Therapeutic efficacy of combined glucocorticoid, intravenous cyclophosphamide, and double-filtration plasmapheresis for skin sclerosis in diffuse systemic sclerosis

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Abstract

We treated skin sclerosis with triple therapy consisting of a glucocorticoid, intravenous cyclophosphamide, and double-filtration plasmapheresis. The objective of this study was to analyze its effectiveness in a case series of patients who received triple therapy.

We enrolled 8 patients with diffuse cutaneous systemic sclerosis (dcSSc) who received triple therapy at our hospital from 2008 to 2016. We analyzed the mean change in the modified Rodnan skin score (mRSS), percentage of the predicted forced vital capacity (%FVC), percentage of the predicted carbon monoxide diffusing capacity (%DLCO), and serum KL-6 levels from baseline to follow-up.

All patients were treated with an intermediate dose of oral prednisolone (30.6 ± 2.1 mg/day) initially. The mean cumulative dose of intravenous cyclophosphamide was 1.4 ± 0.2 g. The mean mRSS decreased significantly at follow-up compared with that at baseline (27.0 ± 3.3 vs 15.8 ± 3.5; P = .03). At the end of the treatment, the mean %FVC and %DLCO were improved moderately, although the differences were not significant. The serum KL-6 levels decreased from 578.9 ± 146.5 to 205.3 ± 43.1 U/ml (P = .02). No significant correlation was found between the change in mRSS or disease duration and the initial skin score severity.

Triple therapy may improve skin sclerosis, with effectiveness equal or superior to other reported treatments. This preliminary case series demonstrates the potential of triple therapy for treating dcSSc. However, prospective studies with long-term follow-up should be performed to assess its role.

Abbreviations: %DLCO = percentage of the predicted carbon monoxide diffusing capacity, %FVC = percentage of the predicted forced vital capacity, dcSSc = diffuse cutaneous systemic sclerosis, DCO = diffusing capacity of the lung for carbon monoxide, IL-6 = interleukin-6, lcSSc = limited cutaneous systemic sclerosis, mRSS = modified Rodnan skin score, MTX = methotrexate, PDGF = platelet-derived growth factor, RNA = ribonucleic acid, SEM = standard error of the mean, SSc = systemic sclerosis, TGF-β = transforming growth factor-beta.

Keywords: double-filtration plasmapheresis, glucocorticoid, intravenous cyclophosphamide, skin sclerosis, systemic sclerosis

Key points/messages

- Triple therapy may improve skin sclerosis in patients with diffuse cutaneous systemic sclerosis.
- A combination of plasmapheresis and immunosuppressants contributed to an immediate effect.
- Treatment for skin sclerosis should be initiated during the early disease phase.

1. Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease of unknown etiology, characterized by progressive skin fibrosis and variable involvement of the respiratory, cardiovascular, renal, and gastrointestinal systems. LeRoy et al classified SSc into diffuse cutaneous systemic sclerosis (dcSSc) and limited cutaneous systemic sclerosis (lcSSc). They also showed that visceral...
involved is more common in patients with dcSSc.\(^1\) Moreover, the skin score is related to disease outcome in patients with dcSSc.\(^2\) Given the lack of disease-modifying therapies for patients with SSc, the mortality associated with the diffuse type is high.\(^3\) The clinical manifestations of SSc are thought to arise from 3 main pathological pathways: vascular damage, immune activation, and excessive synthesis of extracellular matrix. Vascular damage is thought to activate the immune system, contributing to tissue fibrosis.\(^4\) Several cytokines have been investigated as potential fibrosis effectors in SSc, including transforming growth factor-beta (TGF-\(\beta\)),\(^5\) connective tissue growth factor,\(^6\) platelet-derived growth factor (PDGF),\(^7\) and interleukin-6 (IL-6).\(^8\) Several immunosuppressive agents have been proposed as treatment options for patients with SSc, including prednisolone,\(^9\) methotrexate (MTX),\(^10\) cyclophosphamide,\(^11\) and mycophenolate mofetil.\(^12\) However, there is no established treatment. In a prospective study, Valentini et al noted significant improvements in the modified Rodnan skin score (mRSS) and diffusing capacity of the lung for carbon monoxide (DLCO) after treatment with low-dose prednisone and intravenous cyclophosphamide in patients with early dcSSc.\(^13\) Other studies have shown that combining immunosuppressive agents with plasma exchange can effectively treat SSc. Akesson et al indicated that an immediate effect can be expected by combining plasmapheresis with prednisolone and oral cyclophosphamide.\(^14\) Therefore, we investigated the efficacy of triple therapy with glucocorticoid, intravenous cyclophosphamide, and double-filtration plasmapheresis in skin sclerosis.

2. Methods

2.1. Patients

We retrospectively reviewed the medical records of 8 patients who were admitted to our hospital to treat skin sclerosis between 2008 and 2016. All patients met the American College of Rheumatology Classification for Systemic Sclerosis (SSc) Criteria.\(^15\) They were diagnosed with dcSSc based on the criteria published by LeRoy et al.\(^16\) Ethical approval for this study (Ethical Committee N° NCGM-G-002344-00) was provided by the Ethical Committee of National Center for Global Health and Medicine, Tokyo, on July 6, 2018.

2.2. Treatment schedule

All patients were treated with an initial 20 to 40 mg/day oral prednisolone, 500 mg/body intravenous cyclophosphamide, and double-filtration plasmapheresis. The initial dosage of prednisolone was administered for 2 to 5 weeks, after which it was tapered to 2.5 to 5.0 mg/day. Intravenous cyclophosphamide was repeated bi-weekly over the treatment duration. Double-filtration plasmapheresis was performed using ACH-\(\Sigma\) (Asahi Kasei Corporation, Japan). The initial dosage of prednisolone and the frequency of intravenous cyclophosphamide or double-filtration plasmapheresis were determined by physicians’ decision based on the severity of skin lesion.

2.3. Clinical assessments

Clinical data were collected at the initiation of treatment and within a week after the last infusion of intravenous cyclophosphamide or double-filtration plasmapheresis. We analyzed the mean change in the mRSS from baseline to follow-up, the percentages of predicted forced vital capacity (\%FVC) and predicted DLCO (\%DLCO), and serum KL-6 levels. The correlations between the initial skin score severity and disease duration with the percentage of change in mRSS were also evaluated.

2.4. Statistical analysis

Pre-and post-treatment clinical values, including mRSS, \%FVC, \%DLCO, and serum KL-6 levels, were compared using the Wilcoxon signed-rank test. Correlations between the initial skin score severity and disease duration with the percentage of change in mRSS were evaluated using Spearman rank correlation coefficient. A level of \(P < .05\) was considered statistically significant.

3. Results

Table 1 presents the patients baseline characteristics. The proportion of females was 75% and the mean ± SD age at treatment initiation was 55.8 ± 9.9 years. The mean disease duration was 9.8 ± 10.0 months and the follow-up duration was 33.5 ± 11.4 days. No patient was anti-RNA (ribonucleic acid) polymerase III positive. Six patients had interstitial pneumonia. Figure 1 shows changes in the mRSS and treatment schedule for each patient. All patients were treated with an intermediate initial dose of oral corticosteroid [prednisolone 30.6 ± 2.1 mg/day (range: 20–40 mg/day)]. The mean cumulative intravenous cyclophosphamide dose was 1.4 ± 0.2 g (range: 1.0–2.0 g). Double-filtration plasmapheresis was performed at an average of 3.8 ± 0.3 times (range: 2–5 times). After 33.5 ± 11.4 days (range: 19–48 days) of follow-up, the mRSS decreased significantly from 27.0 ± 3.3 to 15.8 ± 3.5 (\(P = .03\)) (Fig. 2a). Only 1 patient (case 6), who started treatment 31 months after disease onset, failed to show mRSS improvement. No significant correlation was found between the initial skin score severity (\(P = .51\)) and disease duration (\(P = .96\)) with the percentage of mRSS change. At the end of treatment, the mean \%FVC and \%DLCO improved moderately but without statistical significance (Fig. 2b and c). KL-6 decreased from 578.9 ± 146.5 to 205.3 ± 43.1 U/ml (\(P = .02\)) (Fig. 2d). As adverse events, gastrointestinal, and vasospastic angina were reported in 1 patient each. No renal crisis was reported despite the use of prednisolone.

| Table 1 |

| Baseline demographic and clinical characteristics of the patients. |

| Age, years, mean ± SD | 55.8 ± 9.9 |
| Sex |  |
| Female, n (%) | 6 (75) |
| Disease duration, months, mean ± SD | 9.8 ± 10.0 |
| Follow-up duration, days, mean ± SD | 33.5 ± 11.4 |
| Autoantibodies present, n (%) |  |
| Anti-Scl-70 | 2/8 (25) |
| Anti-U1-snRNP | 0/7 (0) |
| Anti-Centromere | 1/8 (12.5) |
| Anti-RNA polymerase III | 0/5 (0) |
| Organ involvement |  |
| Interstitial pneumonia | 6/8 (75) |

RNA = ribonucleic acid, Scl = scleroderma, SD = standard deviation, snRNP = small nuclear ribonucleoproteins.
4. Discussion
This study shows the effectiveness of a regimen combining glucocorticoid, intravenous cyclophosphamide, and double-filtration plasmapheresis in patients with SSc. Several studies have suggested that the correlation between vascular changes and early immunological events leads to the generation of activated fibrogenic fibroblasts.\cite{16,17} Although vascular and immunologic pathways are believed to be key to the pathogenesis of SSc, the trigger for the series of events remains poorly understood. The mediating role of several cytokines in skin fibrosis has been investigated.\cite{5-8}
Considering its immunology-based pathogenesis, several immunosuppressants have been investigated in the treatment of skin sclerosis. Two randomized controlled trials examined the effectiveness of MTX for treating SSC. Pope et al reported slightly favorable results for the MTX group [mean ± standard error of the mean (SEM) mRSS 21.4 ± 2.8 in the MTX group vs 26.3 ± 2.1 in the placebo group (P = .0002)].

Several studies have also shown the efficacy of mycophenolate mofetil. A prospective open-label study reported a significant improvement in the mean mRSS at the 12-month follow-up (22.5 at study entry, 13.6 at 6 months, and 8.4 at study end). Recent studies have reported good treatment responses with rituximab or tocilizumab. Jordan et al demonstrated that rituximab improved skin sclerosis, decreased lung fibrosis progression. In that study, mRSS decreased significantly from 18.1 ± 1.6 to 14.4 ± 1.5 (P < .0002).

Another study showed the efficacy of subcutaneous tocilizumab in SSC and the mean change in mRSS from baseline was −3.1 for placebo and −5.6 for tocilizumab at week 48.

Similar to other immunosuppressants, prednisolone has been shown to treat skin involvement effectively. Sharada et al demonstrated that intravenous pulse dexamethasone was useful for treating dcSSc, decreasing the total skin score from 28.5 ± 12.2 to 25.8 ± 12.8.

Others reported that oral corticosteroids was effective for early dcSSc, decreasing the total skin score both 1 year from treatment start (20.3 ± 9.3 vs 12.8 ± 7.0) and at the final evaluation (20.3 ± 9.3 vs 8.7 ± 6.1).

However, prednisolone can precipitate a renal crisis in patients with SSC. Therefore, we measured the anti-RNA polymerase III antibody before starting treatment and closely monitored blood pressure and renal function over the course of triple therapy. None of our patients experienced a renal crisis.

Cyclophosphamide, an alkylating agent, suppresses lymphokine production, and regulates lymphocyte function. It is widely used to treat autoimmune diseases such as systemic lupus erythematosus, microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis. Tashkin et al showed that oral cyclophosphamide had a significant effect on skin sclerosis (−3.6 ± 0.8 cyclophosphamide group vs −0.9 ± 1.2 placebo group).

Regarding the long-term complications of daily oral therapy, intermittent cyclophosphamide, usually administered intravenously every 2 to 4 weeks, is the treatment used most commonly for systemic rheumatic diseases.

Although prednisolone and cyclophosphamide alone can effectively treat skin sclerosis, several studies have reported the efficacy of their combined use. Valentini et al reported a significant improvement in mRSS and DLCO after treatment with intravenous cyclophosphamide (500mg/pulses) and low-dose prednisone (10mg/day prednisone equivalent) in patients with early dcSSc. In this study, mRSS decreased from 23 to 10 after 12 months of treatment.

Therapeutic apheresis is used to treat conditions in which a pathogenic molecule is present in the blood. Several reports have demonstrated its efficacy for treating SSC. In a 2-year prospective trial in which 15 patients (12 with dcSSc) were treated with a combination of prednisolone (20–40mg/day), oral cyclophosphamide (2–2.5mg/kg/day), and plasmapheresis, Akesson et al reported that the patients showed a faster improvement in skin score than those treated without plasmapheresis. Similarly, we found that mRSS improved within 33.1 ± 10.9 days, providing further evidence that the combination of plasmapheresis and immunosuppressants contributes to an immediate effect. We performed double-filtration plasmapheresis rather than simple plasma exchange. The main disadvantage of simple plasma exchange is the nonselective removal of all plasma proteins; thereby, requiring a replacement fluid. Therefore, it carries a risk of infection from fresh frozen plasma, or protein allergy to the replacement fluid, which is not required in double-filtration plasmapheresis.

After being separated by the first filter, the plasma is then fractionated into large- and small-molecular-weight components by the second filter. Small molecules such as...
albumin are returned to the patient, minimizing albumin loss and the risk of infection or allergic reaction from fresh frozen plasma.

In this study, the dose of prednisolone, intravenous cyclophosphamide/number of double-filtration plasmapheresis and follow-up period varied between patients. The dose of prednisolone was determined by physicians' decision based on patients body weight, and it ranged from 0.4 mg/kg to 0.7 mg/kg. Three cases (Case3, Case 6, and Case7) were administered intravenous cyclophosphamide only twice. Several reasons were behind this.

1. Due to past administration of intravenous cyclophosphamide for the pulmonary involvement in before triple therapy, Case 3 was given intravenous cyclophosphamide only twice considering the total amount of cyclophosphamide received;
2. Because we observed signs of efficacy on skin involvement in the early phase of treatment, Case 7 was treated with intravenous cyclophosphamide only twice;
3. For Case 6, double-filtration plasmapheresis was stopped because of vasospastic angina; thus, intravenous cyclophosphamide was also stopped taking its cardiac toxicity into consideration. Since mRSS was scored only during hospitalization, follow-up period varied among patients.

Although disease duration did not correlate with the percentage of mRSS change, 1 case showed no signs of improvement. This patient was treated 31 months after onset, which was the longest period from onset among the included cases. This result is consistent with a report suggesting that the opportunity to prevent fibrosis in dcSSc occurs during the early inflammatory phase.[22] In short, treatment for skin sclerosis should be initiated during the early disease phase.

Regarding respiratory function, both %FVC and %DLCO improved slightly, although there was no statistical significance. Only the serum KL-6 level improved significantly. Several studies have demonstrated the effectiveness of cyclophosphamide for lung involvement in SSc patients.[23,24] KL-6 has been recognized as a predictor of early progression in SSc-related interstitial lung disease.[25] Besides, the inclusion of cyclophosphamide in the treatment protocol is expected to have an effect on lung lesions. Indeed, in this study, a decrease in KL-6 was observed after treatment.

Our study has several limitations. First, this is a retrospective study, which results in some missing data regarding respiratory functional tests. Since spontaneous skin score improvement may occur in early SSc, the single-arm trial could not properly evaluate the efficacy of the triple therapy. Additionally, the number of patients enrolled in this study is small. A larger, randomized placebo-controlled trial is desirable to assess the effect of this therapy. Again, we only evaluated KL-6 as a prognostic parameter, with the exception of other serum markers such as IL-6, PDGF, and TGF-β, which are considered potential fibrosis effectors of SSc. Finally, it is thought that the prevalence of renal crisis in Japanese patients is lower than in other countries,[26] therefore, the risk of renal crisis might have been underestimated.

In conclusion, this preliminary study demonstrates the potential of triple therapy for skin sclerosis in patients with dcSSc. The advantages of triple therapy are its immediate effect and equal or superior effectiveness to treatments with other immunologic agents. Although a larger, prospective, and placebo-controlled trial is needed to evaluate the effectiveness of triple therapy, our results suggest that it might achieve good clinical results in patients with SSc.

Author contributions
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