RHEUMATOLOGY

Rheumatology 2010;49:2408–2412
doi:10.1093/rheumatology/keq275
Advance Access publication 5 September 2010

Concise report

The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab

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Abstract

Objective. SSc is an autoimmune disease characterized by fibrosis of the skin and internal organs. Although the aetiology remains uncertain, many reports have suggested that IL-6 is involved in SSc pathogenesis. Tocilizumab, an anti-IL-6 receptor antibody, is an anti-arthritis medicine that works through the blockade of IL-6 functions. To examine the effect of tocilizumab on SSc, we administered tocilizumab to two SSc patients.

Methods. Two dcSSc patients were administered tocilizumab at 8 mg/kg once a month for 6 months. One patient had pulmonary fibrosis assessed by CT and spirometry, and the other had chronic renal failure caused by scleroderma renal crisis. Their skin condition was monitored with a Vesmeter and the modified Rodnan total skin score (mRTSS). Skin biopsies were obtained before and after the tocilizumab treatment to investigate the histological changes.

Results. After tocilizumab treatment, both patients showed softening of the skin with reductions of 50.7 and 55.7% in the total *z*-score of Vesmeter hardness and 51.9 and 23.0% in the mRTSS, respectively. Histological examination showed thinning of the collagen fibre bundles in the dermis. The creatinine clearance in the patient with chronic renal failure improved from 38 to 55 ml/min. However, the fibrotic changes in the lung in the other patient remained unchanged.

Conclusions. In the two cases of SSc that we report here, softening of the skin was observed during the treatment with tocilizumab.

Key words: Systemic sclerosis, Scleroderma, Interleukin-6, Anti-IL-6 receptor antibody, Tocilizumab, Skin score, Vesmeter.

CLINICAL SCIENCE

Introduction

SSc is a disease of uncertain aetiology, and is characterized by fibrotic changes in not only the skin but also internal organs. Currently, immunosuppressants, e.g. MTX, are recommended for the treatment of SSc and CYC has also been proved to be effective for interstitial lung diseases and skin thickening [1]. However, the benefits are modest and no highly effective therapy exists. MTX sometimes causes adverse pulmonary reactions. Consequently, it is difficult to use MTX for SSc patients with lung involvement. A new therapeutic method is therefore required to improve the condition of the skin or internal organs that have become harder than normal. To this end, IL-6, one of the pro-inflammatory cytokines, has been implicated in the pathogenesis of SSc. IL-6 expression is reportedly high in both the skin and serum of SSc patients [2], and its elevation depends on the skin score [3]. Tocilizumab, an anti-IL-6 receptor antibody, blocks

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Submitted 3 November 2009; revised version accepted 20 July 2010.

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the function of IL-6, and its efficacies for the treatment of RA and Castleman disease have been verified [4]. If IL-6 contributes to the pathological condition of SSc, tocilizumab therapy may be effective for this disease. To clarify the effects of tocilizumab on SSc, we administered tocilizumab to two patients with refractory states of SSc.

Patients and methods

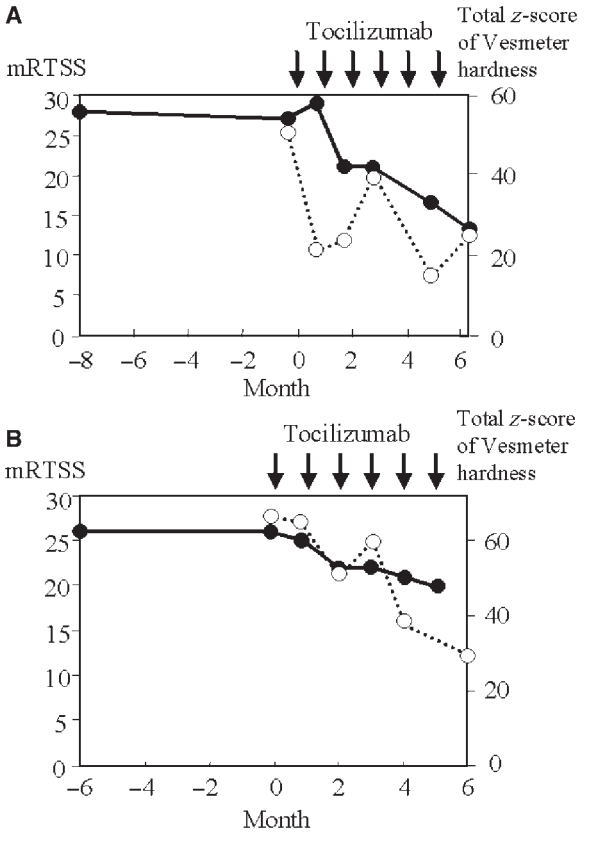
Two patients underwent tocilizumab treatment with the approval of the Ethics Committee of Osaka University Hospital after providing informed consent. The patients met the classification criteria for SSc established by the ARA in 1980 [5]. Tocilizumab was administered at 8 mg/kg every 4 weeks, which is equal to the dosage used for RA. Before this study, the skin condition was evaluated at 17 locations according to the modified Rodnan skin score using a Vesmeter by a single examiner [6]. The modified Rodnan total skin score (mRTSS) was calculated at the same time [7]. Each crude Vesmeter hardness was converted to a *z*-score, which represents the standardized degree of deviation from the normal average, because normal skin hardness varies between body sites. The normal values necessary to calculate *z*-score were referred from our previous study [6]. For identification of histological changes, skin biopsies were obtained from the left forearm before tocilizumab administration. Spirometric evaluation was conducted to assess the restrictive ventilatory impairment as well as lung CT to determine pulmonary fibrosis and an oesophageal radiographic contrast study to evaluate lower oesophageal dilatation. A HAQ for disability index (HAQ-DI) was used to evaluate the activities of daily living [8]. The skin biopsy, spirometry, chest CT and oesophagus radiographic contrast study were performed again after the tocilizumab treatment. Paraffin-embedded biopsy tissues were subjected to haematoxylin and eosin staining as well as immunohistochemical staining using mouse anti-human α-smooth muscle actin (αSMA) antibody 1A4 (DAKO Cytomation, Glostrup, Denmark) to evaluate the number of myofibroblasts using an enzyme-labelled antibody method [9,10]. Briefly, PBS supplemented with 2% BSA was used as a blocking reagent, a 1 : 50 dilution of the anti-αSMA mAb was used as the primary antibody and a peroxidase-conjugated goat anti-mouse/rabbit immunoglobulin antibody (DAKO Cytomation) was used as the secondary antibody.

Patient 1 was a 42-year-old man who had been suffering from swelling of the fingers and RP since 2005. Treatment with prednisolone at 0.5 mg/kg/day was initiated, but his skin sclerosis continued to spread from his hands to his forearms, upper arms and feet. Serological examinations found neither anti-topo I nor ACAs. ANA was positive with homogeneous and speckled stained patterns. The patient was diagnosed with dcSSc, and because of the severe skin sclerosis (maximum mRTSS: 32), he was treated with ciclosporin at 150 mg/day plus predonisolone at 10 mg/day in 2006. His serum creatinine level suddenly increased from 0.7 to 2.7 mg/dl within 1 month in association with an elevation in blood pressure from 144/84 to 160/104 mmHg in 2007. Laboratory data showed neither fragmented red blood cells nor ANCAs. Daily urine volume was not reduced, but urine analysis showed positive for protein and occult blood. The plasma level of rennin activity in the morning showed 51.9 ng/ml/h (normal: 0.5–2.0 ng/ml/h). Consequently, administration of telmisartan at 80 mg/day was started to suppress his blood pressure in view of a diagnosis of scleroderma renal crisis. Before the first administration of tocilizumab, spirometry and chest CT excluded the presence of pulmonary fibrosis, while a radiographic contrast study of the oesophagus demonstrated lower oesophageal dilatation. The serum creatinine level (1.40 mg/dl) and creatinine clearance (38 ml/min) indicated a decrease in renal function, although proteinuria and urine occult blood were negative when started. The peripheral blood cell count and urinalysis were within normal limits. The CRP level was $<$0.04 mg/dl (normal: $<$0.2 mg/dl) and the serum IL-6 concentration was 6.19 pg/ml (normal: $<$4.0 pg/ml). Administration of prednisolone at 10 mg/day and telmisartan at 80 mg/day was continued. Patient 2 was a 57-year-old woman who had been suffering from RP and swelling of the fingers since 2004. She became aware of dyspnoea on effort and sclerotic changes in the skin of the bilateral hands and forearms as well as the chest. Serological examination was positive for anti-topo I antibodies and ANA with homogeneous and speckled stained patterns. She was diagnosed with SSc and treatment with prednisolone at 10 mg/day was initiated in 2005. However, skin sclerosis spread to her face and upper arms, consistent with diffuse cutaneous disease. It continued to worsen, even though ultraviolet A treatment with 1% psoralen lotion was started in 2007. Owing to the progression of skin sclerosis, the patient was admitted to our hospital. Before initiation of tocilizumab therapy, her peripheral blood cell count, urinalysis and serum creatinine level were confirmed to be within the normal limits. The CRP level was $<$0.04 mg/dl (normal: $<$0.2 mg/dl) and the serum IL-6 concentration was 2.77 pg/ml. A CT study revealed patchy infiltrates associated with ground-glass opacities in the bilateral lower lung areas. Spirometry showed that the vital capacity (VC) was 71.6% of the predicted value, forced expiratory volume in 1 s as a percentage of forced VC (FVC) [forced expiratory volume (FEV) 1.0%] was 84.5% and the diffusing capacity for carbon monoxide (DL_{co}) was 35.5%. No lower oesophageal dilatation or retention of contrast agent was observed in a radiographic contrast study. Administration of prednisolone at 10 mg/day was continued.

Results

During the 6-month tocilizumab therapy, both the total *z*-score of Vesmeter hardness and mRTSS decreased (Fig. 1). In Patient 1, the total *z*-score of Vesmeter hardness decreased from 50.1 to 24.7 (50.7% reduction) and mRTSS decreased from 27 to 13 (51.9% reduction). In Patient 2, the total *z*-score of Vesmeter hardness

FIG. 1 Clinical courses of Patient 1 (**A**) and Patient 2 (**B**). (○): total *z*-score of Vesmeter hardness; (●): mRTSS.



decreased from 67.5 to 29.9 (55.7% reduction) and mRTSS decreased from 26 to 20 (23% reduction). The decrease in HAQ-DI was observed in both patients as follows: from 0.375 to 0.125 in Patient 1 and from 1.50 to 1.00 in Patient 2. As a result of skin softening, joint mobility also improved. The distance between the palm and third fingertip during forceful gripping was shortened from 17 mm (right) and 13 mm (left) to 0 mm on each side in Patient 1, and from 30 mm (right) and 23 mm (left) to 12 mm and 0 mm, respectively, in Patient 2. The distance between the lips during forceful mouth opening became vertically elongated from 30 mm to 50 mm in Patient 2. Although histological studies did not show any marked changes in the thickness of the dermis, thinning of the collagen fibre bundles in the dermis was observed (Fig. 2A–D). Immunohistochemical staining for αSMA showed positivity in several cells in the dermis and vascular walls, and the number of positive cells in the dermis decreased after the treatment in both patients (Fig. 2E–H). Patient 1 showed kidney involvement, and the serum creatinine level and clearance improved from 1.40 to 1.18 mg/dl and from 38 to 55 ml/min, respectively, during the treatment period, although they had showed only minor changes from 1.31 to 1.40 mg/dl and from 45 to 38 ml/min during the preceding 6 months. Patient 2 did not show kidney involvement and the kidney function did not change during the treatment period. The oesophageal radiographic contrast studies did not show any pronounced changes, although a minor improvement in contraction after the contrast agent had passed was observed in Patient 1. Patient 2 had lung involvement, and re-examination by chest CT showed the same degree of pulmonary fibrosis as in the first examination. Re-examination of the %VC and %DL_{co} showed that they remained low at 69.8 and 35.5%, respectively. Adverse reactions were not observed during the periods of this study. Other laboratory data including peripheral blood cell counts, aminotransferases, cholesterols or protein did not show marked change. Patient 1 showed leucocytosis and hypertriglyceridaemia before the study, but they did not show significant change.

Discussion

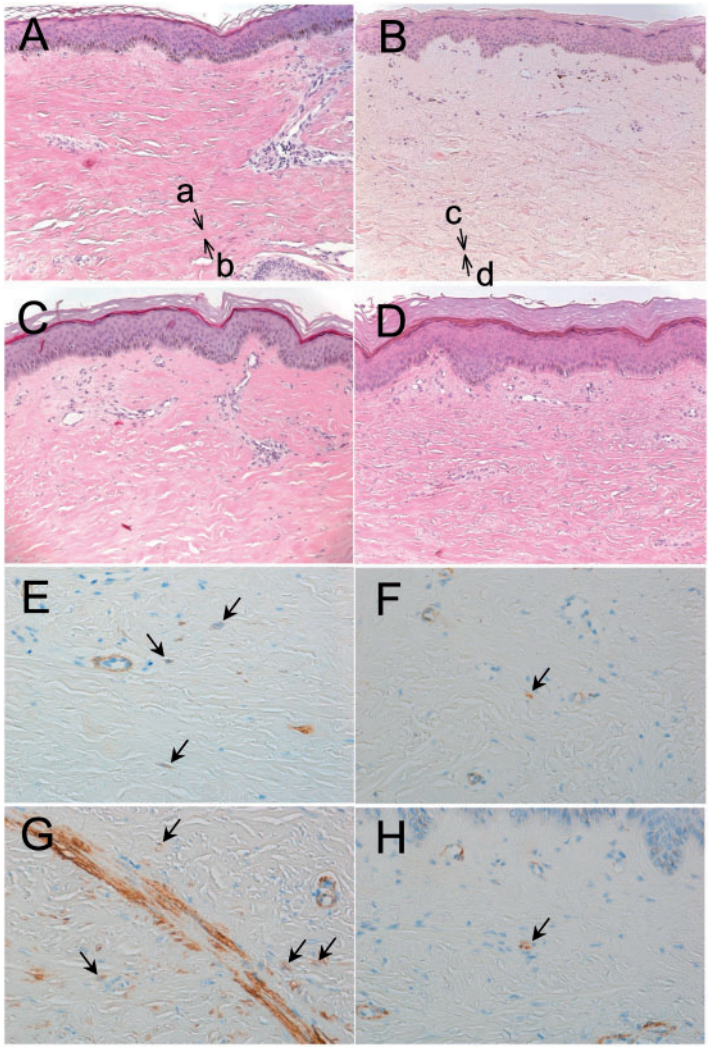
This is the first report on treating SSc patients with tocilizumab. Although the role of IL-6 in SSc remains unclear, many studies have reported that abnormalities in IL-6 are related to SSc. For example, the culture supernatants of peripheral blood mononuclear cells from patients with SSc were reported to contain higher concentrations of IL-6 than those from normal subjects [11]. Similarly, the culture supernatants of skin tissues from SSc patients were also found to contain elevated IL-6 levels [12]. Furthermore, the serum IL-6 concentrations of SSc patients were reported to be increased and the degree of the increase was dependent on the skin thickness score [2, 3]. Although the patients described here did not show marked elevation of CRP or serum IL-6, overproduction of IL-6 by the affected skin even in the case of SSc patients with normal serum IL-6 levels was reported [12]. It has already been reported that anti-IL-6 antibody suppressed pro-collagen production in fibroblasts from SSc patients [13]. Therefore, tocilizumab, an anti-IL-6 receptor antibody that blocks the function of IL-6, is worth studying for the clinical effect on SSc.

Histological examination revealed changes in the collagen fibre bundles, and immunohistochemical staining showed a decrease of αSMA-positive cells in the dermis. αSMA-positive myofibroblasts have been reported to exhibit abundant production of collagen [9], and their number in the dermis was reported to be correlated with skin hardness [10]. IL-6 may affect the production of extracellular matrix through down-regulation of collagen-producing cells.

The interaction between IL-6 and internal organs has been the subject of discussion [14]. Patient 1 did not show marked change in his oesophageal involvement, and Patient 2 did not show any improvement in her pulmonary fibrosis, although both patients showed an improving tendency for their skin. In this study, the vascular involvement including RP was not evaluated. To clarify these points, the present study needs to be extended to other patients with involvement of internal organs.

The Vesmeter is a new device that can measure the physical properties of skin. We previously reported

FIG. 2 (**A**, **B**) Haematoxylin and eosin-stained skin biopsy specimens from the left arm of Patient 1 obtained before tocilizumab therapy (**A**) and after administration of the therapy for 6 months (**B**). Thinning of the collagen fibre bundles in the dermis is observed. A representative example is indicated by the difference between the distances from a to b and c to d. (**C**, **D**) Haematoxylin and eosin-stained skin biopsy specimens from the left arm of Patient 2 obtained before tocilizumab therapy (**C**) and after administration of the therapy for 6 months (**D**). (Original magnification ×100.) (**E–G**) Immunohistochemical staining with an anti-αSMA antibody of skin biopsy sections obtained from Patient 1 before (**E**) and after (**F**) the tocilizumab therapy, and from Patient 2 before (**G**) and after (**H**) the tocilizumab therapy. αSMA-positive cells outside the vascular wall are indicated by (→). (Original magnification ×200.)



that this device is useful for evaluating the skin condition of patients with SSc [6]. Vesmeter hardness is well correlated with the hardness standards authorized by the American Society for Testing and Materials. We used the Vesmeter for serial evaluations of the skin to assess the drug efficacy, and the results demonstrated that this machine was useful for such studies. As shown in Fig. 1, the total *z*-score of Vesmeter hardness tended to be more highly sensitive than mRTSS. This is the first report of a clinical follow-up study using the Vesmeter.

In this report, we have described two patients with SSc who showed skin softening during the treatment with tocilizumab. Further controlled studies are necessary to properly evaluate its efficacy.

Rheumatology key messages

- The skin of patients with SSc softened during treatment with tocilizumab.
- Vesmeter was useful for assessment of therapy for SSc.

Acknowledgements

Funding: This work was supported by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation.

Disclosure statement: T.K. holds a patent for tocilizumab. A.O. has received an adviser expense as a medical adviser of the subcutaneous injection clinical trial for RA of Chugai Pharmaceutical Co., Ltd from April 2010. All other authors have declared no conflicts of interest.

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