TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 886-891
© TÜBİTAK
doi:10.3906/sag-1707-88

Octreotide ameliorates dermal fibrosis in bleomycin-induced scleroderma

Sibel OYUCU ORHAN[1], Ahmet TEKTEMUR[2], Nevzat GÖZEL[1], İbrahim Hanifi ÖZERCAN[3], Servet YOLBAŞ[4], Ahmet YILDIRIM[4], Ebru ÖNALAN[2], Süleyman Serdar KOCA[4,*]

[1]Department of Internal Medicine, Faculty of Medicine, Fırat University, Elazığ, Turkey
[2]Department of Medical Biology, Faculty of Medicine, Fırat University, Elazığ, Turkey
[3]Department of Pathology, Faculty of Medicine, Fırat University, Elazığ, Turkey
[4]Department of Rheumatology, Faculty of Medicine, Fırat University, Elazığ, Turkey

Received: 15.07.2017 • **Accepted/Published Online:** 19.05.2018 • **Final Version:** 16.08.2018

Background/aim: Insulin-like growth factor (IGF)-I is a differentiation and growth factor. Antifibrotic action of octreotide has been reported in pulmonary fibrosis. The present study aimed to research the prophylactic and therapeutic potential of octreotide on a bleomycin (BLM)-induced experimental scleroderma model.

Materials and methods: Sixty *Balb/c* female mice were divided into 6 groups. Daily subcutaneous BLM (100 μg) was injected for 3 weeks in groups II and III and for 6 weeks in groups V and VI. Octreotide (100 μg/kg per day) was injected subcutaneously for the first 3 weeks in group III (prophylactic) and the second 3 weeks in group VI (therapeutic). Mice in groups I, II, and III were sacrificed at the end of the third week, while mice in groups IV, V, and VI were sacrificed at the end of the sixth week.

Results: Repeated BLM applications increased dermal inflammatory cell counts and dermal thickness, and led to dermal fibrosis at both the third and sixth weeks. Moreover, mRNA expressions of TGF-β1 and IGF binding protein (IGFBP)-3 and -5 were higher in the BLM-injected sham groups. On the other hand, IGFBP-3 and -5 mRNA expressions were significantly decreased in both the prophylactic and therapeutic octreotide groups. Similarly, octreotide decreased dermal inflammatory infiltrations and dermal thickness.

Conclusion: Octreotide has antifibrotic actions on experimentally induced dermal fibrosis. It can be suggested that IGF-I plays pathogenic roles, and octreotide is a candidate for research in the treatment of scleroderma.

Key words: Scleroderma, dermal fibrosis, insulin-like growth factor-I, octreotide

1. Introduction

Systemic sclerosis (SSc) is a chronic inflammatory disease characterized by widespread fibrosis of skin and several visceral organs (1). The pathogenesis of SSc is not yet fully understood; however, fibrosis, immune activation, and vasculopathy are known to be the main determinants of pathogenesis (2). Endothelial and inflammatory cells activate the fibroblasts directly via cell–cell interactions or indirectly via cytokines and growth factors (3,4). Activated fibroblasts, also known as myofibroblasts, are the main actors for the excessive production of extracellular matrix (ECM). Myofibroblasts also produce profibrotic cytokines (interleukin [IL]-6) and growth factors (TGF-β1, connective tissue growth factor, and platelet-derived growth factor) (3,4). These properties suggest the autonomy of the myofibroblasts in SSc. It has been reported that the infiltration of inflammatory cells in skin lesions decreases over time (5). Therefore, fibroblasts and growth factors are responsible for sustained fibrosis.

Insulin-like growth factor (IGF)-I is a growth and differentiation factor (6,7). Rothe et al. (8) have reported increased serum IGF-I level in SSc patients. Moreover, increased IGF-I level has been demonstrated in bronchoalveolar fluids of SSc patients with pulmonary fibrosis (9). Serum and dermal tissue IGF-I levels have been documented to be increased in patients with morphea, a localized scleroderma (10). IGF-I binds 1 of 6 different binding proteins (IGF binding protein [IGFBP]); thus, IGFBP affects the bioactivity of IGF-I (6,7). IGFBP-3 and -5 expressions from scleroderma dermal fibroblasts have been reported to be increased (11,12). IGF-I enhances the proliferation of fibroblasts and raises the production of ECM components (13). Similarly, IGFBP-3 and -5 exacerbate fibrotic actions of fibroblasts in an in vitro setting (14), and IGFBP-5 induces dermal fibrosis in an in vivo experimental model (15).

* Correspondence: kocassk@yahoo.com

Growth hormone (GH) induces the hepatic production of IGF-I, while many actions of GH are orchestrated by IGF-I (6,7). Octreotide is a somatostatin analogue that regulates GH secretion. The expression of somatostatin receptor subtype 2A has been shown to be increased in patients with idiopathic pulmonary fibrosis (IPF) (16). Subsequent studies (17–19) have demonstrated that octreotide has antifibrotic potential on a bleomycin (BLM)-induced pulmonary fibrosis model (17,18) and in IPF patients (19). Therefore, in the present study, we aimed to evaluate the prophylactic and therapeutic potentials of octreotide on an experimental model of scleroderma induced by BLM.

2. Materials and methods

2.1. Animals and experimental protocols

Sixty female *Balb/c* mice (8 weeks old, and weighing 25 to 30 g) were included in the study. The mice were divided into 6 groups as prophylactic-early (group I [control I], group II [sham I], and group III [octreotide I]) and therapeutic-late (group IV [control II], group V [sham II], and group VI [octreotide II]) groups (Figure 1). The upper back skin was shaved for subcutaneous injections. The study was approved by the Animal Care and Ethics Committee of Fırat University.

Mice in groups I and IV received only phosphate-buffered saline (PBS) subcutaneously (100 µL/day) into the shaved back skin. The remaining 4 groups subcutaneously received 100 µg of BLM (Bleocin, bleomycin hydrochloride; Nippon Kayaku, Tokyo, Japan) dissolved in PBS (100 µL) to induce dermal fibrosis (20). BLM was injected into the shaved upper back skin for 3 weeks in groups II and III and for 6 weeks in groups V and VI. In addition to BLM, octreotide (100 µg/kg per day) was injected subcutaneously to the dorsal front of the neck for the first 3 weeks in group III (prophylactic) and during the second 3 weeks in group VI (therapeutic) (21).

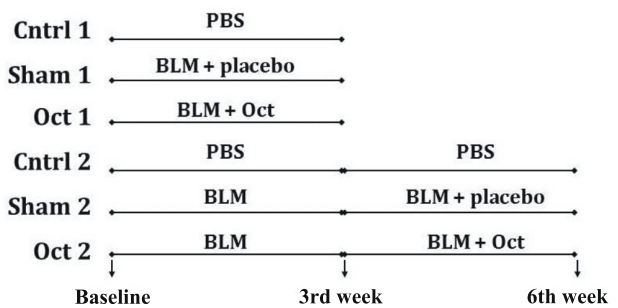

Figure 1. The schemes of experimental applications in the study groups.
Cntrl: control; Oct: octreotide; PBS: phosphate buffered saline; BLM: bleomycin.

Mice in groups I, II, and III were sacrificed at the end of the third week, while mice in groups IV, V, and VI were sacrificed at the end of the sixth week. After final experimental applications, the animals were sacrificed by cervical dislocation under anesthesia with ketamine hydrochloride, and the back skins were collected for further examination. The harvested skin samples were divided into 2 parts. One part was fixed with 10% formalin solution, embedded in paraffin, and used for histopathological examinations, and the other part was stored immediately at –80 °C for real-time PCR (RT-PCR) analysis.

2.2. Histopathological examinations

The skin specimens that were embedded in paraffin were sectioned using a microtome. Subsequently, the skin specimens were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT). Dermal thickness was measured from the dermal–fat junction to the epidermal–dermal junction, and was determined from 5 randomly selected sites of 2 or more skin sections for each animal, at 40×, 100×, and 200× magnifications. Histopathological examinations were performed under a light microscope (Olympus BX-50).

2.3. Real-time RT-PCR analysis

Total RNA was isolated from skin tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNAs were generated by reverse-transcription of total RNA using a cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). PCR reactions were prepared in triplicate, and heated to 50 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 1 min. Standard curves were prepared for each target gene (Applied Biosystems, Foster City, CA, USA) as the endogenous reference in each sample. A real-time PCR analysis was performed with the ABI Prism 7500 Fast Real Time PCR Instrument (Applied Biosystems), using Tag Man Master Mix (Applied Biosystems). The results were standardized to the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The samples were quantified for TGF-β, IGFBP-3, and IGFBP-5 mRNA (Applied Biosystems) using the comparative delta *Ct*, as described in the user's manual (Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

Statistical evaluations were performed using IBM-SPSS-22 (Chicago, IL, USA). The data were expressed as mean ± standard deviation. Kruskal–Wallis one-way analysis of variance and post-hoc Mann–Whitney U test were applied for statistical comparisons. A P value of <0.05 was accepted to be significant.

3. Results

Repeated BLM applications led to skin fibrosis beginning at week 3 (Figures 2 and 3). Among the prophylactic application groups, the mean dermal inflammatory cell

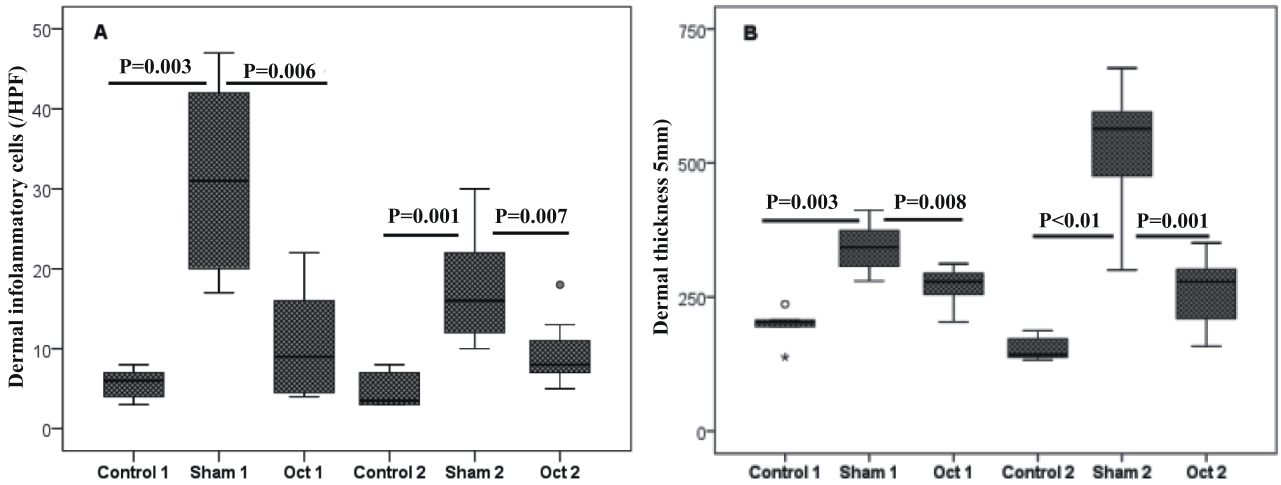

Figure 2. Dermal inflammatory cell counts (A) and dermal thicknesses (B) in the study groups.
Oct: octreotide; HPF: high power field.

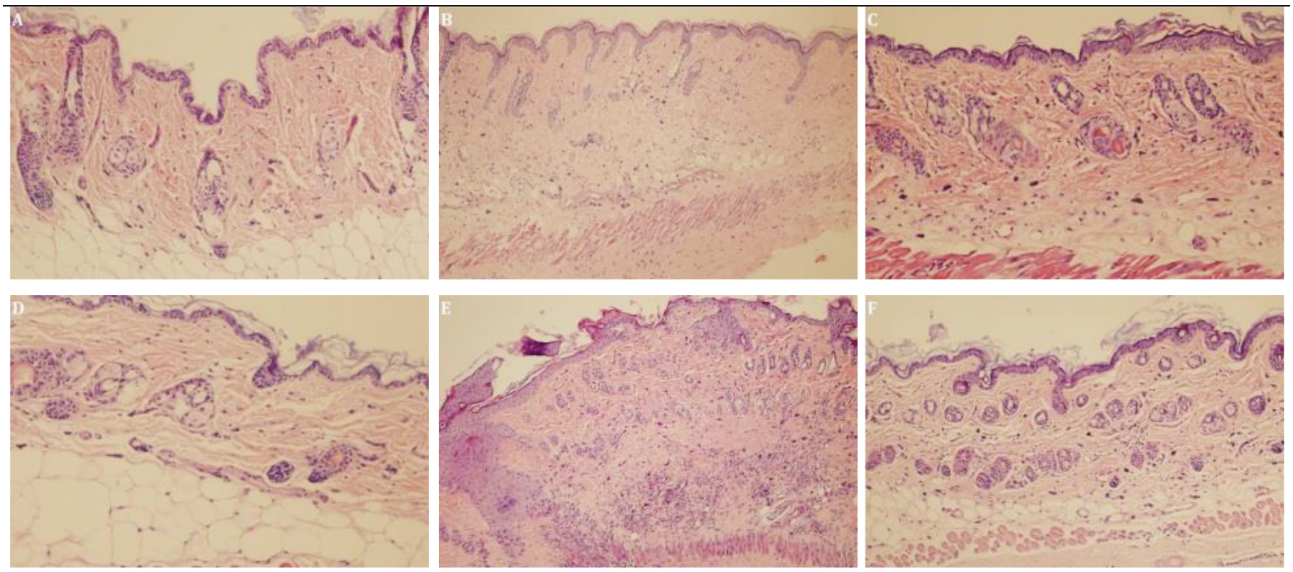

Figure 3. Histopathological appearances of skin sections (H&E, 200×).
Histopathological appearance was normal in the control groups (A and D). Bleomycin caused thickened collagen bundles in dermis and definite skin fibrosis in the sham groups (B and E). Prophylactic and therapeutic applications of octreotide ameliorated dermal fibrosis and decreased infiltrations of inflammatory cells (C and F, respectively).

counts (31.3 ± 12.7 vs. 5.7 ± 1.9, P = 0.003) (Figure 2A) and the mean dermal thickness (342.6 ± 47.3 vs. 197.2 ± 32.5 μm, P = 0.003) (Figure 2B) were higher in the Sham I group when compared to the Control I group. At week 6, the mean inflammatory cell count (17.4 ± 6.8 vs. 4.7 ± 2.3, P = 0.001) and dermal thickness (524.7 ± 116.9 vs. 152.5 ± 21.8 μm, P < 0.001) were higher in the Sham II group compared to the Control II group (Figure 2). Moreover, when compared to the own control group, the BLM injection increased mRNA expressions of TGF-β1, IGFBP3, and IGFBP5 133-, 3.5-, and 29-fold respectively at the third week and 969-, 44-, and 31-fold respectively at the sixth week (Figure 4).

In the prophylactic octreotide group, the mean inflammatory cell count (31.3 ± 12.7 vs. 10.6 ± 6.9, P = 0.006) and the mean dermal thickness (342.6 ± 47.3 vs. 271.5 ± 34.4 μm, P = 0.008) were decreased when compared to the Sham I group (Figures 2 and 3). Moreover, therapeutic octreotide applications decreased the mean inflammatory cell count (17.4 ± 6.8 vs. 9.3 ± 4.2, P = 0.007) and dermal thickness (524.7 ± 116.9 vs. 253.8 ± 67.9 μm, P = 0.001) when compared to the Sham II group

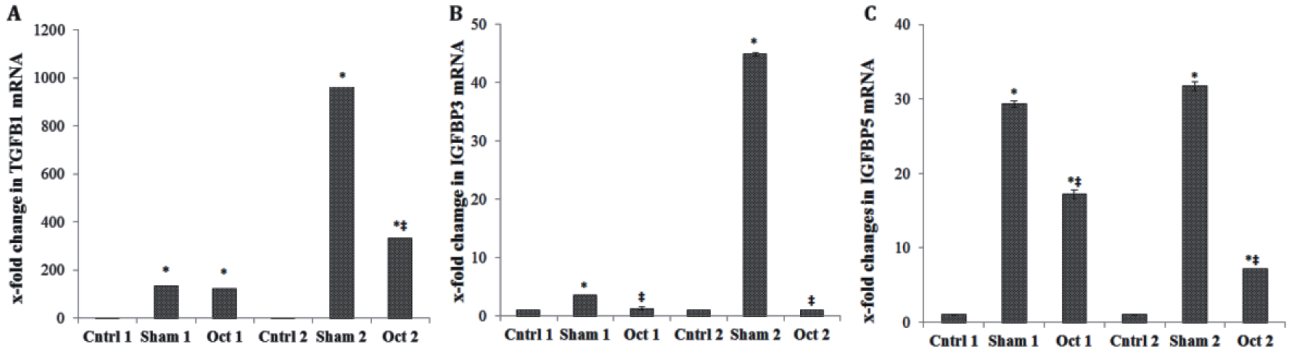

Figure 4. Dermal tissue mRNA expressions of TGFβ1 (A), IGFBP3 (B), and IGFBP5 (C).
Results are expressed as mean ± SD. The gene values were normalized to the GAPDH level.
Cntrl: control; Oct: octreotide; TGF: transforming growth factor; IGFBP: insulin-like growth factor binding protein.
*P value was <0.05 when compared to the Cntrl group.
‡P value was <0.05 when compared to the Sham group.

(Figure 2). On the other hand, prophylactic octreotide application decreased IGFBP-3 and -5 mRNA expressions but not TGF-β1 mRNA expression. However, therapeutic applications decreased TGF-β1, IGFBP-3, and IGFBP-5 mRNA expressions (Figure 4).

4. Discussion

The findings of early and late skin fibrosis in an experimental model induced by BLM, and the prophylactic and therapeutic potentials of octreotide on skin fibrosis were investigated in the present study. Repeated BLM applications led to dermal fibrosis starting at week 3, and dermal fibrosis was marked at week 6. On the other hand, our study showed that octreotide prevented dermal fibrosis and ameliorated established fibrosis.

Scleroderma is characterized by skin fibrosis. However, its pathogenesis needs to be clarified. The BLM-induced dermal fibrosis model is one of the experimental scleroderma models. In our study, repeated BLM injections led to dermal fibrosis and increased IGFBP-3 and -5 mRNA expressions. On the other hand, octreotide treatment ameliorated dermal fibrosis as well as decreased IGFBP-3 and -5 mRNA expression. These results suggest that the IGF–IGFBP axis has a role in the pathogenesis of dermal fibrosis.

It has been reported that IGF-I levels in sera (8) and bronchoalveolar fluids (9) harvested from patients with SSc are increased. Serum and dermal tissue IGF-I levels have been documented to be increased in patients with morphea (10). Moreover, dermal tissue IGF-I level has been positively correlated with Rodnan skin score (10).

The IGF axis includes IGF (I and II), receptors (type 1 and 2), and IGFBP (IGFBP-1 to -6) (22). Almost all IGF-I are in the IGFBP-bound form in the circulation. For instance, more than 90% of the circulating IGF-I binds IGFBP-3 (23). IGFBP-3, and -5 expressions from scleroderma dermal fibroblasts have been reported to be increased (11,12,24). Similarly, IGFBP-3 and -5 exacerbate fibrotic actions of fibroblasts in an in vitro setting (14), and IGFBP-5 induces dermal fibrosis in the in vivo experimental models (15,24). In patients with SSc, IGFBP-5 mRNA expression is shown to be higher in dermal fibroblasts harvested from affected skin than in fibroblasts harvested from unaffected skin and healthy controls (11). In patients with IPF, bronchoalveolar lavage fluid IGFBP-3 level is documented to be increased (23). In vitro lung fibroblast culture harvested from patients with IPF is reported to produce higher IGFBP-3 and -5 (14).

In our study, in addition to dermal fibrosis, BLM injections increased dermal tissue mRNA expressions of IGFBP-3 and -5. These results suggest the pathogenic role of the IGF–IGFBP axis on dermal fibrosis. It has been shown that IGF-I enhances the proliferation of fibroblasts and raises the production of ECM components (13). Moreover, it has been reported that IGF-I enhances the proliferation of fibroblasts and aggravates the expression of pro-collogene-1 mRNA (25).

It is known that IGF-I is mainly produced by the liver. However, it has been shown that it is also expressed by dermal fibroblasts (26). Fawzi et al. (10) have demonstrated that IGF-I level is higher in affected skin tissue than in unaffected sites in patients with localized scleroderma. It can be concluded that the source of IGF-I is fibroblasts in dermal fibrosis.

In our study, octreotide prevented and ameliorated dermal necro-inflammation and dermal fibrosis in a BLM-induced experimental scleroderma model. Octreotide, an analogue of somatostatin, depresses the IGF-I level. It is not uncommonly used in the treatment of several neuroendocrine tumors and upper gastrointestinal

bleeding. On the other hand, it has been reported that intralesional octreotide injections improve pretibial myxedema due to Graves' disease (27). A subsequent study has demonstrated that octreotide leads to regression in the sclerodermatous skin lesions of carcinoid syndrome (28). Moreover, the antifibrotic potential of octreotide on both BLM-induced pulmonary fibrosis (17,18) and IPF patients (19) has been demonstrated in previous studies. These studies (17–18,27,28) suggest the antifibrotic effect of octreotide.

In our study, the fibrosis and mRNA levels of TGF-β1 are more prominent in week 6 compared to week 3. However, there was no significant difference between prophylactic and therapeutic octreotide applications in terms of antifibrotic potential.

The present study has several limitations. The first is that the hypothesis of study should also be investigated in an in vitro setting. Secondly, serum levels of GH and IGF-I should be analyzed.

The present study documents that octreotide has antifibrotic actions on experimentally induced dermal fibrosis. It can be suggested that IGF-I plays a pathogenic role, and that octreotide is a candidate for research in the treatment of scleroderma.

Acknowledgment
This study was supported by the Fırat University Scientific Research Projects Coordination Unit.

References

1. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007; 117: 557-567.
2. Denton CP, Black CM, Abraham DJ. Mechanisms and consequences of fibrosis in systemic sclerosis. Nat Clin Pract Rheumatol 2006; 2: 134-144.
3. Krieg T, Abraham D, Lafyatis R. Fibrosis in connective tissue disease: the role of the myofibroblast and fibroblast-epithelial cell interactions. Arthritis Res Ther 2007; 9: S4.
4. Postlethwaite AE, Shigemitsu H, Kanangat S. Cellular origins of fibroblasts: possible implications for organ fibrosis in systemic sclerosis. Curr Opin Rheumatol 2004; 16: 733-738.
5. Koca SS, Ozgen M, Dagli F, Tuzcu M, Ozercan IH, Sahin K, Isik A. Proteasome inhibition prevents development of experimental dermal fibrosis. Inflammation 2012; 35: 810-817.
6. Veraldi KL, Feghali-Bostwick CA. Insulin-like growth factor binding proteins-3 and -5: central mediators of fibrosis and promising new therapeutic targets. Open Rheumatol J 2012; 6: 140-145.
7. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16: 3-34.
8. Rothe MJ, Altman RD, Falanga V. Plasma somatomedin-C levels in systemic sclerosis. Br J Dermatol 1988; 119: 639-642.
9. Harrison NK, Cambrey AD, Myers AR, Southcott AM, Black CM, du Bois RM, Laurent GJ, McAnulty RJ. Insulin-like growth factor-I is partially responsible for fibroblast proliferation induced by bronchoalveolar lavage fluid from patients with systemic sclerosis. Clin Sci (Lond) 1994; 86: 141-148.
10. Fawzi MM, Tawfik SO, Eissa AM, El-Komy MH, Abdel-Halim MR, Shaker OG. Expression of insulin-like growth factor-I in lesional and non-lesional skin of patients with morphoea. Br J Dermatol 2008; 159: 86-90.
11. Feghali CA, Wright TM. Identification of multiple, differentially expressed messenger RNAs in dermal fibroblasts from patients with systemic sclerosis. Arthritis Rheum 1999; 42: 1451-1457.
12. Knauer AV, Medsger TA. Wright TM, Feghali CA. Insulin-like growth factor binding proteins contribute to the increased extracellular matrix production by fibroblasts from patients with systemic sclerosis (SSc). Arthritis Rheum 2001; 44: 378.
13. Krein PM, Winston BW. Roles for insulin-like growth factor I and transforming growth factor-beta in fibrotic lung disease. Chest 2002; 122: 289-293.
14. Pilewski JM, Liu L, Henry AC, Knauer AV, Feghali-Bostwick CA. Insulin-like growth factor binding proteins 3 and 5 are overexpressed in idiopathic pulmonary fibrosis and contribute to extracellular matrix deposition. Am J Pathol 2005; 166: 399-407.
15. Yasuoka H, Zhou Z, Pilewski JM, Oury TD, Choi AM, Feghali-Bostwick CA. Insulin-like growth factor-binding protein-5 induces pulmonary fibrosis and triggers mononuclear cellular infiltration. Am J Pathol 2006; 169: 1633-1642.
16. Lebtahi R, Moreau S, Marchand-Adam S, Debray MP, Brauner M, Soler P, Marchal J, Raguin O, Gruaz-Guyon A, Reubi JC et al. Increased uptake of 111In- octreotide in idiopathic pulmonary fibrosis. J Nucl Med 2006; 47: 1281-1287.
17. Borie R, Fabre A, Prost F, Marchal-Somme J, Lebtahi R, Marchand-Adam S, Aubier M, Soler P, Crestani B. Activation of somatostatin receptors attenuates pulmonary fibrosis. Thorax 2008; 63: 251-258.
18. Tug T, Kara H, Karaoglu A, Karatas F, Turgut NH, Ayan E, Boran C, Tug E. The effect of octreotide, an analog of somatostatin, on bleomycin-induced interstitial pulmonary fibrosis in rats. Drug Chem Toxicol 2013; 36: 181-186.
19. Crestani B, Chapron J, Wallaert B, Bergot E, Delaval P, Israel-Biet D, Lacronique J, Monnet I, Reynaud-Gaubert M, Tazi A et al. Octreotide treatment of idiopathic pulmonary fibrosis: a proof-of-concept study. Eur Respir J 2012; 39: 772-775.
20. Yamamoto T. Animal model of sclerotic skin induced by bleomycin: a clue to the pathogenesis of and therapy for scleroderma. Clin Immunol 2002; 102: 209-216.

21. Jia WD, Xu GL, Wang W, Wang ZH, Li JS, Ma JL, Ren WH, Ge YS, Yu JH, Liu WB. A somatostatin analogue, octreotide, inhibits the occurrence of second primary tumors and lung metastasis after resection of hepatocellular carcinoma in mice. Tohoku J Exp Med 2009; 218: 155-160.

22. Jogie-Brahim S, Feldman D, Oh Y. Unraveling insulin-like growth factor binding protein-3 actions in human disease. Endocr Rev 2009; 30: 417-437.

23. Aston C, Jagirdar J, Lee TC. Enhanced insulin-like growth factor molecules in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1995; 151: 1597-1603.

24. Yasuoka H, Larregina AT, Yamaguchi Y, Feghali-Bostwick CA. Human skin culture as an ex vivo model for assessing the fibrotic effects of insulin-like growth factor binding proteins. Open Rheumatol J 2008; 2: 17-22.

25. Thiebaud D, Ng KW, Findlay DM, Harker M, Martin TJ. Insulin-like growth factor I regulates mRNA levels of osteonectin and pro-alpha-I (i)-collagen in clonal proosteoblastic calvarial cells. J Bone Miner Res 1990; 5: 761-767.

26. Barreca A, De Luca M, Del Monte P, Bondanza S, Damonte G, Cariola G, Di Marco E, Giordano G, Cancedda R, Minuto F. In vitro paracrine regulation of human keratinocyte growth by fibroblast-derived insulin like growth factors. J Cell Physiol 1992; 151: 262-268.

27. Shinohara M, Hamasaki Y, Katayama I. Refractory pretibial myxoedema with response to intralesional insulin-like growth factor I antagonist (octreotide): down regulation of hyaluronic acid production by the lesional fibroblasts. Br J Dermatol 2000; 143: 1083-1086.

28. Pavlovic M, Saiag P, Lotz JP, Marinho E, Clerici T, Izrael V. Regression of sclerodermatous skin lesions in a patient with carcinoid syndrome treated by octreotide. Arch Dermatol 1995; 131: 1207-1208.