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SÎBEL OYUCU ORHAN
AHMET TEKTEMUR
NEVZAT GÖZEL
İBRAHİM HANİFİ ÖZERCAN
SERVET YOLBAŞ

See next page for additional authors

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Recommended Citation
ORHAN, SÎBEL OYUCU; TEKTEMUR, AHMET; GÖZEL, NEVZAT; ÖZERCAN, İBRAHİM HANİFİ; YOLBAŞ, SERVET; YILDIRIM, AHMET; ÖNALAN, EBRU; and KOCA, SÜLEYMAN SERDAR (2018) "Octreotide ameliorates dermal fibrosis in bleomycin-induced scleroderma," Turkish Journal of Medical Sciences: Vol. 48: No. 4, Article 27. https://doi.org/10.3906/sag-1707-88
Available at: https://journals.tubitak.gov.tr/medical/vol48/iss4/27

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Authors
SİBEL ÖYUCU ORHAN, AHMET TEKTEMUR, NEVZAT GÖZEL, İBRAHİM HANİFI ÖZERCAN, SERVET YOLBAŞ, AHMET YILDIRIM, EBRU ÖNALAN, and SÜLEYMAN SERDAR KOCA

This article is available in Turkish Journal of Medical Sciences: https://journals.tubitak.gov.tr/medical/vol48/iss4/27
Octreotide ameliorates dermal fibrosis in bleomycin-induced scleroderma

Sibel OYUCU ORHAN1, Ahmet TEKTEMUR2, Nevzat GÖZEL1, İbrahim Hanifi ÖZERCAN3, Servet YOLBAŞ4, Ahmet YILDIRIM4, Ebru ÖNALAN2, Süleyman Serdar KOCA4,*

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

886
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
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 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).
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-collagen-I 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 intraleisonal 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. Second, 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.

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