Scleroderma Lung Fibrosis and Biologic Drugs

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Abstract

Biologic agents have revolutionized the management of some inflammatory diseases like rheumatoid arthritis, seronegative spondyloarthropathies and Crohn's disease; the selective inhibition of mediators involved in systemic chronic processes have allowed to expand the knowledge among the complex pathogenic pathways that are involved in inflammation and damage.

The off-label use of biologic drugs is expanding. This review highlights the theoretical basis that may support the use of biologic drugs in the treatment of Systemic Sclerosis (SSc), a disabling and sometimes devastating disease whose treatment remains largely unsatisfactory. Some progresses have been made concerning the vascular complications of the disease, but the skin and visceral fibrosis, which is the hallmark of the disease, is still orphan of effective therapy. Moreover the results of the limited clinical experience about biologic agent administration in SSc and SSc-interstitial lung disease are presented.

Introduction

Interstitial lung disease often complicates the course of Systemic Sclerosis (SSc), mainly in the diffuse subset with anti-Scl70 antibody positivity. Severe interstitial lung disease usually develops during the early phase of the disease and it negatively impacts on survival; in fact lung fibrosis together with pulmonary artery hypertension is the most frequent cause of SSc-related death [1]. Therefore evaluation of lung involvement, close monitoring of lung function and treatment of pulmonary interstitial disease are among the principal tasks in the management of SSc.

Cyclophosphamide is the only drug that has demonstrated a small but significant benefit in the treatment of scleroderma interstitial lung disease; the drug orally administered for 1 year in comparison with placebo ameliorated lung function and dyspnoea index together with skin thickening and quality of life [2]. However the 1 year follow-up after stopping treatment showed that the beneficial effects of cyclophosphamide on lung function persisted until 18 months and then waned, whereas the positive effect on dyspnoea was still present at the end of the follow-up [3]. The toxicity of the drug prevents a more prolonged duration of the treatment.

At present no evidence of efficacy exists for either other immune suppressants or antifibrotic agents, in spite of exciting data obtained in experimental models with drugs like imatinib [4] and bosentan [5].

Resources should be allocated and aggressive searches have to be conducted to develop better therapeutic strategies to counteract this fearful complication.

The Era of Biologics

During the last decade many biologic drugs have become available for the treatment of both rheumatic and non-rheumatic diseases; they selectively block one cytokine as TNFα, IL-1 or IL-6 by a direct binding with a mechanism antigen/antibody or ligand/receptor or by the inhibition of its receptor or they act by blocking a specific molecule exposed on cellular surface as CD20 or by inhibiting the costimulatory signal.

The results obtained in the treatment of rheumatoid arthritis and seronegative spondyloarthropathies have favored the extension of the use of the biologic drugs in the management of other systemic rheumatic disorders, sometimes with brilliant results as, for example, in case of severe or refractory Behcet's disease by anti-TNFα agent administration [6].

We review the pathogenetic role of proinflammatory cytokines targeted by the licensed biologic drugs as well as the role of B cells in scleroderma damage and the clinical data concerning the use of biologics in SSc with particular attention to interstitial lung involvement.

Pathogenic role of TNFα and anti-TNFα blockers

There are *in vitro* many evidences of an antifibrotic effect of TNFα. TNFα induces a dose-dependent inhibition of type I and type III collagen and fibronectin synthesis in human dermal fibroblasts [7]. Another study conducted on the same cell type showed that TNFα interferes with TGFβ signaling cascade through activation of AP-1 transcription factors forming complexes with Smad3 within cell nucleous that compete with the binding of Smad3 to DNA [8].

Moreover many *in vitro* studies on human fibroblasts obtained at different sites demonstrated that TNFα interacts with the process of matrix degradation by inducing the synthesis of matrix metalloproteinases (MMPs) and reducing the production of tissue inhibitors of matrix metalloproteinases (TIMPs). TNFα stimulates the synthesis of collagenase in dermal and synovial fibroblasts [9], promotes the synthesis of collagenase, gelatinase and stromelysin together with inhibition on TIMPs in gingival and uterin cervical fibroblasts [10,11], downregulates TIMP production and upregulates MMP1 and MMP2 expression in vocal fold fibroblasts [12], promotes the synthesis of MMP2 in lung fibroblasts [13]. Analogous induction of MMPs by TNFα was observed in other cell types as cardiomyocites and cardiomyofibroblasts [14], confirming a role of TNFα in cardiac remodeling. These multiple observations clearly showed that TNFα increases the MMP/TIMP ratio, favouring collagen degradation.

In addition TNFα is able to suppress the TGFβ-induced release of CTGF, one of the crucial mediators of fibrosis in SSc [15].

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Together these results indicate that TNFα plays an antifibrotic role either inhibiting the synthesis of collagen or modulating the degradation of matrix deposition by fibroblasts.

On the other hand these data contrast with other observations that suggest instead a profibrotic effect of TNFα. A recent *in vitro* study showed that TNFα induces a profibrotic phenotype in murine intestinal fibroblasts promoting collagen synthesis, inhibiting MMP2 activity and overexpressing TIMPs [16]. Another investigation on a murine fibroblast cell line indicated that TNFα promotes fibrosis by induction of TGFβ expression [17].

These contrasting data of the effects of TNFα on fibrosis were also observed in *in vivo* models of tissue fibrosis, as recently reviewed [18]. Transgenic mouse overexpressing TNFα were resistant to develop lung fibrosis induced by TGFβ or after exposition to bleomycin [19]. Other investigators instead reported that TNFα-transgenic mouse developed a chronic lymphocytic alveolitis whose histologic examination resembled that of idiopathic lung fibrosis [20].

The discrepant results of these experimental data might be interpreted as the different effect of TNFα on different cell types or organs; another interesting hypothesis is that TNFα may explicate either profibrotic or antifibrotic effects according to the presence or to the absence of an inflammatory component, respectively [18]. Translating these contradictory results into the clinical setting anti-TNFα blockers might be useful in the very early phase of the SSc when inflammatory infiltrates are present in involved tissues as skin and lung, but not in case of established disease when the inflammatory tissue component dissipates and fibrosis may progress in the lack of a proinflammatory stimulus [18]; therefore in longstanding disease anti-TNFα may exaggerate established fibrosis.

In vivo data show evidence of an increased level of serum TNFα in both limited and diffuse forms of SSc which correlates with the presence of pulmonary fibrosis [21]; in addition alveolar macrophages of patients with scleroderma pulmonary disease produce bigger amount of TNFα than those of both patients affected by SSc with no lung involvement or healthy controls [22]

No randomized clinical trials have been conducted on the use of anti-TNFα blockers in patients affected by SSc; only small case series and single cases have been reported. In the majority of the cases arthritis was the main indication.

Etanercept has been administered to 18 SSc patients, 6 with diffuse and 12 with limited form of the disease, all with joint involvement for a period varying from 2 to 66 months obtaining clinical benefit in 15 cases; forced vital capacity (FVC) and diffusion lung capacity for carbon monoxide (DLCO) values slightly declined during treatment but in a similar manner as SSc patients who did not receive the drug. The Authors concluded that these data likely reflect the natural decline of lung function due to SSc and not secondary to etanercept treatment. However etanercept was withdrawn in two patients; in one case for severe deterioration of lung function and in the second one for the appearance of a lupus-like syndrome [23].

Bosello et al. treated with anti-TNFα inhibitors 4 patients affected by longstanding SSc, all with lung involvement and erosive polyarthritis, 2 with a diffuse subset and 2 with a limited subset. At first infliximab was given at the dosage of 3 mg/Kg respectively at weeks 0, 2, 6 and 14, followed by etanercept 25 mg twice a week; after 6 months the authors observed a strong reduction of skin thickening in all cases together with healing of digital ulcers (2 cases) and amelioration of hand motion in one case with severe joint contractures [24]. Unlucky the study did not report any data concerning lung function evaluation before and after the treatment. In the same way no data on lung function were reported in an open-label study on infliximab treatment that was given to 16 patients affected by diffuse SSc, characterized by a mean disease duration of 15.7 months, a progressive skin involvement, the absence of anticentromere antibodies and the lack of significant pulmonary or heart involvement; as a matter of fact FVC < 55% of predicted values or estimated left ventricular ejection fraction < 50% were exclusion criteria. The drug was administered at the dosage of 5 mg/Kg respectively at weeks 0, 2, 6, 14 and 22. Skin involvement did not significantly reduce at 26 weeks whereas fall of serum level of aminoterminal propeptide of type III collagen as well as reduced release of type I collagen by dermal fibroblasts was registered; however the expression of TGFβ1 in cutaneous biopsy of involved skin was more intense after treatment in comparison with baseline and the serum level of IL-2 receptor resulted unchanged, suggesting that infliximab does not interfere with T-cell activation in SSc. But the most interesting data observed in this study concerned the safety. First of all during the trial serious adverse events directly attributable to SSc were reported like development of renal crisis, severe Raynaud's phenomenon, digital ischaemia, infected digital ulcers and atrial fibrillation. In addition, in spite of relatively high dose of the drug, 5 cases developed anti-infliximab antibodies with neutralizing activity that were significantly correlated with infusion reactions; moreover these antibodies may have interfered with drug efficacy [25]. On the ground of this experience the co-administration of methotrexate with infliximab as a new future experience on treatment of SSc may be warranted.

The effect of anti-TNFα inhibitors on skin involvement of the two above-mentioned studies [24,25] were contradictory, but the limits of the modified Rodnan skin score (mRSS) in the evaluation of cutaneous thickening are well known; in addition the frequent spontaneous amelioration of skin involvement in the diffuse subset of the disease raises a matter in the evaluation of the data.

Two SSc patients died from pulmonary involvement during treatment with anti-TNFα antagonists; one patient with lung fibrosis treated with adalimumab showed a rapid progression of fibrosing alveolitis with worsening of the lung function and died from respiratory distress without infective complications [26]; another subject developed a fatal pulmonary actinomycosis during infliximab treatment [27]. Similarly dramatic worsening of lung function was observed in cases of asymptomatic pulmonary fibrosis secondary to rheumatoid arthritis in the lack of infection or other causes of respiratory decline during infliximab treatment [28].

On the other hand a SSc patient with severe interstitial lung disease and pulmonary hypertension showed a stabilization of lung function tests and pulmonary fibrosis as assessed by high-resolution computed tomography after 1 year of infliximab administration; the withdrawal of the drug, due to personal reasons, was followed by a dramatic progression of pulmonary involvement and the patient died 11 months later [29].

Therefore the few clinical data on the use of anti-TNFα blockade in scleroderma lung involvement are contradictory.

Considering both experimental and clinical results there is some concern about the use of anti-TNFα blockers in SSc, above all in case of interstitial lung disease; a thorough safety evaluation is imperative. Recently an EUSTAR expert consensus did not recommend the routine use of anti-TNFα inhibitors in SSc, because there is no evidence that these drugs may successfully counteract fibrotic process; arthritis may benefit from such treatment and therefore may be a potential indication [30].

Pathogenic role of B cells and rituximab

During the recent years B cells have been recognized as one of the multiplayers involved in the complex pathogenesis of scleroderma damage.

B cells were found in skin biopsy of SSc patients, mostly located around small vessels, whereas there is no evidence of the presence of B cells in skin of normal subjects [31]. Moreover B cell infiltration was found in scleroderma interstitial lung disease [32].

The number of peripheral B cells is increased in SSc patients; in addition abnormal B cell compartments were demonstrated, which are characterized by an increase of naïve B cells and a reduction of memory B cells; both the subsets overexpress CD19, a pivotal regulator of B cell maturation [33]. Also B cells release IL-6, a cytokine which shows a profibrotic effect [34] and contributes to the development of a Th2 milieu [35].

B lymphocytes produce autoantibodies; in almost all SSc patients autoantibodies are detected in serum and some of them might play a pathogenic role, as antibodies against endothelial cells [36], platelet derived growth factor receptor [37], fibroblasts [38] and topo-isomerase I [39].

In SSc the levels of B-lymphocyte stimulator (BLyS), a potent B cell survival factor, are elevated, above all in diffuse subset and correlate with disease severity [40]. BLyS might represent a possible therapeutic target; its inhibition may modulate overexpression of B cells in SSc.

Additionally, there is a robust body of evidence that B cells are implicated in animal models of scleroderma, like tight-skin mice, as recently reviewed by Daoussis et al. [41]. These experimental and clinical data suggest that B lymphocytes are involved in skin and lung scleroderma damage and prompted many research groups to use rituximab, a chimeric monoclonal antibody against human CD20, in the treatment of SSc.

An open label, randomized controlled study was conducted to evaluate the efficacy of rituximab in scleroderma patients with interstitial lung disease. Besides pulmonary involvement all the enrolled patients had a diffuse pattern of the disease, the positivity for anti-Scl70 antibody and a stable therapy during the previous 12 months. Rituximab was administered at the dosage of 375mg/m^2 once a week for 4 consecutive weeks at baseline and repeated after 24 weeks. Eight patients were randomized to rituximab treatment and 6 to placebo. After 1 year in the rituximab arm lung function improved: FVC increased from 68.13 ± 19.69 to 75.63 ± 19.73% of predicted values, p = 0.0018 and DLCO increased from 52.25 ± 20.71 to 62 ± 23.21% of predicted values, p = 0.017; in the placebo control group both parameters slightly declined. The comparison of FVC and DLCO changes after 1 year revealed that patients receiving rituximab significantly improved with respect to control group. None of the patients receiving rituximab exhibited a deterioration of lung function, whereas 5 out of 6 control cases showed worsening of FVC and/or DLCO. In addition skin thickening evaluated by mRSS, collagen deposition in skin specimens and health assessment quality all ameliorated in rituximab treated patients but not in the placebo arm [42]. Interestingly, lung function tests of the 8 cases who were given rituximab continued to improve after 2 additional courses of the drug [43], suggesting that repeated cycles may allow to obtain increasing benefit, as it was shown in rheumatoid arthritis [44]. The limitations of this experience were the small number of the enrolled patients and the heterogeneity of the cases, concerning disease duration, disease severity, previous and concomitant treatments.

The amelioration of lung function after rituximab therapy was also reported in three single SSc cases [45,47].

Three open-label trials on rituximab treatment of SSc globally enrolled 32 patients, all affected by diffuse subset of the disease [48,50]; all but 2 cases had a disease duration less than four years. Patients with severe organ involvement were excluded. Rituximab was given at the dose of 1 g two weeks apart. Stable lung function tests were observed in all the 3 studies; during the treatment none of the patients developed severe lung, heart or renal involvement. This result is noteworthy considering that patients with early diffuse disease have a higher risk of developing severe visceral complications. Skin involvement evaluated by mRSS ameliorated in two studies [48,49], whereas it was reported stable in the third experience [50]. In addition other interesting results were reported after rituximab administration: the decrease of serum IL-6 level [49], the reduction of activity index [49], the skin depletion of B cells [50], the fall of the number of dermal myofibroblasts [48,50] and the decrease of the hyalinised collagen in skin biopsies [48].

In all the studies rituximab treatment resulted well tolerated: Even if these preliminary experiences did not furnish unequivocal results, it is interesting to point out that lung function ameliorated or remained stable during rituximab treatment and these results are noteworthy considering that pulmonary involvement is the major cause of morbidity and mortality in SSc patients. Large controlled randomized trials comparing rituximab to placebo or to cyclophosphamide are required to better understand the role of B cell depletion therapy in the context of a disease like SSc characterized by a great clinical heterogeneity; patients with early diffuse disease should be the best target. Moreover the potential therapeutic effect of the combination of rituximab plus cyclophosphamide or mycophenolate should be explored as well as the possible role of a sequential therapy. Moreover the best dosage, the indication to repeated courses and the duration of the treatment should be defined.

Pathogenic role of IL-6 and tocilizumab

IL-6 is a multifunctional cytokine which is implicated in the pathogenesis of multiple chronic inflammatory diseases; it is essential for B cell differentiation, induces acute phase response and shows a profibrotic effect stimulating the production of collagen and glycosamonoglycan.

Many investigations indicate that IL-6 may be involved in the pathogenesis of SSc. IL-6 levels were found to be increased in serum of SSc patients; the values were higher in diffuse versus limited subset of the disease [34] and in patients with alveolitis in comparison with patients without lung involvement [51]. In addition IL-6 levels resulted inversely correlated with vital capacity values [52]. Moreover immunochemistry evaluation on skin biopsies showed that IL-6 expression is increased in endothelium and fibroblasts of SSc patients in comparison with normal subjects [53] and cultured fibroblasts of SSc subjects produce more IL-6 than controls [54]. Also IL-6 serum concentration correlated with skin thickening evaluated by mRSS [55]. Additionally *in vitro* experiments showed that IL-6 blockade by a IL-6 antibody provoked a strong reduction in procollagen type I production by cultured SSc fibroblasts [56].

To the best of our knowledge, only one very limited experience was reported concerning the administration of tocilizumab in systemic sclerosis [57]. Two patients affected by diffuse SSc received tocilizumab 8 mg/kg every 4 weeks for 6 months. The main visceral involvement was chronic renal failure due to scleroderma renal crisis in one case and pulmonary fibrosis in the other one. Both the cases experienced skin involvement amelioration, as assessed by mRSS and by Vesmeter, a device that can measure the physical property of the skin; moreover after treatment cutaneous biopsy with immunohistochemistry

evaluation showed thinning of the dermal collagen fibre bundles and decrease of the number of αSMA positive cells, a molecule expressed on myofibroblasts, the mesenchymal cell type most responsible for the excessive extracellular matrix component production in tissue of SSc patients. The kidney function of the first patient ameliorated during tocilizumab administration, whereas the lung function of the second patient remained stable. Side effects were not registered.

Other Biologic Drugs

IL-2 receptor levels are increased in SSc patients in comparison with normal controls and the serum concentration is strongly associated with mortality [58]; in addition evaluation of cytokines in bronchoalveolar lavage fluid showed that IL-2 receptor is detectable in patients with pulmonary fibrosis secondary to SSc and that high levels of IL-2 together with TNFα predict progressive and end-stage lung involvement [59].

Basiliximab is a chimeric monoclonal antibody that selectively inhibits αchain of IL-2 receptor expressed on activated T and B cells. The drug has been approved for the treatment of kidney allograft rejection; moreover it has been successfully used in steroid-resistant graft-versus host disease [60], a disorder that exhibits some similarities with SSc.

A small open-label trial on the use of basiliximab in SSc has recently been published by Becker et al. [61], after a previous single case reported by the same group [62]. Ten patients with anti-Scl70 positive, rapidly progressed diffuse disease in an early phase were enrolled; all patients had a stable immunosuppressive and concomitant medication. Pulmonary fibrosis was present in 8 cases. Basiliximab was administrated for 6 months; the data are available for seven cases. Concerning lung involvement globally both FVC and DLCO slightly ameliorated; the authors underlined that 4 cases showed an increase of FVC > 10% and 2 cases an increase of DLCO > 10%. Moreover skin thickening reduced. The drug was quite well tolerated.

Clinical experience concerning the use in SSc patients of abatacept, a co-stimulation blocker agent, are not reported, but a randomized, double-blind, placebo-controlled clinical study is ongoing (http://clinicaltrials.gov/ct2/show/NCT00442611?term= abatacept +scleroderma&rank=1).

Conclusions

The progress in the understanding of the pathogenic mechanisms involved in inflammatory rheumatic disease led to the development of several biological drugs. With regards to SSc treatment rituximab seems to be the drug with the strongest rational and with the most promising clinical data of efficacy among the available biologic agents. During the last years the management of specific organ-based complications has ameliorated with the introduction in clinical practice of new drugs, in particular pulmonary vasodilators. Nevertheless a therapy which may halt the development/progression of fibrosis is still lacking.

Take Home Messages

Scleroderma management needs a therapeutic strategy able to prevent/reverse fibrosis.

Among the biologic drugs borrowed by rheumatoid arthritis paraphernalia rituximab seems to be the most promising agent.

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