Treatment with Megestrol Acetate Improves Human Immunodeficiency Virus-Associated Immune Thrombocytopenia

Francisco Gomez,* Pedro Ruiz, Rafaela Lopez, and Consuelo Rivera

Department of Medicine, Hospital Universitario Puerto Real/Servicio Andaluz de Salud, University of Cadiz School of Medicine, Cadiz, Spain

Received 7 May 2001/Returned for modification 5 October 2001/Accepted 12 November 2001

Splenic macrophage Fcy receptors participate in the pathophysiology of immune cytopenias, and in such disorders, the beneficial effects of glucocorticoids are in part mediated by decreased expression of macrophage Fcγ receptors. In the animal model, progesterones, like glucocorticoids, inhibit expression of these receptors. Megestrol acetate (MA) is a progesterone frequently used for treating human immunodeficiency virus (HIV)-associated anorexia-cachexia. Twenty-eight patients with HIV-associated thrombocytopenia with shortened platelet survival and increased platelet-associated immunoglobulin G (IgG) who were being treated with MA for anorexia-cachexia were prospectively studied for a 6-month period to assess the potential role of progesterones in the treatment of immune thrombocytopenia. Treatment with MA for nonconsecutive periods of 2 months and 1 month significantly increased platelet count and platelet survival without significant alteration of platelet-associated immunoglobulin G levels. Of the 28 patients studied, 22 presented a complete response, 19 presented a complete response 1 month after finishing the MA treatment regimen, and 12 remained in complete response for a further month. Expression of Fcy receptors (FcyRI and FcyRII) by peripheral blood monocytes and the in vitro recognition of IgG-sensitized cells by monocytes were significantly decreased by the MA treatment. Decreased expression and functioning of these receptors significantly correlated with platelet counts and survival times, but no relationship was found with platelet-associated immunoglobulin G, circulating immune complexes, body mass index, plasma HIV load, or CD4 lymphocyte levels. These results suggest that treatment with progesterones, like MA, may be an alternative therapy for immune cytopenias, with few side effects.

Preliminary data have suggested that treatment with megestrol acetate (MA) enhances the platelet count of malnourished patients with human immunodeficiency virus (HIV)-associated thrombocytopenia (21), most of whom present immunoglobulin G (IgG) antiplatelet antibodies (13, 27). Receptors for the Fc fragment of IgG (FcγRs) on macrophages play an important role in host defense against infection (10, 19), particularly in the pathophysiology of immune cytopenias (3, 5, 7, 8, 22, 23). Hence, regulation of the expression of these splenic receptors is an important target in the immunotherapeutic treatment of such disorders.

Glucocorticoid treatment is the standard therapy for immune cytopenias such as immune thrombocytopenic purpura and immune hemolytic anemia (1, 8), but its usefulness is limited by significant side effects. Glucocorticoids inhibit the expression of splenic macrophage Fcγ receptors and increase cell survival (6, 8, 22, 23). In an animal model (the guinea pig), progesterones have been shown to decrease the clearance of IgG-sensitized cells (11, 24) through their effect on the expression of these receptors. However, this effect of progesterone has not been reported before in humans.

MA is a progesterone already approved for the treatment of HIV-associated anorexia-cachexia (14, 25, 26) but not yet for thrombocytopenia. We have performed a prospective study of 28 patients presenting HIV-associated thrombocytopenia, with shortened platelet survival and elevated platelet-associated IgG, who were being treated with MA for anorexia-cachexia. The objective was to assess the role of MA in the specific treatment of HIV-associated thrombocytopenia by monitoring the platelet count and platelet survival and the surface expression and functioning of peripheral blood monocyte FcyRI and FcyRII.

MATERIALS AND METHODS

Patients. We prospectively studied patients with HIV-associated thrombocytopenia treated in the outpatient clinic of our hospital between January 1992 and December 1995. Data on 28 of these patients who were taking MA for anorexia-cachexia (4 females and 24 males; age, 29 ± 12 years) and who fulfilled the inclusion criteria and completed the 6-month follow-up period were analyzed (Table 1).

The patients eligible for this study were between 18 and 60 years old, with at least three platelet counts of less than 50,000/ml during the previous 6 months, shortened platelet survival, and elevated platelet-associated IgG levels. We excluded patients with renal disease (plasma creatinine ≥2 mg/dl), liver disease (prothrombin or prothrombin time below 80% of the levels of controls, serum albumin <3.5 mg/dl, or bilirubin ≥3 mg/dl), cirrhosis, ultrasonographic signs of portal hypertension, active infection, sepsis, neoplasia, and autoimmune disorders. Patients with an AIDS-defining event or those receiving immunosuppressive treatment during follow-up were also excluded. Patients gave informed consent before enrollment. The study was approved by the Ethic and Clinical Trials Committee of our university hospital.

The characteristics of the patients are given in Table 1. None of the patients were excluded because they developed an AIDS-defining event. Two patients were excluded because they failed to comply with MA treatment during the first month of treatment. These two patients were not considered for analysis because they generated no more data than the parameters before commencement of the treatment.

Seventeen patients acquired HIV infection by intravenous drug abuse (60.71%), 6 patients by heterosexual transmission (21.43%), and 5 patients by homosexual or bisexual practices (17.86%). Six patients were being treated with

* Corresponding author. Mailing address: Department of Medicine, Hospital Universitario Puerto Real/Servicio Andaluz de Salud, University of Cadiz School of Medicine, Avenida de la Paz, 16, 11500, El Puerto Santa Maria, Cadiz, Spain. Phone and fax: 34-956-56 27 14. E-mail: fgomez@comcadiz.org.
two nucleoside reverse transcriptase analogs (four with zidovudine [AZT] plus zalcitabine [DDC] and two with AZT plus didanosine [DDI]), and 22 patients were being treated with AZT monotherapy when this study commenced. No patients had received protease inhibitors, and all of them had detectable virus loads (200 HIV RNA copies/ml) in plasma on enrollment or during follow-up.

### Treatment and response

<table>
<thead>
<tr>
<th>Time of treatment</th>
<th>MA dose (mg/kg/day)</th>
<th>Response at end of mo (no. of patients)</th>
<th>Body mass index (kg/m²)</th>
<th>CD4 count (cells/µl)</th>
<th>HIV RNA load in plasma (RNA copies/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Complete</td>
<td>Partial</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td>15</td>
<td>8</td>
<td>5</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Mo 1</td>
<td>640</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Mo 2</td>
<td>826 ± 51</td>
<td>30</td>
<td>5</td>
<td>20 ± 3</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>Mo 3</td>
<td>None</td>
<td>16</td>
<td>3</td>
<td>3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Mo 4</td>
<td>826 ± 51</td>
<td>22</td>
<td>3</td>
<td>3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Mo 5</td>
<td>None</td>
<td>32</td>
<td>8</td>
<td>4</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Mo 6</td>
<td>None</td>
<td>32</td>
<td>8</td>
<td>4</td>
<td>32 ± 3</td>
</tr>
</tbody>
</table>

*Twenty-eight patients (4 female, 24 male; mean age ± SEM, 29 ± 7 years) with HIV-associated thrombocytopenia, with shortened platelet survival and elevated platelet-associated IgG, treated with MA for anorexia-cachexia, participated in the study. The characteristics of patients on enrollment (before treatment), the dose of MA and the evolution of HIV infection during follow-up are shown. Results are expressed as means ± SEM. HIV-pVL = HIV-RNA load in plasma.

#### Flow cytometry

Monoclonal antibodies (MAbs) directed against FcγRI (MAb32.2), FcγRII (MAbHIV.3), and FcγRIII (MAbS08) were used in indirect immunofluorescence binding studies to assess protein expression of these receptors on monocytes. Experiments were performed as previously described (9, 17, 18). Cells were incubated with antibodies for 30 min at 4°C and washed twice with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin and 0.02% sodium azide. Bound antibodies were labeled by incubation with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse Ig antibody (Tago, Inc., Burlingame, Calif.) for 30 min at 4°C. Cells were washed twice, fixed with 4.0% paraformaldehyde, and analyzed by flow cytometry. Fluorescence was measured by a FACScan cytometer with Consort 32 software (Becton-Dickinson, Madrid, Spain). For all samples, 10,000 events were recorded on a logarithmic fluorescence scale, and the actual mean fluorescence intensity (MFI) for each sample was determined. In order to correct for autofluorescence, the MFI of a nonreactive murine IgG1 antibody (P3×63) was subtracted from the MFI of the anti-FcγR-stained cells. Changes in mean fluorescence intensity was calculated as ΔMFI (change in MFI) = (MFI of anti-FcγR MA-treated cells) − (MFI of P3×63 MA-treated cells) − (MFI of anti-FcγR control cells) − (MFI of P3×63 control cells).

#### Preparation of IgG-sensitized RBCs

Antibody-sensitized sheep erythrocytes were prepared as previously described (9, 17, 18). In brief, 1 × 10⁸ sheep RBCs in 1.0 ml of 0.01-mol/liter EDTA buffer were sensitized by adding mouse monoclonal antibody Sp2/HL, subclass IgG2b (Serotec Ltd., Bicester, Oxon, England), in 0.1 ml at 37°C for 1 h. We used the optimal antibody dilutions found in previous studies, 1:20 and 1:80 (9, 17, 18). The IgG-sensitized sheep RBCs were washed twice and resuspended in Hank's balanced salt solution to a final concentration of 10⁶ cells/ml.

#### Monocyte recognition of sheep IgG-sensitized RBCs

In vitro recognition of sheep IgG-sensitized red blood cells RBCs by peripheral blood monocytes was assessed by Wilcoxon rank correlation test. The in vitro recognition of sheep IgG-sensitized red blood cells RBCs by peripheral blood monocytes was assessed by Wilcoxon rank correlation test. The in vitro recognition of sheep IgG-sensitized red blood cells RBCs by peripheral blood monocytes was assessed by Wilcoxon rank correlation test. The in vitro recognition of sheep IgG-sensitized red blood cells RBCs by peripheral blood monocytes was assessed by Wilcoxon rank correlation test.
respectively) were significantly higher than pretreatment values \( (P < 0.001) \). Two months after MA withdrawal (i.e., in month 6), the mean platelet count \( (41,170 \pm 2,436 \text{ per ml}) \) did not differ significantly from baseline values \( (P = \text{not significant}) \). Twenty two patients (78.57\%) presented a complete response with MA treatment (i.e., in month 4). One month after terminating MA treatment (i.e., in month 5), 19 patients (67.86\%) presented a complete response, 6 patients a partial response, and 3 patients no response, while a month later (i.e., in month 6) 12 patients (42.86\%) were still in complete response.

Platelet survival (mean \( \pm \) SEM) was significantly shortened on enrollment \( (T_{1/4} = 118.50 \pm 28 \text{ min}) \) \( (P < 0.001) \). Two months of MA treatment increased the platelet survival in all patients; 17 of the 28 patients (60.07\%) attained normal platelet survival. Mean platelet survival increased significantly to \( T_{1/4} = 350.10 \pm 43 \text{ min} \) after 2 months of MATreatment (i.e., by month 2) \( (P < 0.001) \). Two months after MA withdrawal (i.e., in month 6), mean platelet survival \( (156.90 \pm 87 \text{ min}) \) did not differ significantly from baseline values (Fig. 1). Platelet-associated immunoglobulins, change in platelet-associated IgG, and change in platelet-associated IgM were not significantly altered by MA treatment at any stage of the study. Results are expressed as mean \( \pm \) SEM. * \( P < 0.001 \).

We assessed the surface expression of both Fcγ receptors expressed by peripheral blood monocytes, FcγRI and FcγRII by using flow cytometry (Table 2). Results are expressed as percent inhibition of the mean fluorescence intensity below pretreatment values \( (\text{mean} \pm \text{SEM}) \). The expression of peripheral blood monocyte FcγRI decreased significantly 1 and 2 months after MA treatment, by 41.13\% \( \pm \) 3.71\% and 51.80\% \( \pm \) 4.73\%, respectively \( (P < 0.001) \). The expression of peripheral blood monocyte FcγRII decreased significantly 1 and 2 months after MA treatment by 36.83\% \( \pm \) 4.21\% and 45.53\% \( \pm \) 4.47\%, respectively \( (P < 0.001) \). Two months after MA withdrawal, the expression of peripheral blood monocyte Fcγ receptors FcγRI and FcγRII did not differ significantly from baseline (Table 2).

The in vitro recognition of IgG2b-sensitized RBCs by peripheral blood monocytes at two ionic strengths \( (0.15 \text{ and } 0.07) \) was determined to assess the functioning of FcγRI \( (\Delta \mu = 0.15) \) and FcγRII \( (\Delta \mu = 0.07) \) (9, 18). Results are expressed as
percent inhibition compared with pretreatment values (mean ± SEM). The recognition of IgG2b-sensitized RBCs by peripheral blood monocyte FcγRI (µ = 0.15) was significantly inhibited after 1 and 2 months of MA treatment, by 22.23% ± 1.71% and 43.30% ± 2.26%, respectively (P < 0.001). The recognition of IgG2b-sensitized RBCs by peripheral blood monocyte FcγRII (µ = 0.07) was significantly inhibited after 1 and 2 months of MA treatment, by 18.42% ± 1.41% and 35.43% ± 2.37%, respectively (P < 0.001). Two months after MA withdrawal, the recognition of IgG2b-sensitized RBCs by peripheral blood monocyte Fcγ receptors FcγRI and FcγRII did not differ significantly from baseline (Table 2).

Platelet survival significantly correlated with platelet count (P < 0.001). No relationship was observed between platelet survival and the level of platelet-associated IgG or IgM. Neither the platelet count nor the expression of monocyte Fcγ receptors was correlated with body mass index, number of CD4 cells, or plasma RNA HIV load, or circulating immune complexes.

MA treatment was well tolerated. No side effects severe enough to discontinue treatment were reported. No changes were observed in hematologic parameters other than platelets during the course of treatment. Increased appetite and weight gain were observed in 85.71% of the patients. No consistent elevation of blood glucose, total cholesterol, or triglyceride levels was observed in this short-term study. No significant alterations of liver enzymes by treatment with MA were observed in the 17 (60.71%) patients with chronic hepatitis C virus liver disease.

**DISCUSSION**

The data obtained in this short-term study suggest that treatment with MA significantly enhances platelet count and survival; it decreases the surface expression of macrophage Fcγ receptors, FcγRI and FcγRII, and does not alter the platelet-associated immunoglobulin. Treatment with MA for 1 month produced a complete response in the majority (78.57%) of the patients studied, while 2 months after MA withdrawal, 42.86% of patients were still in complete response.

Platelet-associated IgG or IgM and circulating immune complexes were not altered by MA treatment. The increased platelet count and survival and decreased expression of peripheral blood monocyte Fcγ receptors, FcγRI and FcγRII, observed do not seem to be due to weight gain or to progression of HIV infection, since those effects of MA were not correlated with the body mass index or with the number of CD4 cells and HIV RNA plasma viral load, respectively. No significant changes were observed in the body mass index, number of CD4 cells, or plasma HIV load during follow-up.

Enhanced platelet production by MA may in part explain the increased platelet count. Nevertheless, our findings of enhanced platelet survival and decreased expression of macrophage Fcγ receptors, with no alteration of platelet-associated immunoglobulin following MA treatment, cannot be explained by an improved bone marrow production of platelets. Therefore, the most consistent mechanism for the observed MA treatment effect is a decreased macrophage Fcγ receptor-dependent phagocytosis, resulting in longer platelet survival and platelet count.

It has recently been observed that the beneficial effects of treatment with intravenous immunoglobulin for immune cytopenias depends upon induction of the surface expression of macrophage FcγRIIB (21). Our finding of decreased monocyte FcγRII expression is not in contradiction with that observation, since our experimental design does not differentiate between the expression of receptor isotypes FcγRIIA and FcγRIIB. Thus, while the surface expression of the monocyte receptor FcγRIIB may indeed be enhanced, the surface expression of the other receptor isotype FcγRIIA may have been decreased. Another pathophysiologically limitation of our study is the lack of data on the macrophage FcγRIII that is also involved in the pathophysiology of immune cytopenias (2, 3, 5–7, 12). We have not performed invasive studies to harvest macrophages (peritoneal, pulmonary, or splenic). Nevertheless, we did determine the surface expression of FcγRIII by peripheral blood monocytes in some patients, but its expression was either very low or absent.

Our results indicate that MA treatment improves platelet count and platelet survival in patients with HIV-associated thrombocytopenia, with shortened platelet survival and with elevated platelet-associated immunoglobulin. Therefore, progestanes such as megestrol acetate may be used in the treat-

---

**TABLE 2. Effect of treatment with MA on platelet count and platelet survival**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>Mo 1</th>
<th>Mo 2</th>
<th>Mo 3</th>
<th>Mo 4</th>
<th>Mo 5</th>
<th>Mo 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA dose (mg/kg/day)</td>
<td>640</td>
<td>826 ± 51</td>
<td>None</td>
<td>826 ± 51</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Platelet count (no./µl)</td>
<td>22,280 ± 2,110</td>
<td>147,522 ± 2,620</td>
<td>232,472 ± 3,122</td>
<td>171,830 ± 2,742</td>
<td>246,378 ± 3,014</td>
<td>170,306 ± 2,916</td>
<td>41,170 ± 2,036</td>
</tr>
<tr>
<td>Responders</td>
<td>152,762 ± 2,813</td>
<td>239,931 ± 3,213</td>
<td>186,244 ± 2,991</td>
<td>257,703 ± 3,312</td>
<td>174,707 ± 3,011</td>
<td>49,933 ± 2,996</td>
<td></td>
</tr>
<tr>
<td>Partial responders</td>
<td>96,331 ± 1,602</td>
<td>98,271 ± 1,917</td>
<td>76,207 ± 1,237</td>
<td>97,326 ± 1,826</td>
<td>94,201 ± 1,348</td>
<td>37,126 ± 2,198</td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>41,670 ± 1,172</td>
<td>43,771 ± 1,237</td>
<td>41,891 ± 1,301</td>
<td>44,212 ± 1,403</td>
<td>42,137 ± 1,205</td>
<td>27,733 ± 2,343</td>
<td></td>
</tr>
<tr>
<td>Platelet survival, T 1/4 (min)</td>
<td>118.50 ± 28</td>
<td>350.12 ± 43</td>
<td>426.32 ± 51</td>
<td>156.91 ± 87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>391.72 ± 39</td>
<td>455.37 ± 58</td>
<td>167.07 ± 89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial responders</td>
<td>267.91 ± 32</td>
<td>297.92 ± 42</td>
<td>132.27 ± 39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>107.73 ± 24</td>
<td>132.33 ± 38</td>
<td>122.56 ± 31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Twenty-eight patients with HIV-associated thrombocytopenia, with shortened platelet survival and elevated platelet associated IgG, treated with MA for anorexia-cachexia, participated in the study. The evolution of the platelet count and the platelet survival on enrollment (before treatment) and during treatment with MA are shown. Results are expressed as mean ± SEM for the 28 patients as well as for those patients showing a complete response (responders), a partial response, or no response.*
ment of immune cytopenias, with few short-term side effects. The effect of MA compared with glucocorticoids and the risk-benefit ratio of glucocorticoids in combination with MA for the treatment of immune cytopenias await confirmation by appropriate clinical trials.

ACKNOWLEDGMENTS

This research was supported by grants from the Spanish Ministerio de Educacion y Ciencia (PM92-0259 and RE-28515062) and the Consejeria de Educacion, Junta de Andalucia (Group 3224).

We are grateful to Royston F. Smart for assistance with the English language presentation of this article.

REFERENCES