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EM703, the new derivative of erythromycin, inhibits transcription of type I collagen in normal and scleroderma fibroblasts

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KEYWORDS
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Summary

Background: Excessive accumulation of collagen in the skin and internal organs in systemic sclerosis (SSc) is considered to result from enhanced transcription of collagen in fibroblasts. Macrolides have been reported to show various pharmacological activities. Recently, it was reported that EM703, a new derivative of erythromycin, improved bleomycin-induced pulmonary fibrosis in mice.
Objective: Therefore, we attempted to examine the effects of EM703 on the type I collagen synthetic activity in normal and SSc dermal fibroblasts.
Methods: Normal and SSc dermal fibroblasts were cultured with various concentrations of Erythromycin A or EM703 for 48 h. Amount of type I collagen in the culture medium was measured with ELISA with anti-type I collagen antibody. Type I collagen mRNA levels were measured by northern blots analysis and type I collagen transcription and regulation of the human COL1A1 promoter activity were examined by transient transfection and luciferase assay. Electrophoretic gel mobility shift assay was also performed for measurement of binding activities of DNA binding factors to the COL1A1 promoter.
Results: We found that EM703 reduced collagen production and the mRNA levels of α1(I) collagen in a dose-dependent manner in the normal fibroblasts. The transcription of COL1A1 was downregulated as detected by the luciferase assay. The downregulation was also detected using DNA containing various short lengths of the COL1A1 promoter region. EM703 did not inhibit COL1A1 transcription when the luciferase

Abbreviations: PBS, phosphate-buffered saline; EGTA, [ethylenebis(oxyethylenenitrilo)] tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride.

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assay was performed using DNA containing the COL1A1 promoter with a short substitution mutation of the CCAAT box. Decreased production of type I collagen at the transcriptional level was also found in SSc fibroblasts treated with EM703.
Conclusion: These results suggest that EM703 inhibits the transcription of type I collagen in both normal and SSc fibroblasts, and that the transcription is inhibited through the CCAAT box of the COL1A1 promoter.
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1. Introduction

Systemic sclerosis (SSc) is a fibrotic systemic connective tissue disorder characterized by excessive accumulation of collagen in the skin and a number of internal organs. Enhanced production of type I collagen from the dermal fibroblasts at the transcription level has been regarded as one of the important pathophysiological processes leading to dermal fibrosis in SSc [1,2]. Although putative antifibrotic or matrix-altering agents tested in SSc have included interferon-α [3], D-penicillamine [4], recombinant human interferon-γ [5], and recombinant human relaxin [6], trials of treatments for fibrosis in SSc have been disappointing [7]. In this context, macrolides have been reported to show various pharmacological activities in addition to their antimicrobial activity, including anti-inflammatory activity [8], inhibition of the *in vitro* proliferation of malignant tumor cells [9], inhibition of the growth of fibroblasts [10]. There have been some reports in recent years suggesting that macrolides, such as azithromycin, may be effective in the treatment of cystic fibrosis, an autosomal recessively inherited disease characterized by visceral fibrosis as one of its pathological features [11,12]. EM703 is a new 12-membered ring derivative of erythromycin A (EM-A) without antibacterial effects prepared by the Kitasato Institute for Life Sciences, Kitasato University which has been reported to suppress the action of nuclear factor (NF)-κB and the production of interleukin-8, demonstrating that the anti-inflammatory actions of macrolides are independent of their antibacterial activity [13,14]. Recently, Li et al. [13] reported that EM703 improved bleomycin-induced pulmonary fibrosis in mice. Therefore, we attempted to examine the effects of EM703 on the type I collagen synthetic activity in normal and SSc dermal fibroblasts. We found that EM703 exerted an inhibitory effect against the production of type I collagen in both normal and SSc fibroblasts. Furthermore, it was also revealed that the production of type I collagen was inhibited at the transcription level by the drug through its action on the CCAAT box of the COL1A1 promoter.

2. Materials and methods

2.1. Patients

All the SSc patients enrolled in the study fulfilled the published criteria for the diagnosis and classification

Table 1 Skin fibroblast strains and descriptions of the patients with systemic sclerosis

Name of the strain	Age (years)	Sex	Duration	Type	Biopsy site	Antinuclear antibody
Systemic sclerosis						
SSc-1	47	F	1 year	D	Forearm	Scl-70 (+)
SSc-2	45	F	15 years	D	Forearm	Scl-70 (+)
SSc-3	47	F	1 year	L	Forearm	Scl-70 (+)
SSc-4	48	F	9 months	D	Abdomen	(−)
SSc-5	61	F	8 months	D	Abdomen	(−)
SSc-6	43	F	2 years	D	Forearm	Scl-70 (+)
SSc-7	42	F	13 years	D	Forearm	Scl-70 (+)
SSc-8	54	F	12 years	D	Forearm	Scl-70 (+)
SSc-9	49	F	20 years	D	Forearm	Scl-70 (+)
Controls						
N-1M	1	M			Forearm	
N-20M	20	M			Buttocks	
N-56M	56	M			Abdomen	
N-48F	48	F			Forearm	
N-49F	49	F			Forearm	

D: diffuse type; L: limited type.

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