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사인추출물의 인슐린 유사 성장인자-I의 합성과 피부 노화 개선에 대한 효과

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Effects of Amomi Semen Extract on Synthesis of Insulin-like Growth Factor-1 and Anti-wrinkle in Skin

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요 약: 본 연구에서 프로모터-리포터 분석방법을 통해 인슐린 유사 성장인자-I의 프로모터를 자극하는 천연물을 선별한 결과 사인 추출물이 가장 좋은 프로모터 자극효과를 나타냈으며, 무모생쥐에서 패쇄첩포 후 RT-PCR로 실험한 결과 IGF-1 mRNA를 35% 증가시키는 것으로 나타났다. 사인 추출물의 피부 주름개선 효과를 알아보기 위하여 인체 섬유아세포에서는 type-I collagen과 MMP-1 합성 변화를 관찰하였으며, 무모생쥐에서는 콜라젠의 증가와 진피 두께를 관찰하였다. 그 결과, 동위원소를 이용한 콜라젠 증가실험에서 type-I collagen은 38% 증가하였으며 무모생쥐에서 실시한 RT-PCR 결과에서는 mRNA가 21% 증가하는 것으로 관찰되었다. MMP-1 효소발현의 경우 ELISA 분석을 통해서 63%의 높은 발현저해능을 확인하였고 Western blot에서도 발현이 저해되는 것을 확인하였다. 추출물을 무모생쥐에 패쇄첩포 하였을 경우 대조군에 비해 진피 두께가 두꺼워지고 콜라젠 양도 증가되는 것으로 조직염색 관찰을 통해 확인하였다. 이상의 결과를 통해 사인추출물은 피부 주름개선에 좋은 효과를 나타내는 것으로 보이며, 여기에는 인슐린 유사성장 인자-I의 발현증가와 관련된 기전이 관여하는 것으로 판단된다.

Abstract: We screened several materials to stimulate IGF-1 promoter activity using luciferase reporter assay and found that Amomi Semen extract (ASE) among them is the most powerful stimulator. We also studied about the anti-wrinkle effect of ethanolic extract of Amomi Semen *in vitro* and *in vivo*. Semi-quantitative RT-PCR showed that the extract elevated the presence level of IGF-1 mRNA. And [^{3}H] proline incorporation and semi-quantitative RT-PCR showed that the extract increased the expression of type-I collagen compared with vehicle *in vitro* and *in vivo*, respectively. Significant inhibition of MMP-1 expression was determined by ELISA and Western blot. Finally, topical treatment of the extract on hairless mouse's dorsal skin expanded the volume of collagen and dermal thickness. These results suggest that Amomi Semen may be a good candidate for improving extracellular matrix through the increase of collagen expression and inhibition of MMP-1 expression. Moreover, this study enables us to guess that IGF-1 stimulated by the extract may be involved in the mechanism of anti-wrinkle effect of it.

Keywords: *amomi semen, IGF-1, wrinkle, collagen, MMP-1*

1. Introduction

Insulin like growth factor-1 (IGF-1) plays a crucial role in formation of bone, muscle, blood vessel and extracellular matrix. Also IGF-1 is involved in regulation of cell cycle, apoptosis and wound healing. Generally, it is reported that the plasma levels of IGF-1 are decreased with aging[1].

Amomi Semen, the dried seed of *Amomum villosum (Zingiberaceae)*, is recognized as a rich source of essential oils, which have spasmolytic, sedative, antimicrobial activities[2]. In addition, it has traditionally been used as a folk remedy for the treatment of diabetes, and it is reported that the antidiabetic action of Amomi

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may be mediated through the stimulation of glucose uptake and the potentiation of insulin action[2].

We researched about the promoting effect of ASE on synthesis of IGF-1 and anti-wrinkle in skin.

2. Materials and Methods

2.1. Cell Culture

Human dermal fibroblasts from the infant foreskin were grown under proper culture conditions (37℃, 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) containing 0.48 mg/mL glutamine, 100 IU/mL penicillin, 50 mg/mL streptomycin, and 10% fetal bovine serum. Between the fourth and seventh passage, cells were used for the experiment.

2.2. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNAs from human dermal fibroblast and SKH-1 hairlsse mouse's dorsal skin were isolated using Trizol reagent (Gibco, USA) according to the manufacturer's instructions. The cDNA coding for target gene was synthesized from the total RNA using first strand cDNA synthesis kit (MBI fermentas, USA) and then was amplified using Ex Taq (Takara, Japan). PCR primers were produced by custom oligonucleotide synthesis service (Bioneer, Korea). GAPDH and β-actin was amplified in parallel and the results were used for nomalization.

2.3. [^{3}H]-Proline Incorporation into Collagen Assay

Collagen production by conflent monolayers was determined by the incorporation of [^{3}H]-proline into collagenase-sensitive protein as described previously by A.M.H. Cornelissen *et al.*[6]. The collagen synthesis was determined by subtraction of the counts released into the blank incubation from those released in the presence of collagenase.

2.4. Enzyme-linked Immunosorbent Assay (ELISA)

The measurement for the expression level of MMP-1 after ASE treatment in cultured foreskin fibroblast was performed by ELISA kit (Amersham, UK).

2.5. Western Blot Analysis

The levels of MMP-1 and type-I procollagen were determined by immunoblot analysis. The target proteins electroblotted onto nitrocellulose membrane and detected by chemiluminescence method. The specific antibody for MMP-1 was purchased from Oncogene and that for type-I procollagen was acquired from hybridoma cell line, SP1D8.

2.6. Topical Application and Histological Analysis

This study was conducted in conformity with the policies and procedures of the Institutional Animal Care and Use Committee of the AmorePacific R&D center. Female albino hairless mice (Skh:hr-1) purchased from Charles River Laboratories (Wilmington, Mass, USA) were used for the topical application of Amomi semen solution (1%) in vehicle (propylene glycol:ethanol=7:3). The solution was occlucsively applied four times to the dorsal skin during 2 weeks. The biopsied skins were performed with hematoxylin and eosin stain (H&E stain) and special stain, vangieson stain with verhoeff stain as a counter stain, for the histological analysis of them.

2.7. Statistics

Statistical analysis was performed with Student's t-test. A P value of 0.05 was selected as the limit of statistical significance.

3. Results and Discussion

3.1. ASE Promotes IGF-1 mRNA Expression

We constructed a stable cell line containing rat IGF-1 promoter gene fused with firefly luciferase reporter gene in tandem. Using the stable cell line, we screened several materials to stimulate IGF-1 promoter activity and found that Amomi Semen extract (ASE) among them is the most powerful stimulator (data not shown). And then we checked the influence of ASE on IGF-1 mRNA expression. The result revealed that ASE increased the presence level of IGF-1 mRNA at initial stage after ASE treatment in fibroblast and also promoted IGF-1 mRNA expression by about 35% compared with vehicle in hairless mice (Figure 1).

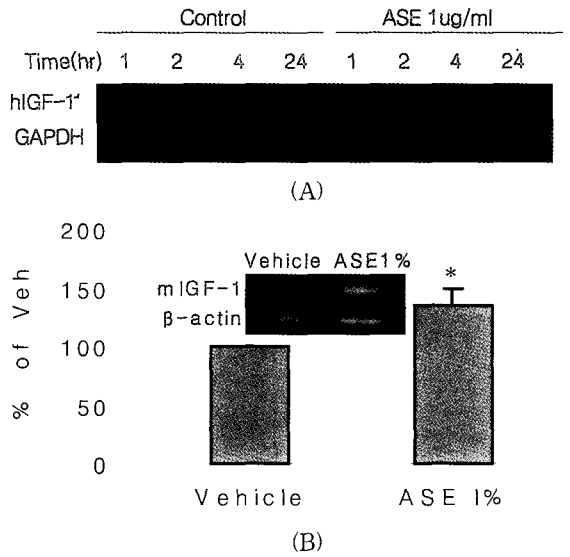

Figure 1. Effects of ASE on the presence level of IGF-I mRNA. (A) The expression profile of IGF-I mRNA by RT-PCR after treatment with ASE in fibroblast (B) Semi-quantitive RT-PCR for IGF-I mRNA after short-term occlusive patch in hairless mice.

3.2. ASE Promotes Type-I Collagen Expression

We examined whether or not ASE affect type-I collagen expression using [^{3}H] proline incorporation assay in cultured fibroblast. The results showed that ASE increased type-I collagen production by about 38% *in vitro*. To inspect *in vivo* effect of ASE, we occlusively treated with ASE on hairless mouse's dorsal skin for 2 weeks. Through semi-quantitative RT-PCR, we also found that ASE promotes the expression of type-I collagen mRNA by about 21% compared with vehicle (Figure 2).

3.3. ASE Inhibits UVA-induced MMP-1 Expression

It is also known that IGF-1 inhibits MMP-1 expression by TGF-β signaling[4]. So we tested the inhibition ability of ASE on MMP-1 expression in cultured fibroblast. UVA (15 J/cm^2) irradiation induced MMP-1 expression in fibroblast and the induced MMP-1 expression was markedly reduced by ASE (Figure 3). Accordingly, we supposed that ASE could act as a anti-wrinkle agent through the inhibition of MMP-1 expression.

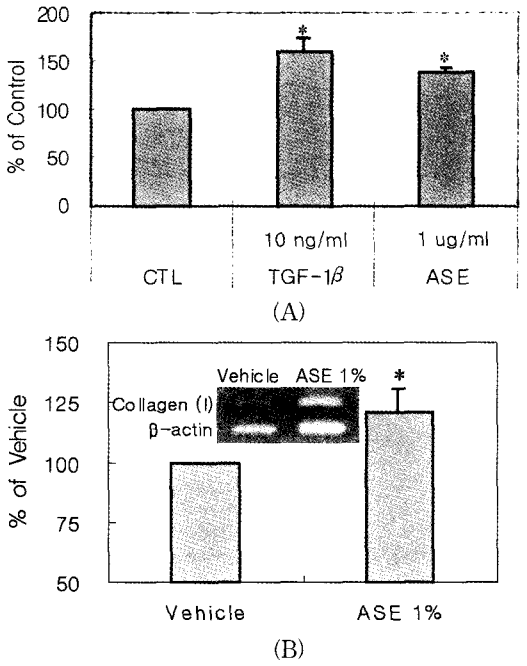

Figure 2. Effects of ASE on type-I collagen expression. (A) The presence level of type-I collagen expressed after treatment with ASE was analyzed by [3H]-proline incorporation assay (B) Semi-quantitive RT-PCR for type-I collagen after short-term occlusive patch in hairless mice.

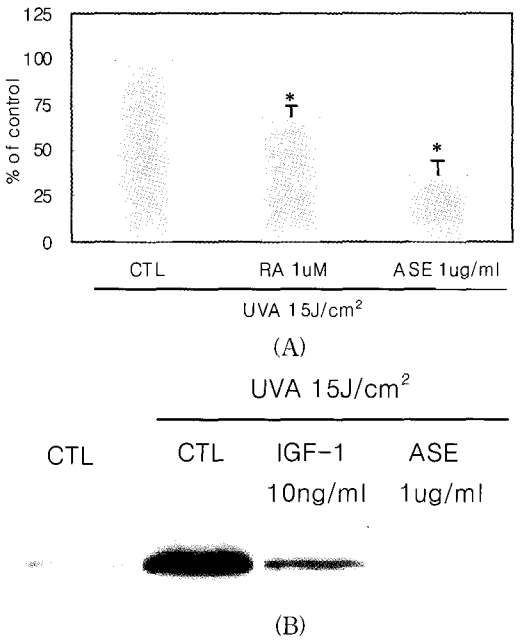

Figure 3. Effects of ASE on UVA-induced MMP-1 expression. The presence level of MMP-1 expressed in each well was analyzed by ELISA (A) and western blot (B). CTL, control; RA, retinoic acid; ASE, Amomi semen extract.

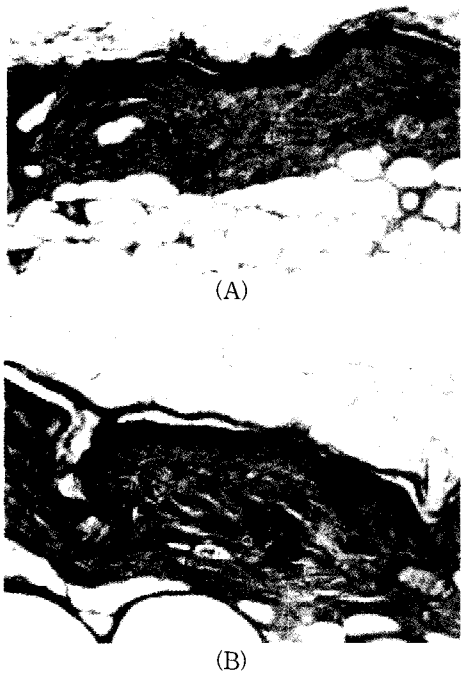

Figure 4. Effects of ASE on ECM in aged hairless mouse. The aged hairless mice were topically treated with vehicle (propylene glycol:ethanol = 7 : 3) (A) and ASE 1% (B). Hairless mouse skin sections were stained by vangieson stain with verhoeff stain as a counter stain.

3.4. ASE Expanded the Volume of Collagen and Dermal Thickness

To determine whether ASE affect ECM in aged hairless mouse or not, topical treatment of the extract was occlusively performed. The result of special stain, vangieson stain with verhoeff stain as a counter stain, showed that the amount of collagen in dermis was increased and dermis was thickened (Figure 4).

4. Conclusion

In this study, Amomi Semen extract (ASE) was chosen as a potent candidate for activating IGF-I promoter by luciferase reporting assay. Through the additional experiment, it was also found that ASE increased the presence level of IGF-1 mRNA *in vitro* and *in vivo*. And so, we guessed that ASE could act as a stimulator in secreting IGF-I.

On the basis of these results, we investigated the anti-wrinkle effect of ASE in cultured fibroblast and animal model. The result showed that ASE upregulated the type-I collagen production and inhibited the MMP-1 expression. In addition, histology analysis revealed that ASE increased the amount of collagen and dermal thickness. In conclusion, this study enabled us to determine that ASE could be a good anti-wrinkle agent making it possible to improve skin disorders.

However, further study about reason for the followings is required; what kind of mechanisms is involved in promoting IGF-1 mRNA expression by ASE and in showing anti-wrinkle effect of ASE.

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