Effects of EM201, the new derivative of erythromycin, on type I and type III collagen gene expression in cultured dermal fibroblasts

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Thin of dermis is a principal histological change of skin atrophic disorders or aged skin. It is reported that amount of collagen in the skin is decreased during aging. It is also known that productions of type I and type III collagen are decreased and collagenase expression is increased in *in vitro* and in *in vivo* aged fibroblasts. Macrolides have been reported to show various pharmacological activities in addition to antimicrobial activity including anti-inflammatory activity, inhibition of tumor angiogenesis, inhibition of the expression of vascular endothelial growth factor (VEGF), immune-modulation of keratinocytes and inhibition of the in vitro proliferation of malignant tumor cells, etc. We would like to present effects of the new erythromycin analog EM201 on collagen I and III production in cultured dermal fibroblasts. Methods: Dermal fibroblasts were cultured with routine methods. When the cell layers had become confluent, the cells were incubated for 48 hours at 37 °C in DMEM containing 0.2% FBS, supplemented with from 10⁻¹⁰ M to 10⁻⁸ M of EM201. Amounts of collagen were measured using ELISA for type I collagen. Total RNA was isolated from cultured fibroblasts by extraction in guanidium isothiocyanate then northern blots analysis were performed using ³²P labeled cDNA of a1(I) collagen, a1(III) collagen and GAPDH. Results: EM201 enhanced collagen production and mRNA levels of a1(I) collagen (to 200% in maximum) and a1(III) collagen (to 170% in maximum) in a dose dependent manner in cultured dermal fibroblasts. These results suggest that EM201 has potential to improve the thin of dermis of skin atrophic disorders or aged skin in future.

**Functional deletion analysis of human α1(I) collagen promoter gene**

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The regulation of type I collagen gene expression is one of the important role in formation of fibrosis or skin atrophy. However, Deletion analysis of human α1(I) collagen gene (COL1A1) 5'-flanking region for the transcription level of human COL1A1 is not satisfactory performed. We present deletion analysis of COL1A1 5'-flanking region for the transcriptional activity with luciferase assay using chimeric genes with various deletion constructs of the human COL1A1 5'-flanking region fused to the luciferase gene. Methods: Human dermal fibroblasts were cultured with routine methods. Cells were transfected employing the FuGene 6 transfection reagent (Roche Applied Science). Fibroblasts were plated and cultured to 50% confluence in 60-mm dishes and then transfected with 1.0 µg of COL1A1 deletion constructs of 2,300 bp, 804 bp, 610 bp, 332 bp, 165 bp and 133 bp fused to the luciferase gene and with 20 ng of CMV renilla luciferase construct (Promega). After 48 hours, cells were harvested and lysates were prepared using passive lysis buffer (Promega). Luciferase activity in equal aliquots was determined by Dual Luciferase Reporter Assay System (Promega). Results: Relative luciferase activity were not so much changed when DNA containing 804 bp or 610 bp of COL1A1 were transfected, whereas when DNA containing 332 bp of COL1A1 were transfected, relative luciferase activity were remarkably reduced. These results suggest that there are cis-DNA elements which activate transcription of COL1A1 between -310 and -610 of the COL1A1 5'-flanking region. Now the work is progress of more precise deletion analysis of that region.

ヒトα1(1)コラーゲン・プロモーター遺伝子の機能的欠損解析

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