Cyclophosphamide reduces neutrophilic alveolitis in patients with scleroderma lung disease: a retrospective analysis of serial bronchoalveolar lavage investigations
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Objectives: To determine whether cyclophosphamide is beneficial for patients with scleroderma lung disease (SLD).
Methods: The effect of 6 months' treatment with intravenous cyclophosphamide on the functional capacity of patients, lung function tests, high resolution computed tomography of the lungs, and cytology of bronchoalveolar lavage was evaluated in 21 patients with SLD.
Results: The treatment was well tolerated and all patients completed 6 months' treatment. Intravenous cyclophosphamide stabilised or improved the patients' functional status and lung function tests. The extent of the lungs affected remained unchanged, as assessed with HRCT of the lungs. Patients with SLD and neutrophilic alveolitis (NA) showed greater improvement than patients with normal levels of granulocytes in the bronchoalveolar lavage fluid (BALF). Significant reduction of neutrophils was also seen in the patients with SLD and NA, whereas no significant change was seen in the level of granulocytes in patients with SLD and an initially normal percentage of granulocytes.
Conclusions: Previous reports that patients with SLD with increased levels of granulocytes in BALF are more likely to benefit from treatment with intravenous cyclophosphamide are confirmed. Additionally, clinical improvement in this group of patients is accompanied by a significant decrease in the percentage of granulocytes in BALF.

Scleroderma interstitial lung disease (SLD) is the main cause of death in patients with systemic sclerosis (SSc). Although the pathophysiology of SLD is not clear, histological examination and analysis of bronchoalveolar lavage fluid (BALF) have shown the presence of inflammation in the lower respiratory tract of patients with SLD. Moreover, an increased percentage of granulocytes in BALF obtained from patients with SSc has been shown to predict greater decline in lung function tests over time, and a worse clinical prognosis.

Based on the inflammatory hypothesis of the pathogenesis of SLD, cyclophosphamide (CYC) was introduced for treatment of SLD. It has been shown to stabilise or improve lung function and reduce mortality in patients with SLD and alveolitis.

Although CYC is believed to exert its beneficial effects through inhibition of inflammation within the respiratory tract, clear evidence for this effect is still lacking. This study aimed at evaluating changes in BALF in patients treated with CYC owing to SLD.

PATIENTS AND METHODS
Patients
We included 21 non-smoking patients in the study, who received intravenous CYC owing to SLD, and evaluated their pulmonary disease before and after 6 months' treatment. All patients fulfilled the American College of Rheumatology preliminary classification criteria for SSc. Evaluation of the pulmonary disease included estimation of exercise capacity (according to New York Heart Association (NYHA) classification), high resolution computed tomography (HRCT) of the lungs, lung function tests, and BALF analysis. Based on HRCT of the lungs, patients were classified as having predominantly ground-glass opacification, predominantly honeycombing, or mixed pattern. The extent of the disease was evaluated as the percentage of abnormal lungs, as described elsewhere.

The patients had not received immunosuppressive or corticosteroid treatment for more than 5 years before CYC was started. Patients with evidence of respiratory infections were excluded.

In all patients antinuclear and anticentromere antibodies were evaluated using an indirect immunofluorescence test and anti-Scl-70 antibodies by means of an enzyme linked immunosorbent assay (ELISA).

Cyclophosphamide treatment
CYC was given based on the presence of SLD features on HRCT scans of the lungs, plus at least one of the following: significant (>10% of initial value) decrease in forced vital capacity (FVC) within the past 6 months or the presence of neutrophilic alveolitis (NA) as judged by cytological analysis of BALF. CYC was given intravenously, 1.0 g every 30 days for 6 consecutive months. In addition, patients received prednisone (<10 mg/day).

Full blood cell count, urine analysis, and determination of the erythrocyte sedimentation rate (ESR) were carried out before the first infusion of CYC and repeated monthly during the treatment. C reactive protein (CRP) was measured before the first infusion and 1 month after the last (sixth) infusion of CYC.

 Bronchoscopy with bronchoalveolar lavage
Bronchoalveolar lavage was performed with the informed consent of the patient as a part of the routine clinical evaluation of patients with SSc, as previously described.

ABBREVIATIONS: BALF, bronchoalveolar lavage fluid; CRP, C reactive protein; CYC, cyclophosphamide; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HRCT, high resolution computed tomography; NA, neutrophilic alveolitis; NYHA, New York Heart Association; SLD, scleroderma lung disease; SSc, systemic sclerosis

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Differential counts of BALFs were made from the cytospin samples after staining with May-Grunwald-Giemsa stain.

Alveolitis was diagnosed when the percentage of neutrophils in the BALF was >3.0%, or the percentage of eosinophils was >2.5%, or both. These cut off values are in agreement with the American Thoracic Society guidelines as well as the reference values obtained in our laboratory.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U test, Fisher’s exact test, and Wilcoxon’s matched pairs test. Statistical analysis was performed using the Mann-Whitney U test.

RESULTS

Patient characteristics

Table 1 presents the clinical characteristics of patients with SLD.

At baseline, cytological analysis of BALF showed increased percentages of neutrophils in 13/21 (62%) patients. The percentage of eosinophils was increased in two patients only, and in each case this was accompanied by an increased percentage of neutrophils. The remaining 8/21 (38%) patients had normal percentages of granulocytes in their BALFs.

The sex, age, disease duration, disease subset, functional capacity, FVC, and serological status of the patients with NA did not differ significantly from those without NA. HRCT of the lungs disclosed honeycombing as the main pattern in only 1/13 patients with NA, and ground-glass opacification as the main pattern in 1/8 patients without NA only. In the group of patients with NA the mean CRP value was significantly higher than in those without NA, although there were no significant differences in the mean ESR values between the groups.

Results of treatment with cyclophosphamide

All patients completed the 6 months’ course of CYC. Side effects included nausea (n = 10) and hair loss (n = 1). In one patient, a woman age 45, introduction of CYC was associated with amenorrhoea.

Evaluation of functional capacity according to the NYHA classification showed that after treatment with CYC 13 patients remained stable, whereas the remaining eight improved. Functional capacity did not deteriorate in any of
The beneficial effect of CYC treatment was seen predominantly in the group of patients with NA. In this group significantly fewer patients were classified as NYHA class III or IV after the treatment (reduction from nine to three patients, Fisher's exact test; p<0.05).

CYC treatment significantly improved the FVC in the total group of patients with SLD. The FVC of patients with NA improved significantly, whereas the FVC in the patients without NA remained stable. The percentage of the lung affected, as evaluated by HRCT, did not change significantly in any of the groups studied.

CYC treatment significantly reduced the ESR and CRP values in the whole group of patients with SLD as well as in those with and without NA (table 1).

**DISCUSSION**

Several studies have shown that CYC treatment stabilises or improves lung function in patients with SLD. It has also been shown that patients with SLD with alveolitis benefit from treatment with CYC more than those without alveolitis. It is presumed that the beneficial effect of CYC in patients with alveolitis is due to inhibition of inflammation within the lower respiratory tract, but few studies have included serial BALF analysis in patients with SLD. Recently, we have shown that treatment with intravenous CYC significantly decreased the total cell number and the percentage of granulocytes in six patients with NA due to SLD. The significant decrease in the percentage of neutrophils was accompanied by a significant decrease in the concentration of leukotriene B4, which is a strong chemokinetic factor for neutrophils.

In this study, we compared changes in the total cell number, the percentages of neutrophils and eosinophils in BALF from another five patients treated with CYC owing to SLD. Schnabel et al found a significant reduction in the percentages of granulocytes in six patients receiving CYC because of interstitial lung disease related to collagen vascular diseases, including two patients with SLD. We found no significant changes in HRCT scans of the lungs after treatment, which may be a result of the relatively high number of patients with fibrotic changes. Cytological analysis of BALF showed a significant reduction in the total number of cells in the BALF after treatment with CYC, which is in agreement with a previous report of Varai et al. Like Giacomelli et al, we found no significant change in the percentages of granulocytes in the whole group of 20 patients. However, when patients with NA were analysed separately, a significant reduction in the percentages of neutrophils and eosinophils was shown. In contrast, in a group of patients without NA there were no significant changes in the percentages of neutrophils.

Together with the significant reduction of granulocytes in the BALF, there was a significant improvement in functional capacity and FVC in patients with NA, whereas in the group of patients without NA functional capacity and lung function remained stable. This is in agreement with the results of White et al, who showed significant improvement in lung function and survival after CYC treatment in patients with NA in comparison with those without NA.

We also found that serum CRP levels were significantly higher in patients with NA than in those without, although the ESR values did not differ significantly between the two groups. Therefore, our results suggest that CRP, as a more specific biochemical marker of inflammation, may be helpful in identifying patients with alveolitis. Our patients with NA improved after CYC treatment, which is in agreement with a previous report of Akesson et al, who showed that patients with SLD with raised acute phase protein levels are more likely to benefit from treatment with CYC. Like Akesson et al, we observed a significant decrease in CRP and ESR levels after treatment with CYC.

In summary, our study suggests that one of the mechanisms responsible for the beneficial effect of CYC in SLD is reduction of NA in the lungs.
References