Cyclosporin A and iloprost treatment of systemic sclerosis: clinical results and interleukin-6 serum changes after 12 months of therapy


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Abstract

Objectives. The main aim was to analyse the long-term therapeutic effects on systemic sclerosis (SSc) patients of treatment with either (i) iloprost alone or (ii) low-dose oral cyclosporin A (CyA) associated with iloprost. A secondary aim was to analyse interleukin-6 (IL-6) serum levels in SSc patients before and after 1 yr of treatment.

Methods. A clinical trial was performed in which 20 consecutive SSc patients were alternately randomized into two homogeneous groups receiving either monthly i.v. iloprost (1 ng/kg/min in 6 h i.v. infusion, for 5 consecutive days, 1 week per month) (Group I) or low-dose CyA (2.5 mg/kg/day) associated with iloprost administration (Group II). IL-6 concentrations were evaluated by ELISA in the sera of each patient before and after 1 yr of therapy and in 20 healthy subjects.

Results. After 1 yr of therapy, a significant improvement of skin (~P = 0.008), microvascular (~P = 0.004) and oesophageal (~P = 0.05) morphological and functional parameters was observed only in Group II patients. Furthermore, after 1 yr of treatment, a significant reduction (~P = 0.007) of IL-6 serum concentration was observed only in Group II patients.

Conclusions. Collectively, our data suggest that the combination of low-dose CyA with iloprost administration may be of clinical utility in SSc and that a mechanism of action of CyA in SSc may include the decrease in IL-6 production.

Key words: Systemic sclerosis, Cyclosporin A, Iloprost, Interleukin 6.

Systemic sclerosis (SSc) is a chronic inflammatory disease characterized by fibrosis of the connective tissue, and involves skin, small vessels and internal organs [1]. Recently, clinical trials have been performed to evaluate the efficacy of immunosuppressive and vasodilator drugs, since microvascular lesions and abnormalities of the immune system have been recognized as being the most precocious alterations [2, 3]. In particular, the effects of cyclosporin A (CyA) as an immunosuppressive agent, and iloprost, as a synthetic analogue of prostaglandin I2, inducing preferential microvascular dilatation, have been analysed.

CyA has been reported to improve both SSc skin lesions [4] and oesophageal function [5]. Unfortunately, administration of ~3 mg/kg/day of CyA elicited concerns due to its renal toxicity [4]. Questions remain unanswered concerning: (a) the optimal CyA dosage in SSc, as lowering the CyA dosage may achieve a decrease in the incidence of side-effects while maintaining the therapeutic effects; (b) the mechanisms of CyA pharmacological action in SSc.

Regarding the latter topic, CyA is known to affect the transcription of genes encoding several cytokines [interleukin (IL)-2, IL-3, IL-4, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF)] [6–8], some of them involved in the pathogenesis of SSc [9]. In particular, IL-6 possesses fibrogenic and immunostimulatory effects.
The variations of IL-6 serum concentrations, in the two mean total NCV score for each subject was calculated
ability and effectiveness. Twenty SSc patients were randomized to receive treatment with either iloprost alone or in association with low-dose (2.5 mg/kg/day) long-term CyA. The tolerability and efficacy of both therapeutic regimens, and the variations of IL-6 serum concentrations, in the two groups of SSc patients are reported.

Patients, materials and methods

Patients

Twenty consecutive patients (12–65 yr, 18 females and two males) affected by SSc were enrolled in the study at the time of their first admission to the Department of Internal Medicine. All patients fulfilled the ACR criteria for classification of SSc [17]. Patients had been affected by diffuse (16 patients) or limited (four patients) forms of SSc for no more than 2 yr. None were being administered either steroid therapy or immunosuppressive drugs. All patients were affected by Raynaud’s phenomenon. Consecutive patients were alternately randomized into two groups which were matched for age, sex and disease pattern (constituted by eight patients with diffuse and two patients with limited SSc). Group I were administered monthly iloprost (1 ng/kg/min in 6 h i.v. infusion, for 5 consecutive days, 1 week per month), while Group II received the same iloprost regimen as Group I as well as oral CyA (2.5 mg/kg/day).

The study was approved by the ethical committee of the Department of Internal Medicine at the University of Genoa and all participants gave informed consent.

Clinical evaluation

Complete clinical evaluations were performed before the beginning of treatment (baseline time or T0) and at the end of 12 months of therapy (end time or T12).

We utilized a battery of tests to evaluate cutaneous, oesophageal, pulmonary, renal and cardiac functions, as well as the nailfold microvascular pattern.

In particular, cutaneous involvement was analysed by plicometry because this procedure seems to guarantee better reproducibility and reduced variability than other skin scoring systems such as the ‘Rodnan’ score, as recently described [18]. A total plicometer skin score was assigned to each patient on the basis of the scores obtained by measuring the plica of each skin area (Table 1).

The small-vessel architecture was studied by nailfold videocapillaroscopy (NCV) [19, 20]. In keeping with previous classifications, the NCV ‘scleroderma patterns’ were divided into three groups, as follows: ‘early’, ‘active’ and ‘late’ [20]. Change in shape (ramified capillaries), altered arrangement of the capillary loops (altered vascular architecture) and decrease in the number of capillaries were chosen as NCV parameters for the scoring of SSc patients. These measures show a progressive evolution during the natural course of the disease. Furthermore, in our experience, the variability index of such an NCV score is <10% when considering both the interassay variation (among tests repeated at short time intervals) and the intra-assay variation (among measurements obtained by different operators at the same time). We adopted a semiquantitative rating scale assigning a score of ‘0’ to the absence of NCV changes and scores of ‘1’, ‘2’, ‘3’ to minimal, intermediate and maximal abnormal changes, respectively [20]. The mean total NCV score for each subject was calculated by summing the mean scores (obtained from all examined fingers; Table 1).

The oesophageal alterations were studied using manometry and 24 h pH-metry. A total oesophageal score was created by summing the partial scores related to: (i) the percentage of tertiary waves among the 24 h oesophageal waves; (ii) the pressure of the lower oesophageal sphincter (LES) (mmHg); (iii) the percentage of time with a pH of <4 during 24 h observation (Table 1). In our experience, the consideration of all these three parameters confers on the oesophageal score a high reproducibility index, although statistical data on the variability index are not available at the present time. However, it is known that pH-metry has elevated reproducibility and very low variability [21]. Patients stopped antacid and eukinetic treatments a week before commencement of the testing.

Lung vital capacity (VC) and diffusive lung carbon monoxide (DLCO) were studied by spirometry. VC and DLCO spirometric scores were calculated. The sum of both scores constituted the total lung score (Table 1).

Flow resistance within small intrarenal vessels was considered to be a major and highly sensitive index of scleroderma kidney involvement, and was measured by intrarenal duplex Doppler sonography [22]. The Resistive Index (RI) was calculated in order to assess the renal intraparenchymal vascular bed. RI values of ≤0.7 were considered normal [22] and scored as ‘0’, while RI values of >0.7 were considered abnormal and scored as ‘1’ (Table 1).

Heart evaluation was performed using echocardiography, a procedure that allows the identification of left ventricular hypertrophy (LVH) and/or pericardial effusion (PE). These conditions are frequent cardiac manifestations of SSc [23, 24].

Sera

Sera of the patients enrolled in the study were collected before the beginning of treatment and after 12 months of therapy. The sera of 20 healthy, age- and sex-matched donors were also collected. All sera were stored at −80°C until analysis.
Table 1. Skin, small-vessel and visceral organ scores utilized in the study

<table>
<thead>
<tr>
<th>Scores</th>
<th>Skin*</th>
<th>Altered architecture</th>
<th>Reduced number</th>
<th>Ramified capillaries</th>
<th>Tertiary waves</th>
<th>LES</th>
<th>pH</th>
<th>Vc</th>
<th>DLCO</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>0</td>
<td>&gt;12</td>
<td>≤5</td>
<td>20</td>
<td>25</td>
<td>≤0.7</td>
</tr>
<tr>
<td>1</td>
<td>&gt;50% cut-off</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
<td>&lt;10</td>
<td>9–11</td>
<td>6–10</td>
<td>21–30</td>
<td>26–35</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>2</td>
<td>&lt;50% cut-off</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>11–20</td>
<td>6–8</td>
<td>11–15</td>
<td>31–40</td>
<td>36–45</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>3</td>
<td>No plica</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
<td>21–30</td>
<td>3–5</td>
<td>16–20</td>
<td>41–30</td>
<td>46–55</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31–40</td>
<td>0–2</td>
<td>21–30</td>
<td>&gt;50</td>
<td>&gt;56</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41–50</td>
<td>31–40</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td></td>
<td></td>
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<td>51–60</td>
<td>41–50</td>
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<td>7</td>
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<td></td>
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<td></td>
<td>61–70</td>
<td>&gt;50</td>
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</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;70</td>
<td></td>
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</tbody>
</table>

*According to ref. [18], the skin scores derived from each of nine skin areas were summed to obtain the total skin score.
*Percentage of tertiary waves among 24 h oesophageal waves.
*Mean score.
LES, lower oesophageal sphincter.
*Percentage of time with pH < 4 during 24 h observation.
*Percentage of reduction compared to expected normal values.
*Resistive index calculated by intrarenal duplex Doppler sonography.

Table 2. Skin, NCV and visceral organ scores before and after 1 yr of therapy in the two groups of SSc patients

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Skin</th>
<th>NCV</th>
<th>Oesophagus</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0b</td>
<td>T12c</td>
<td>T0</td>
<td>T12</td>
<td>T0</td>
</tr>
<tr>
<td>I</td>
<td>14.4 ± 2.1</td>
<td>12.3 ± 1.8</td>
<td>4.8 ± 1.0</td>
<td>4.9 ± 0.9</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>(P = 0.1)</td>
<td></td>
<td>(P = 0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>15.2 ± 2.0</td>
<td>11.3 ± 1.8</td>
<td>5.6 ± 2.2</td>
<td>5.8 ± 0.5</td>
<td>5.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>(P = 0.008)</td>
<td></td>
<td>(P = 0.004)</td>
<td></td>
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</table>

*Group I, iloprost; Group II, iloprost + CyA.
*Before therapy.
*After 12 months of therapy.
*Mean score ± s.d.
*P level relative to the statistical difference between T0 and T12.

Determination of IL-6 concentrations

Serum IL-6 concentrations were determined by the human Interleukin 6 ELISA kit from Endogen Inc. (Woburn, MA, USA). The assay was performed in duplicate and the results were expressed as pg/ml. The intra-assay and inter-assay coefficients of variations were <10%.

Statistical analysis

The presence of statistically significant differences between means was analysed by the Mann-Whitney U-test for non-parametric values. All statistical evaluations were performed using GraphPad InstatTM Version 2 and GraphPad PrismTM Version 2 software.

Results

Clinical evaluation at baseline and after 12 months

The results reported in this study were collected before and after 12 months of therapy. All patients reported good tolerance to both therapeutic regimens, and no one withdrew from the study. The most frequent side-effects observed during treatment with iloprost were headache, flushing and nausea which were well controlled by decreasing the rate of i.v. infusion. No relevant CyA side-effects were seen in our series. In particular, no signs of renal toxicity were detected.

No statistically significant differences were observed between the baseline and end time skin, NCV and internal organ scores in Group I (Table 2).

On the contrary, patients in Group II showed a significant reduction of skin (P = 0.008), NCV (P = 0.004) and oesophageal scores (P = 0.05), but not of lung and renal scores, after 12 months of therapy (Table 2).

The patients also self-reported subjective amelioration of their clinical status on a questionnaire in which any improvements of different disease symptoms and signs were scored qualitatively (not shown).

None of the patients in our series showed echocardiographic alterations at baseline.

IL-6 serum levels in SSc patients and in healthy donors

Baseline IL-6 serum levels were significantly higher in SSc patients than in healthy donors: 17.03 ± 17.9 and 17.5 ± 13.1 pg/ml in Group I and II, respectively, vs 1.1 ± 1.5 in controls (P = 0.001). Baseline and end time IL-6 serum concentrations in both Groups I and II were
compared. A significant reduction of IL-6 serum concentrations was observed after 12 months of treatment with the association of CyA and iloprost (3.8 ± 2.5 pg/ml, \( P = 0.007 \)), while no significant variations were detectable in patients treated with iloprost alone (12.3 ± 14.9 pg/ml, \( P = 0.3 \)).

Since IL-6 is implicated in liver synthesis of acute-phase reactants, we measured the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the two groups at baseline and after 12 months of therapy. No significant differences were observed (mean baseline ESR = 21 ± 18 mmHg and mean 12 months ESR = 22 ± 20 mmHg; mean baseline CRP = 4 ± 6 mg/dl and mean 12 months CRP = 4 ± 5 mg/dl).

Discussion

The results of our study show that SSc patients treated for 12 months with low-dose CyA and iloprost showed improvements of skin, microvascular and oesophageal alterations. Furthermore, CyA treatment significantly decreased IL-6 serum levels in SSc patients.

Specific therapy for SSc is still under debate since no ideal treatment has yet been identified. In the last decade, immunosuppressive drugs and vasodilators have frequently been utilized [25]. Among vasodilator drugs, iloprost seems beneficial in reducing skin alterations as well as the severity and frequency of Raynaud’s phenomenon when administered i.v. but not by the oral route [13–15, 26, 27]. However, in our study, no objective improvement of all analysed clinical indexes could be demonstrated following iloprost alone. Therefore, we suggest that iloprost treatment could be selectively proposed as a support therapy that may affect the Raynaud’s-related symptoms of SSc positively.

On the other hand, since CyA seems to interfere with the immune system activation and related vascular lesion mechanisms, the value of the therapeutic association with iloprost was related to both basic and symptomatic treatment of skin, microvascular and oesophageal alterations.

The major concerns for CyA use in SSc are related to the incidence of side-effects [4, 28], and the finding that CyA might induce the secretion from different cell types of transforming growth factor (TGF) with a fibrogenic effect [29, 30]. However, recent studies have pointed out that CyA is able to antagonize TGF effects biochemically and functionally at the same time [31–33].

Among cytokines, IL-6 is likely to play an important role in SSc pathogenesis since it: (a) stimulates fibroblast proliferation and collagen production [9–11]; (b) causes both B-lymphocyte polyclonal activation (thus inducing hypergammaglobulinaemia and autoantibody production) and T-cell activation [12]; and (c) induces synthesis of a metalloproteinase-inhibiting collagenase and, hence, reduces the degradation of the newly synthesized matrix [34]. Accordingly, elevated IL-6 serum levels have been observed in SSc patients by us and other investigators [35]. We hypothesized that therapeutic effects of CyA could be at least partially mediated by direct or indirect inhibition of IL-6 production [36, 37]. The results of our study were consistent with this hypothesis since CyA treatment significantly decreased IL-6 serum concentrations while iloprost was unable to induce such an effect.

In conclusion, the association of CyA and iloprost seems at the present time to be one of the most promising approaches to the long-term treatment of SSc.

Acknowledgement

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References


