Reagents

- Twenty kinds of free amino acids and twenty kinds of amidated amino acids were used in this study.

2. Cell Culture

- Human dermal fibroblasts (HEFs) were cultured in a closed incubator at 37 °C in a humidified atmosphere containing 5% CO_2. The growth medium was Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, USA) and 1% antibiotics (100 U mL^{-1} penicillin, 0.1 mg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B) (Thermo Fisher, Waltham, MA, USA).

3. Cell Viability Assay

- The viability of cells was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

4. Measurement of Collagen Secreted

- The collagen protein levels of the conditionded medium were assessed using the Sircol™ Collagen Assay (Biocolor, Antrim, UK) according to the manufacturer's instructions..

5. Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) Analysis

Gene Name	GenBank Accession Number	Forward (F) and Reverse (R) Primer Sequences
Collagen type I alpha 1 chain (COL1A1)	NM_000088.4	F: 5'-GGGATTCCCTGGACCTAAAG-3' R: 5'-GGAACACCTCGCTCTCCA-3'
Collagen type III alpha 1 chain (COL3A1)	NM_000090.4	F: 5'-GGACCTCCTGGTGCTATAGGT-3' R: 5'-GGGGTCTACCTGATTCTCCAT-3'
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_002046.3	F: 5'- ATGGGGAAGGTGAAGGTCG -3' R: 5'- GGGGTCATTGATGGCAACAA -3'

6. Wound Healing Assay

- Wound healing assay was performed by using Oris™ Universal Cell Migration Assembly kit (Platypus Technologies, WI, USA) according to the manufacturer's instructions

7. Statistical Analysis

- The experimental results are presented as the mean ± standard deviation (SD) of three or more independent experiments. SigmaStat v.3.11 Statistical Analysis Software (Systat Software Inc, San Jose, CA, USA) was used in the statistical analysis of data. A one-way analysis of variance (ANOVA) at $p < 0.05$ level was performed to determine the existence of different group means. All experimental groups were compared to each other using Duncan's multiple range tests.

Results & Discussion:

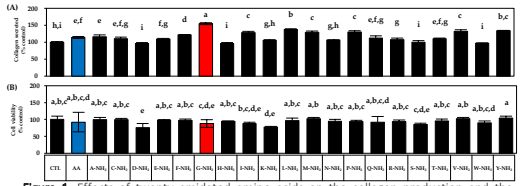


Figure 1. Effects of twenty amidated amino acids on the collagen production and the viability of human dermal fibroblasts (HDFs).

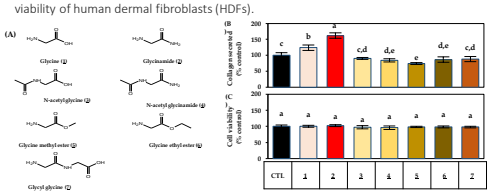


Figure 2. Effects of glycine analogs on collagen production and the viability of HDFs.

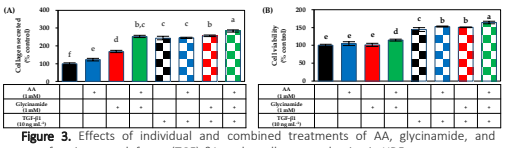


Figure 3. Effects of individual and combined treatments of AA, glycinamide, and transforming growth factor (TGF)-β1 on the collagen production in HDFs.

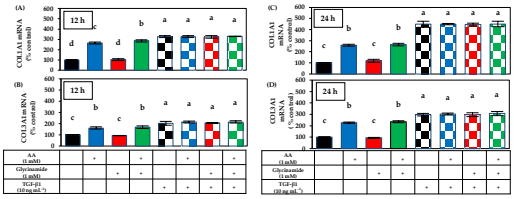


Figure 4. Effects of individual and combined treatments of AA, glycinamide, and TGF-β1 on the mRNA expression of procollagen genes in HDFs.

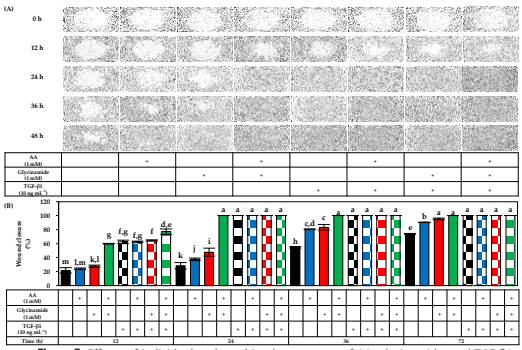


Figure 5. Effects of individual and combined treatments of AA, glycinamide, and TGF-β1 on the wound closure in HDFs.

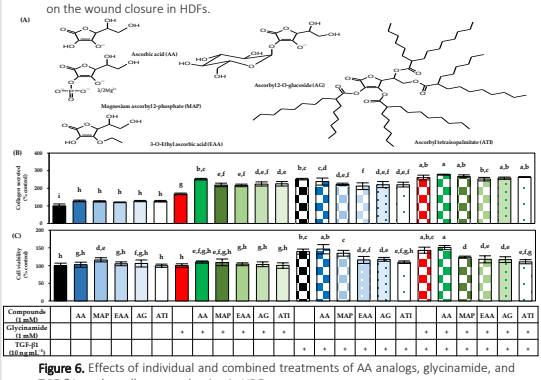
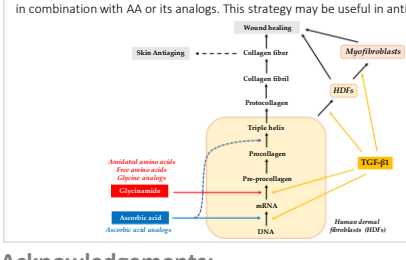


Figure 6. Effects of individual and combined treatments of AA analogs, glycinamide, and TGF-β1 on the collagen production in HDFs.

Conclusions:

This study provided a new strategy to enhance cell collagen production using glycinamide in combination with AA or its analogs. This strategy may be useful in antiaging cosmetics.



Acknowledgements:

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