Prolonged Complete Clinical Remission in Patients With Severe Pemphigus Vulgaris After Cycles of Intravenous Cyclophosphamide

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Abstract. Background. Corticosteroids are the systemic treatment of choice in patients with pemphigus vulgaris, but chronic administration is associated with side effects. Intravenous treatment with cyclophosphamide can improve the clinical signs of pemphigus vulgaris.

Material and methods. We prospectively studied 8 patients diagnosed with pemphigus vulgaris. Six of these had mucocutaneous pemphigus vulgaris and 2 had mucosal pemphigus vulgaris. Treatment consisted of 10 cycles of cyclophosphamide at a dose of 10-15 mg/kg separated by 15 days, while maintaining the initial corticosteroid and immunosuppressant dose. Clinical efficacy was assessed and the anti-epidermal intercellular substance (EIS) and anti-desmoglein (DSG) 3 and 1 antibody titers were monitored (by indirect immunofluorescence and enzyme-linked immunosorbent assay, respectively).

Results. All patients with pemphigus vulgaris responded excellently to treatment. Five of the 8 patients achieved complete remission of pemphigus lesions after 10 cycles of cyclophosphamide. In the other 3 patients, the skin lesions disappeared a few weeks after the last cycle of cyclophosphamide. A substantial reduction in immunosuppressant dose was possible in all patients. Furthermore, an improved immunologic response was observed in all cases after cyclophosphamide treatment, with decreased anti-DSG1 and anti-DSG3 antibody titers and well as decreased circulating anti-EIS antibody titers. During the mean 15.1 month follow-up (range, 1-25 months), no new lesions appeared and no side effects of cyclophosphamide therapy were reported.

Conclusions. fortnightly cycles of intravenous cyclophosphamide may be a useful therapeutic option in patients with severe pemphigus vulgaris. A reduction of corticosteroid dose was possible with this therapeutic approach and the cumulative cyclophosphamide dose was lower than with daily oral administration. Our findings also show that the therapeutic approach induces clinical and immunologic remission in most patients.

Key words: autoantibodies, cyclophosphamide, desmoglein, immunosuppressants, pemphigus vulgaris.
immunológiadespúes del tratamiento con CF, con disminución en el título de anticuerpos frente a las Dsg 1 y 3, así como del título de anticuerpos circulantes frente a la SIE. Tras un seguimiento medio de 15,1 meses (1-25 meses) ningún paciente ha presentado nuevas lesiones de PV. A su vez, no se han observado efectos secundarios por la CF.

Conclusiones. La administración de pulsos quincenales de CF intravenosa puede ser una opción terapéutica útil en pacientes con PV grave. Este esquema terapéutico permite disminuir la dosis de corticosteroides con una menor dosis acumulada de CF que en la administración oral diaria. Además, nuestros resultados muestran que este esquema terapéutico se acompaña de una remisión clínica e inmunológica en la mayoría de los pacientes.

Palabras clave: autoantígenos, ciclofosfamida, desmogleína, fármacos inmunosupresores, pénfigo vulgar.

Introduction

Pemphigus vulgaris (PV) is an autoimmune blistering disease mediated by immunoglobulin (Ig) G-type autoantibodies directed against desmogleins (Dsg) in the dermosomes of skin and mucosal keratinocytes.¹ There are 2 clinical variants of the disease: mucocutaneous PV and mucosal PV. Mortality from PV, which is currently estimated at between 5% and 15%,² was high until systemic corticosteroid therapy became the treatment of choice. In many patients, the cause of death is associated with adverse reactions related to the long-term administration of corticosteroids rather than with PV itself.³,⁴

PV can be treated effectively with a wide range of drugs, which also serve to reduce the cumulative corticosteroid dose. Noteworthy among these drugs are antiinflammatories, immunomodulators, and immunosuppressants such as azathioprine, mycophenolate mofetil, cyclosporin, and cyclophosphamide. The combined use of corticosteroids and immunosuppressants has reduced both the morbidity and mortality associated with PV.⁵,⁶

The main treatment goal in patients with PV is to achieve optimum clinical outcomes with minimum morbidity. Numerous studies have demonstrated the clinical efficacy of intravenous pulse cyclophosphamide therapy.⁷-¹¹ This route of administration has proven to be as efficacious as the administration of oral cyclophosphamide on a daily basis but it has the added advantage that it is associated with lower morbidity.¹²,¹³

Following recommendations in the literature on the administration of intravenous pulse cyclophosphamide therapy,¹⁰,¹¹ we treated 8 patients, all with severe PV, with 2-weekly cycles of cyclophosphamide. The patients had all received previous treatment with oral prednisone and azathioprine or mycophenolate mofetil. The primary aim of this study was to evaluate the clinical and immunologic efficacy of intravenous cyclophosphamide administered every 2 weeks and to determine how this treatment regimen might help to reduce the use of corticosteroids and immunosuppressants.
Results

All 8 patients included in the study exhibited excellent clinical and immunologic response (according to the criteria published by Fleischli et al\(^\text{11}\)) following the administration of ten 2-weekly cycles of intravenous cyclophosphamide. Overall, the lesions healed in a mean period of 5.1 months (range, 3–7 months) (Table 2). Clinical response to cyclophosphamide was observed in either an initial phase, in which lesions resolved completely during cyclophosphamide therapy, or in a later phase, in which patients continued to improve despite completion of therapy and tapering of prednisone and immunosuppressant doses.

Initial Response

Five patients (patients 1, 2, 6, 7, and 8) (62.5%) had developed adverse reactions due to the administration of systemic corticosteroids (Table 1).
Late Response

Patients 3, 4 and 5 achieved complete clinical response in a mean of 6.5 months from the start of pulse therapy and a mean of 1.5 months from the completion of therapy. It is interesting to note that each patient experienced progressive clinical improvement after completion of therapy and with the progressive tapering of prednisone (to a dose of 10 mg/d). Adjuvant immunosuppressive decrease was detected in antibody titers against Dsg1, Dsg3, and EIS in all patients. In patient 6, we were unable to find evidence of a PV immune response and in patients 2 and 4, we detected no anti-Dsg1 or anti-Dsg3 antibodies, although we did find low circulating levels of anti-EIS antibodies (Table 2). None of the patients developed active PV lesions on the skin or mucous membranes during follow-up. Clinical response, therefore, can be considered excellent in all cases (Table 3).

Table 2. Response to 2-Weekly Pulse Intravenous Cyclophosphamide (Cy) Therapy

<table>
<thead>
<tr>
<th>Patients</th>
<th>No. of Cy Cycles</th>
<th>Starting Cy Dose, mg</th>
<th>Maximum Cy Dose, mg</th>
<th>Cy Dose Before First Cycle of Cy, mg</th>
<th>Prednisone Dose Before First Cycle of Cy, mg</th>
<th>Prednisone Dose After Last Cycle of Cy, mg</th>
<th>Disease Activity After Last Cycle of Cy</th>
<th>EIS-Dsg1 Before Last Cycle of Cy, 1:10</th>
<th>EIS-Dsg3 After Last Cycle of Cy, 1:10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>70</td>
<td>15</td>
<td>No lesions after seventh cycle</td>
<td>1:20</td>
<td>1:10</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1100</td>
<td>1100</td>
<td>1000</td>
<td>60</td>
<td>10</td>
<td>No lesions after tenth cycle</td>
<td>1:20</td>
<td>1:10</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>900</td>
<td>1500</td>
<td>14 100</td>
<td>100</td>
<td>60</td>
<td>Mild</td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1500</td>
<td>1500</td>
<td>15 000</td>
<td>60</td>
<td>20</td>
<td>Mild</td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1800</td>
<td>1800</td>
<td>18 000</td>
<td>60</td>
<td>20</td>
<td>Mild</td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1000</td>
<td>1000</td>
<td>10 000</td>
<td>60</td>
<td>20</td>
<td>No lesions after sixth cycle</td>
<td>1:20</td>
<td>neg</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1800</td>
<td>1800</td>
<td>18 000</td>
<td>90</td>
<td>50</td>
<td>No lesions after tenth cycle</td>
<td>1:320</td>
<td>1:80</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1700</td>
<td>1700</td>
<td>17 000</td>
<td>40</td>
<td>15</td>
<td>No lesions after tenth cycle</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

Abbreviations: Az, azathioprine; Dsg, desmoglein; EIS, epidermal intracellular substance; MM, mycophenolate mofetil; neg, negative; P, prednisone; PL, plasmapheresis.

*Response was excellent in all patients based on criteria described by Fleischli et al. (Excellent indicates severe or moderate disease improving to absence of disease, none; good, severe disease improving to moderate or mild disease; minimal, moderate disease improving to mild disease; and none, no change or worsening of disease).

*According to scale described by Fleischli et al. (For MC PV: no lesions; mild, fewer than 20 blisters; moderate, 20-40 blisters; severe, over 40 blisters. For MU PV: mild, 1-5 erosions; moderate, 5-10 erosions; severe, over 10 erosions or extensive erosions).

*Determined by indirect immunofluorescence.
reduce the starting dose of immunosuppressants in 4 patients (patients 3, 4, 5, and 6) (Table 3).

The Figure shows the results of treatment in 1 of the patients.

### Adverse Reactions

We observed no significant adverse reactions in any of the 8 patients during the 2-weekly cyclophosphamide cycles. Five patients experienced nausea despite antiemetic therapy but the symptoms disappeared within a few days of the infusion. One patient developed transient neutropenia (800/µL), which required treatment with colony-stimulating factor; the neutrophil count improved

<table>
<thead>
<tr>
<th>Patients</th>
<th>Follow-up Duration After Last Cycle of Cy, mo</th>
<th>Current Prednisone Dose</th>
<th>Current Adjuvant Imunosuppressive Therapy</th>
<th>Current PV Severity</th>
<th>Levels of Antibodies Against EIS/Dsg1/Dsg3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>10 mg/d</td>
<td>Az, 100 mg/d</td>
<td>No lesions</td>
<td>neg/neg/83</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>15 mg/d</td>
<td>MM, 3 g/d</td>
<td>No lesions</td>
<td>1:20/neg/neg</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>5 mg/d</td>
<td>MM, 2 g/d</td>
<td>No lesions</td>
<td>neg/29/neg</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>2.5 mg/d</td>
<td>MM, 1.5 g/d</td>
<td>No lesions</td>
<td>neg/neg/neg</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>2.5 mg/d</td>
<td>MM, 1.5 g/d</td>
<td>No lesions</td>
<td>1:10/neg/57</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>2.5 mg/d</td>
<td>MM, 1 g/d</td>
<td>No lesions</td>
<td>neg/neg/neg</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>30 mg/d</td>
<td>MM, 3 g/d</td>
<td>No lesions</td>
<td>1:20/45/203</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>15 mg/d</td>
<td>MM, 3 g/d</td>
<td>No lesions</td>
<td>1:40/178/29</td>
</tr>
</tbody>
</table>

**Abbreviations:** Az, azathioprine; Dsg, desmoglein; EIS, epidermal intracellular substance; MM, mycophenolate mofetil; neg, negative; PV, pemphigus vulgaris.

Figure. Clinical manifestations in patient 1. A, before cyclophosphamide cycles administered in association with corticosteroids and azathioprine. B, 1 month after last cycle of cyclophosphamide.
and remained within the normal range for the remaining cycles (Table 2). No other adverse reactions due to cyclophosphamide have been reported to date. We observed no adverse reactions associated with the administration of azathioprine or mycophenolate mofetil, or secondary infections due to immunosuppression.

Discussion

Systemic corticosteroid therapy is the most effective treatment in the initial phases of PV. The treatment is normally administered in combination with corticosteroid-sparing immunosuppressants such as azathioprine and mycophenolate mofetil. Cyclophosphamide, however, has also proven to be efficacious in PV but more adverse reactions have been reported when the drug is administered in low oral doses than when administered as intravenous pulse therapy, hence the establishment of diverse treatment regimens aimed at minimizing toxicity, reducing cumulative doses, and achieving better clinical response. Of note among the regimens described in the literature are the coadministration of cyclophosphamide and corticosteroids in monthly or weekly cycles, the monthly administration of cyclophosphamide in association with low doses of oral cyclophosphamide, the coadministration of cyclophosphamide and dexamethasone cycles, cyclophosphamide pulse therapy and plasmapheresis, concomitant cyclophosphamide and vincristine, and immunoablative high-dose cyclophosphamide. Cyclophosphamide pulse therapy has also been administered in association with azathioprine and prednisone but only in isolated cases.

Clinical response was excellent in all 8 patients included in our study; with a 2-weekly regimen of intravenous cyclophosphamide, all the patients achieved complete clinical response (absence of active lesions) within a mean of 5.1 months. Five (62.5%) of the patients had no skin or mucous membrane lesions on completion of the 10 cycles, while the 3 remaining patients (37.5%) achieved complete clinical response after the tenth cycle, with a mean healing time of 6.5 months from the start of the regimen and 1.5 months from the last session. In a study that used pulse intravenous cyclophosphamide therapy at monthly intervals, clinical response was found to be excellent in 66% of patients and minimal in 22%. In all the patients who responded clinically to treatment, disease control was achieved in 2 to 5 months. In another study that used the same treatment regimen, Bhat et al reported a mean healing time of 2.1 months for skin lesions and 3.6 months for mucosal lesions. The patients included in that study, however, had lesions on less than 10% of their body surface. Pasricha et al reported complete healing of PV lesions in 32% of patients treated with pulse dexamethasone and cyclophosphamide therapy in combination with low doses of oral cyclophosphamide. In those patients, it was possible to withdraw all treatment. Several years later, the same research group found that 72% of patients with PV achieved complete remission within a year of treatment with the above regimen (48.5% in 6 months and 23.5% in 12 months).

Current literature suggests administering intravenous cyclophosphamide as monthly pulses. This recommendation is based on the observation that a patient’s leukocyte count begins to decrease gradually 2 to 3 days after initiation of treatment and reaches minimum levels after 10 to 12 days, which is when antibody production is most affected. The body begins to produce antibodies again within 1 to 2 weeks, which is when the repeated administration of cyclophosphamide might be most effective. Nonetheless, our initial experience showed us that patients experienced severe flares in disease activity 4 weeks after receiving intravenous cyclophosphamide and just before the next cycle. Considering this and reports of improved clinical response in patients with lymphomas treated with cyclophosphamide maintained at higher concentrations for longer, we decided to assess clinical response to the administration of 2-weekly rather than monthly cyclophosphamide in our patients. A similar regimen to ours, proposed by Pasricha et al, involved administering pulse cyclophosphamide therapy at 2-to-3-week intervals in patients with severe PV in order to induce earlier clinical remission and shorten the active disease phase. Intravenous cyclophosphamide therapy administered as 2-weekly pulses should therefore only be used in patients with highly active PV.

None of our patients have experienced flares in disease activity to date. Furthermore, titers of antibodies against and EIS, Dsg1, and Dsg3 showed a substantial progressive decrease both during and after pulse therapy. Even though patient 5 showed a slight increase in anti-Dsg1 and anti-Dsg3 antibody titers after 2 years of testing negative, he has not experienced any cutaneous or mucosal flare-ups to date. This patient will, however, require more frequent monitoring than the other patients due to an increased risk of relapse. Three patients (patients 1, 7, and 8) were still free of symptoms several months after completion of cyclophosphamide therapy, even though they had positive anti-Dsg1 and anti-Dsg3 titers. No flare-ups have been observed in any of the other patients either. Fleischli et al reported flares in disease activity in almost 50% of patients administered pulse intravenous cyclophosphamide therapy at monthly intervals. In the study by Bhat et al, the corresponding percentage in a series of patients that received the same treatment was 34.6% 3 weeks to 8 months after completion of treatment. Pandya and Sontheimer
reported similar results. Our findings therefore demonstrate that pulse intravenous cyclophosphamide, in association with corticosteroid-sparing immunosuppressants, can eliminate B-cell clones more efficiently when administered every 2 weeks than every month. While some patients may retain circulating levels of antibodies against Dsg1 and Dsg3, these antibodies appear to have a weak or possibly even inexistent role in the development of new PV lesions.

Adverse reactions to cyclophosphamide were negligible in our series, a finding which coincides with results reported for patients treated with monthly cycles of cyclophosphamide.7,10,11 Our patients will, nonetheless, need to be monitored regularly as we cannot rule out the possibility of future complications.

With our regimen, we were able to reduce the initial dose of prednisone by at least 50% in the majority of our patients and also to reduce the dose of immunosuppressants in a substantial proportion of patients. Only patient 7 is still receiving a moderate dose of prednisone. This 2-weekly regimen, therefore, also allows immunosuppressant doses to be reduced rapidly, considerably reducing the risk of adverse reactions while maintaining clinical efficacy.

Intravenous pulse cyclophosphamide therapy administered every 2 weeks could be indicated in patients with severe PV who do not respond to conventional immunosuppressive therapy, who develop adverse reactions due to medication (mostly corticosteroids), and who experience flares in disease activity despite high-dose corticosteroid therapy. More controlled studies, however, are required to evaluate the true role of cyclophosphamide in the management of PV and to assess the long-term safety of the drug. Furthermore, clear guidelines are needed to direct the approach and to make clear when we should use chemotherapy drugs such as intravenous cyclophosphamide or other drugs such as anti-CD20 monoclonal antibody29-31 in association with other immunosuppressants in patients with severe PV.

Conflicts of Interest
The authors declare no conflicts of interest.

References


