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Regulation of Type I Collagen and Interstitial Collagenase mRNA Expression in Human Dermal Fibroblasts by Colchicine and D-penicillamine

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Abstract

Sclerosis is a disease process in which idiopathic hardening occurs in the skin and/or internal organs as a result of the accumulation of type I collagen, induced mainly by transforming growth factor-β. Colchicine and D-penicillamine are widely used for its treatment. Their effects are known to be due to post-translational down-regulation of type I collagen synthesis, with colchicine also up-regulating interstitial collagenase. To determine whether or not they have any pre-translational effect on type I collagen and MMP-1, and also to observe their effects on the action of TGF-β, cultured neonatal foreskin fibroblasts were treated with colchicine and D-penicillamine, singly and together. The amount of type I collagen and MMP-1 mRNA were quantitated by Northern blot hybridization. Colchicine suppresses the basal level of type I collagen mRNA but minimally stimulates the mRNA expression of MMP-1, whereas D-penicillamine does not have any significant effects on either. Colchicine was also able to significantly suppress the TGF-β-induced up-regulation of type I collagen mRNA expression.

Key Words: Sclerosis, type I collagen, interstitial collagenase, mRNA, TGF-β, colchicine, D-penicillamine

INTRODUCTION

Scleroses of the skin and/or internal organs are induced by the uncontrolled accumulation of type I collagen. This condition, when involving skin, may be classified clinically into circumscribed scleroderma (morphea) and systemic scleroderma (progressive systemic sclerosis).[1,2]

D-penicillamine and colchicine are both used for the treatment of sclerosis. D-penicillamine inhibits the formation of intermolecular and intramolecular collagen cross-links[3,4] and also possesses immunosuppressive behavior.[5,6] Colchicine has an inhibitory effect on the transport and secretion of collagen by microtubular assembly inhibition[7-9] and it also increases collagenase activity *in vitro*.[10] However, the pre-translational actions of both agents on type I collagen and interstitial collagenase (matrix metalloproteinase-1; MMP-1), including their mRNA expression, has not been elucidated. Some clinical studies[11,12] have reported beneficial effects of D-penicillamine and colchicine in the treatment of scleroderma, while others[13,14] have reported their ineffectiveness or treatment failure due to systemic toxicities.

In scleroderma, chronic inflammation generally precedes fibrosis, and inflammatory cell-derived cytokines are crucial mediators of fibrogenesis, of which transforming growth factor-β (TGF-β) is the most important cytokine.[15] It can stimulate the transcription of type I collagen[16] but inhibit that of MMP-1.[17,18] In this study, using cultured human dermal fibroblasts, we examined the effects of colchicine and D-penicillamine on the basal expression of type I collagen and MMP-1 mRNA, as well as their effects on the TGF-β-induced up-regulation of type I collagen mRNA expression.

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MATERIALS AND METHODS

Cell culture

Human dermal fibroblasts were obtained from neonatal foreskin. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Life Technologies Inc., Stafford, TX, U.S.A.) supplemented with 20% fetal calf serum (FCS; Life Technologies Inc.) in a humidified atmosphere of 5% CO_2 for 7−10 days. Cells were subcultured using DMEM supplemented with 10% FCS and they were used between the third and sixth passages for this experiment. The fibroblasts were stimulated with various concentrations of colchicine and D-penicillamine when they were more than 80% confluent in culture dishes. TGF-β (PeproTech Inc., Rocky Hill, NJ, U.S.A.), 5 ng/ml, and tumor necrosis factor-α (TNF-α; Boerhinger Mannheim, Mannheim, Germany), 10 ng/ml, were used as positive controls.

Isolation of RNA and Northern blot

Total RNA from the cells was isolated by the guanidinium isothiocyanate procedure.[19] Total RNA, 20 μg, was fractionated in formaldehyde containing 0.8% agarose gel and transferred to a nylon membrane (Zeta-Probe; Bio-Rad, Richmond, CA, U.S.A.) by a membrane-transfer device (PosiBlot; Stratagene, St. Paul, Minnesota, U.S.A.). RNA was bound to the membrane by a UV cross-linker (Spectrolinker; Stratagene). The filters were prehybridized at 42°C for 2 hours in hybridization buffer and subsequently hybridized overnight with ^{32}P-labelled cDNAs for human α1 (I) collagen and human MMP-1. The filters were washed with 2 standard saline citrate (SSC) and 0.1×SSC. The ^{32}P-labelled cDNA-mRNA hybrids were visualized with autoradiography, quantitated with densitometry (Fuji Medical Systems U.S.A. Inc., Stamford, CT, U.S.A.) and corrected for the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

RESULTS

Effects of colchicine and D-penicillamine on basal expression of type I collagen mRNA expression

Neonatal foreskin fibroblasts were stimulated with TNF-α and various concentrations of D-penicillamine and colchicine. As shown in Fig. 1, TNF-α suppressed the basal expression of type I collagen (below 50% of basal expression level) whereas D-penicillamine did not. Colchicine suppressed the basal expression of type I collagen mRNA (Fig. 1) in proportion to its concentration (data not shown) down to 30−33% of basal expression level. When both agents were added, the result was identical to that when colchicine alone was used and they did not seem to affect each other at the mRNA level.

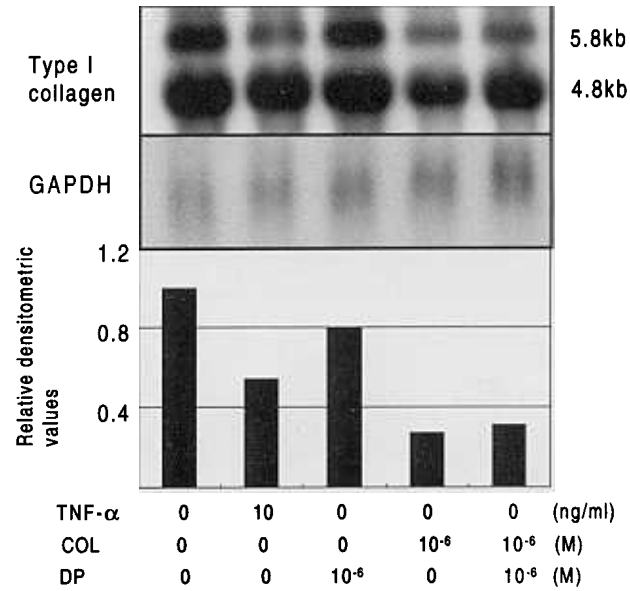

Fig. 1. Suppression of the basal expression of type I collagen mRNA by colchicine but not D-penicillamine. Neonatal foreskin fibroblasts were stimulated with TNF-α and combinations of colchicine and D-penicillamine. Their total RNA was extracted and the amount of α1(I) procollagen mRNA was quantitated by Northern blotting. Density of each blot was measured with a densitometer and corrected against the value of the corresponding GAPDH expression.

Effects of colchicine and D-penicillamine on TGF-β-induced type I collagen mRNA expression

Neonatal foreskin fibroblasts were stimulated by TGF-β and various concentrations of D-penicillamine and colchicine. TGF-β alone increased type I collagen mRNA expression as previously reported[16] but D-penicillamine did not suppress the TGF-β-induced type I collagen mRNA expression (Fig. 2). Colchicine suppressed TGF-β-induced type I collagen mRNA expression (Fig. 2) in direct proportion to its con-

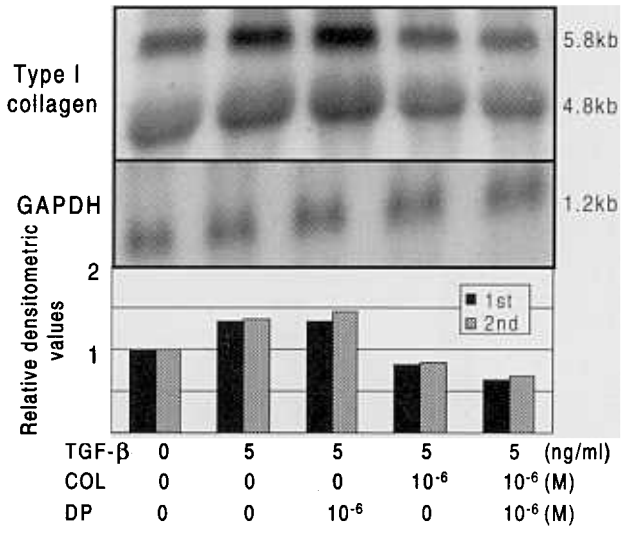

Fig. 2. Suppression of TGF-β-induced type I collagen mRNA expression by colchicine but not D-penicillamine. Neonatal foreskin fibroblasts were stimulated with TGF-β and combinations of colchicine and D-penicillamine. Their total RNA was extracted and the amount of α1 (I) procollagen mRNA was quantitated by Northern blotting. Density of each blot was measured with a densitometer and corrected against the value of the corresponding GAPDH expression. Identical experiment was repeated and shown above is the result of the first experiment.

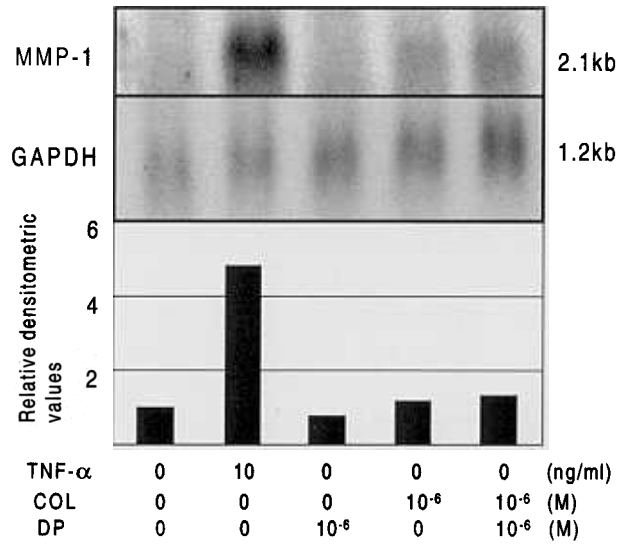

Fig. 3. Minimal upregulation of the mRNA expression of interstitial collagenase (MMP-1) by colchicine. Neonatal foreskin fibroblasts were stimulated with TNF-α and combinations of colchicine and D-penicillamine. Their total RNA was extracted and the amount of MMP-1 mRNA was quantitated by Northern blotting. Density of each blot was measured with a densitometer and corrected against the value of the corresponding GAPDH expression.

centration (data not shown) down to 50% of control. When colchicine was combined with D-penicillamine, the former also suppressed the mRNA expression without showing any signs of interaction with the latter at the mRNA level.

Effects of colchicine and D-penicillamine on the MMP-1 mRNA expression

Total RNA of foreskin fibroblast was extracted and the amount of MMP-1 mRNA was quantitated by Northern blotting. As shown in Fig. 3, TNF-α increased the MMP-1 mRNA expression (4.8-fold) but D-penicillamine did not have any effects. Colchicine minimally upregulated the mRNA expression of MMP-1 (1.18 to 1.32-fold).

Comparison of the inhibitory potency of colchicine and dexamethasone on TGF-β-induced type I collagen mRNA expression

To elucidate the potency of colchicine on the suppression of type I collagen mRNA expression, neonatal fibroblasts were stimulated with TGF-β and various concentrations of colchicine and dexamethasone. As shown in Fig. 4, both colchicine and dexamethasone suppressed TGF-β-induced type I collagen mRNA expression in direct proportion to their concentrations. Therefore, the potency of colchicine on the suppression of TGF-β-induced type I collagen mRNA expression was approximately equivalent to that of dexamethasone.

DISCUSSION

Chronic inflammation generally precedes fibrosis in the early stages of scleroderma and fibrogenesis is mostly induced by the inflammatory cell-derived cytokines. Of these, TGF-β is the most important cytokine.[15] This cytokine can stimulate the transcription of type I collagen[16] and inhibit that of MMP-1.[17,18] *In vitro* studies with scleroderma fibroblasts have shown that the stimulation of collagen production is dependent on TGF-β[20] and that fibroblasts in idiopathic pulmonary fibrosis produce TGF-β along with abnormally-elevated levels of collagen.[21] TGF-β activates transcription of the Jun oncogene, resulting in the increased formation of

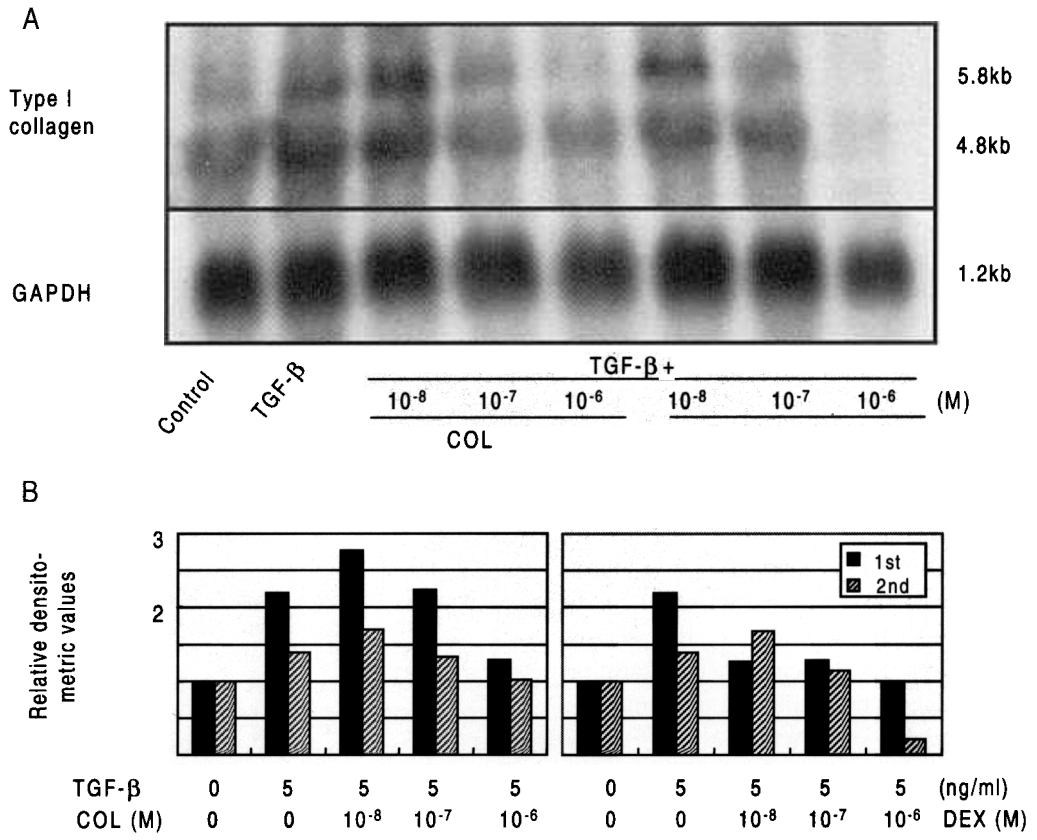

Fig. 4. Suppression of TGF-β-induced type I collagen mRNA expression by colchicine and dexamethasone. Neonatal foreskin fibroblasts were stimulated with TGF-β, colchicine and dexamethasone. Their total RNA was extracted and the amount of α1(I) procollagen mRNA was quantitated by Northern blotting. Density of each blot was measured with a densitometer and corrected against the value of the corresponding GAPDH expression. (A) Autoradiography of the second experiment. (B) Graphical representation of the two identical experiments.

activator protein-1 (AP-1), which is formed by Jun-Jun homodimer or Jun-Fos heterodimer. AP-1 in turn binds to the AP-1 binding sequences in the promoters of type I collagen and MMP-1 genes, and it regulates the transcription of the corresponding genes.[16] On the other hand, TNF-α, released by macrophages, is known to possess functions opposite to those of TGF-β, causing suppression of collagen production via the NF-κB pathway,[22] while stimulating synthesis of metalloproteinase.[23-27]

D-penicillamine and colchicine are widely used for the treatment of sclerosis. Their post-translational effect on the regulation of type I collagen and MMP-1 has been confirmed, but pre-translational aspects of their action as well as their influence on TGF-β, the main cytokine responsible for the fibrosis, have not been elucidated. To examine their effect on mRNA expression of type I collagen and MMP-1, neonatal foreskin fibroblasts were stimulated with TGF-β, TNF-α and various concentrations of D-penicillamine and colchicine. Total RNA was extracted from the fibroblasts and the amount of α1 (I) procollagen and MMP-1 mRNA were quantitated by Northern blotting.

Colchicine suppressed the basal as well as the TGF-β-induced expression of type I collagen mRNA expression in direct proportion to its concentration. However, D-penicillamine did not inhibit the basal expression or the increased expression of type I collagen mRNA induced by TGF-β nor did it interact with colchicine at the mRNA level. Colchicine minimally increased the expression of MMP-1 mRNA expression (1.18 and 1.32-fold) in two separate experiments but the effect was not as obvious as that on type I collagen mRNA. *In vitro* study has shown that collagenase activity was 2 to 10 times greater

when colchicine was added to culture media in a cultured rheumatoid synovium.[10] In our experiment, the increment of MMP-1 mRNA expression was so minimal that there is a possibility that colchicine activates collagenase via other mechanisms such as converting collagenase to an active form, or inhibiting the degradation of collagenase. The potency of colchicine on the suppression of TGF-β-induced type I collagen mRNA expression was almost equivalent to that of dexamethasone, a finding that places colchicine as a bona fide anti-fibrotic agent worthy of further evaluation.

Combined administration of D-penicillamine and colchicine did not result in any appreciable effects on mRNA expression of type I collagen and MMP-1. However, the two agents have different mechanisms for their post-translational actions and co-administration of the two may have a beneficial effect in the treatment of sclerosis. In addition to the already known inhibitory effect of colchicine on the transport and secretion of collagen by inhibiting the microtubular assembly[7-9] and having a stimulatory effect on collagenase activity,[10] in this study colchicine suppressed the basal as well as the TGF-β-induced up-regulation of mRNA expression of type I collagen. Therefore, combined administration of colchicine and D-penicillamine, although it did not show any significant advantages in our *in vitro* study at the mRNA level, may have an additional clinical benefit over single-drug treatment.

In conclusion, our study revealed that colchicine significantly suppresses basal type I collagen mRNA expression, as well as the action of TGF-β in addition to its already known post-translational modulatory effect. Colchicine also minimally stimulates the mRNA expression of MMP-1 whereas combined administration of the two agents did not result in any appreciable effects at the mRNA level.

REFERENCES

1. Elder D, Elenitsas R. Benign pigmented lesions and malignant melanoma. In: Elder D, Elenitsas R, Jaworsky C, Johnson B Jr, editors. Lever's Histopathology of the Skin. 8th ed. Philadelphia: Lippincott-Raven Publishers; 1997. p.626-30.
2. Rowell NR, Goodfield MJD. The connective tissue diseases. In: Champion RH, Burton JL, Ebling FJG, editors. Textbook of Dermatology. Oxford. 5th ed. Oxford: Blackwell Scientific Publications; 1992. p.2225-66.
3. Uitto J, Helin P, Rasmussen O, Lorenzen I. Skin collagen in patients with scleroderma: Biosynthesis and maturation in vitro and the effect of D-penicillamine. Ann Clin Res 1970;2:228-34.
4. Nimni ME. Penicillamine and collagen metabolism. Scand J Rheumatol Suppl 1979;28:71-8.
5. Schumacher K. Basis of immunosuppressive effect of D-penicillamine. Internist 1975;16:450-65.
6. Epstein O, De Villiers D, Jain S, Potter BJ, Thomas HC, Sherlock S. Reduction of immune complexes and immunoglobulins induced by D-penicillamine in primary biliary cirrhosis. N Engl J Med 1979;300:274-8.
7. Dehm P, Prockop J. Time lag in the secretion of collagen by matrix-free tendon cells and inhibition of the secretory process by colchicine and vinblastine. Biochim Biophys Acta 1972;264:75.
8. Diegelmann RF, Peterkofsky B. Inhibition of collagen from bone and cultured fibroblasts by microtubular disruptive drugs. Proc Natl Acad Sci USA 1972;69:892-6.
9. Ehrlich HP, Bornstein P. Microtubules in transcellular movement of procollagen. Nature New Biol 1972;238:257-60.
10. Harris ED Jr, Krane SM. Effects of colchicine on collagenase in cultures of rheumatoid synovium. Arthritis Rheum 1971;14:669-84.
11. Alarcon-Segovia D, Ramos-Niembro F, Ibanez de Kasep G, Alcocer J, Tamayo RP. Long-term evaluation of colchicine in the treatment of scleroderma. J Rheumatol 1979;6:705-12.
12. Jimenez SA, Sigal H. A fifteen-year prospective study of treatment of rapidly progressive sclerosis (PSS) with D-penicillamine (D-PEN). Arthritis Rheum 1989;32:S34.
13. Steen VD, Blair S, Medsger Jr TA. The toxicity of D-penicillamine in systemic sclerosis. Ann Intern Med 1986; 104:699-705.
14. Uitto J, Jimenez S. Fibrotic skin disease. Arch Dermatol 1990;126:661-4.
15. Varga J, Jimenez SA. Modulation of collagen gene expression: Its relation to fibrosis in systemic sclerosis and other disorders. Ann Intern Med 1995;122:60-2.
16. Chung KY, Agarwal A, Uitto J, Mauviel A. An AP-1 binding sequence is essential for regulation of the human $\alpha 2$ (I) collagen (COL1A2) promoter activity by transforming growth factor-β. J Biol Chem 1996;271:3272-8.
17. Edwards DR, Murphy G, Reynolds JJ, Whitham SE, Docherty AJ, Angel P, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J 1987;6:1899-904.
18. Uria JA, Jimenez MG, Balbin M, Freije JMP, Lopez-Otin C. Differential effects of transforming growth factor-β on the expression of collagenase-1 and collagenase-3 in human fibroblasts. J Biol Chem 1998;273:9769-77.
19. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
20. Kikuchi K, Hartl CW, Smith EA, LeRoy EC, Trojanowska M. Direct demonstration of transcriptional activation of collagen gene expression in systemic sclerosis fibroblasts:

insensitivity to TGF beta 1 stimulation. Biochem Biophys Res Commun 1992;187:45-50.
21. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at site of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad USA 1991;88:6642-6.
22. Kouba DJ, Chung KY, Nishiyama T, Vindevoghel L, Kon A, Klement JF, et al. Nuclear Factor-B mediates TNF-inhibitory effect on α2 (I) collagen (COL1A2) gene transcription in human dermal fibroblasts. J Immunol 1999; 162:4226-34.
23. Solis-Herruzo JA, Brenner DA, Chojkier M. Tumor necrosis factor α inhibits collagen gene transcription and collagen synthesis in cultured human fibroblasts. J Biol Chem 1988;263:5841-5.
24. Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M. Prolonged activation of *jun* and collagenase genes by tumor necrosis factor-α. Nature 1989;337:661-3.
25. Lefebvre V, Peeters-Joris C, Vaes G. Modulation by interleukin 1 and tumor necrosis factor α of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophysica Acta 1990;1052:366-78.
26. Mauviel A, Heino J, Kahari VM, Hartmann DJ, Loyau G, Pujol JP, et al. Comparative effects of interleukin-1 and tumor necrosis factor-α on collagen production and corresponding procollagen mRNA levels in human dermal fibroblasts. J Invest Dermatol 1991;96:243-9.
27. Takeda K, Hatamochi A, Arakawa M, Ukei H. Effects of tumor necrosis factor-α on connective tissue metabolism in normal and scleroderma fibroblast cultures. Arch Dermatol Res 1993;284:440-4.