Effect of Myleran and 6-Mercaptopurine (6-MP) on the Serum Level of Vitamin B₁₂ in Chronic Myelocytic Leukemia

By V. M. Doctor, D. E. Bergsagel and C. C. Shullenberger

A gradual decline in serum vitamin B₁₂ levels is reported to occur following the reduction of the leukocyte count in patients with chronic myelocytic leukemia treated with 6-mercaptopurine (6-MP), Myleran or x-irradiation.¹ ²

In the present study, serum vitamin B₁₂ determinations and various clinical and hematologic measurements were made on patients with chronic myelocytic leukemia before and during 6-MP or Myleran therapy and also at regular intervals during the first remission period. The results indicate that a reduction in total leukocytes following therapy is followed by a gradual lowering of serum vitamin B₁₂. A rough correlation was observed between the serum B₁₂ levels at the completion of therapy and the duration of the first remission.

METHODS

Vitamin B₁₂ Assay: Blood was obtained by venipuncture and allowed to clot. Serum was removed following centrifugation and stored at -20°C until assayed. Vitamin B₁₂ assays were run on the serum according to the procedure described earlier.¹

Hematologic: The routine methods used are described by Cartwright.⁴ The diagnosis of leukemia was based on clinical features, leukocyte counts and the examination of Wright-stained smears of the peripheral blood and bone marrow aspirate. Patients diagnosed as having chronic myelocytic leukemia showed marked myelocytic hyperplasia, and all cases showed less than 10 per cent blasts in the peripheral blood or marrow smears at the time they were first started on treatment.

RESULTS

The effect of 6-MP or Myleran on the leukocyte count and serum vitamin B₁₂ concentration in chronic myelocytic leukemia is presented in table 1 and figures 1 to 3. It is apparent from the results that a reduction in the total leukocyte count following therapy is followed by a gradual lowering of serum vitamin B₁₂ concentration. In the six cases reported in table 1, the initial pretherapy serum vitamin B₁₂ concentrations range from 1100 μg/ml to 21000 μg/ml and thus are uniformly higher than the average values reported (184, 362, 387 and 275 μg B₁₂/ml.) for normals by four different laboratories.¹ ³ ⁴ ⁵ As a result of therapy with 6-MP or Myleran, the total leukocyte counts fell to

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From the Department of Medicine, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

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a range between 4,100 and 24,500 cells/mm$^3$, and serum $B_{12}$ levels dropped
to a range between 300 $\mu$g/ml and 3600 $\mu$g/ml. Therapy was continued in
all the six cases until such time as the total leukocyte counts dropped
to around 10,000 cells/mm$^3$. Duration of remission in table 1 is defined as the
period in days beginning from the termination of therapy to the time the
total leukocyte count was first recorded to be higher than 20,000 cells/mm$^3$.
There is no apparent relationship between the duration of remission and
the duration of therapy, or between the duration of remission and the pre-
therapy or post-therapy leukocyte count, or between the duration of remission
and the pretherapy serum $B_{12}$ concentrations. It is apparent from the results
of table 1 that the patients with the lower serum $B_{12}$ concentrations at the
termination of therapy have had the longer remissions. In addition the au-
thors are following a 37-year-old-white male patient with chronic myelocytic

Figs. 1. (top) and 2.—Effect of 6-MP and Myleran on the total leukocyte counts and
serum vitamin $B_{12}$ levels in chronic myelocytic leukemia patients.
leukemia who was treated with triethylene melamine (7 doses of 5 mg. each) and x-ray therapy prior to coming to this hospital. His serum vitamin B$_{12}$ was 380 $\mu$g/ml and the total leukocyte count was 19,000 cells/mm$^3$ when he was first seen. This patient has been in remission for 17 months without any further therapy and there has been no appreciable increase in his serum vitamin B$_{12}$ or the total leukocyte count. The results on Case 7 (fig. 3) indicate that following 6-MP therapy this patient's leukocyte count dropped to below 10,000 cells/mm$^3$, and his spleen was palpable 2 cm. below the costal margin, while his serum vitamin B$_{12}$ was 900 $\mu$g/ml, which is significantly higher than the average reported for normals.$^{1,3,5}$ In spite of continued therapy, his total leukocyte count increased to 187,000 cells/mm$^3$ and his serum B$_{12}$ increased to 1900 $\mu$g/ml. Thus, consideration of these two clinical situations suggest that a failure of the serum B$_{12}$ level to approach the average reported for normals may indicate a failure of the control of the leukemic process despite the production of a normal leukocyte level.
TABLE 1.—Effect of 6-MP and Myleran on the Leukocyte Count and Serum Vitamin B\textsubscript{12} Concentration in Chronic Myelocytic Leukemia

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of Therapy* Days</th>
<th>Serum B\textsubscript{12} Before Therapy (\mu g/ml)</th>
<th>Pretherapy Total Leukocyte cells/mm\textsuperscript{3}</th>
<th>Serum B\textsubscript{12} at End of Therapy (\mu g/ml)</th>
<th>Post-therapy Total Leukocyte cells/mm\textsuperscript{3}</th>
<th>Duration of Remission Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 (6-MP)</td>
<td>4,650</td>
<td>250,000</td>
<td>3,600</td>
<td>10,800</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>180 (6-MP and Myleran)</td>
<td>1,700</td>
<td>377,000</td>
<td>1,400</td>
<td>24,500\textsuperscript{f}</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>88 (6-MP)</td>
<td>21,000</td>
<td>216,500</td>
<td>1,100</td>
<td>4,100</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>98 (Myleran)</td>
<td>5,800</td>
<td>386,000</td>
<td>700</td>
<td>8,550</td>
<td>148</td>
</tr>
<tr>
<td>5</td>
<td>125 (6-MP and Myleran)</td>
<td>—</td>
<td>400,000</td>
<td>700\textsuperscript{f}</td>
<td>20,000\textsuperscript{f}</td>
<td>455</td>
</tr>
<tr>
<td>6</td>
<td>90 (Myleran)</td>
<td>1,100</td>
<td>193,600</td>
<td>300</td>
<td>10,100</td>
<td>&gt;264</td>
</tr>
</tbody>
</table>

*Details on the dosages of therapy for Cases 1, 2, 5 and 6 are shown in figures 1 and 2. \textsuperscript{f}Note that during remission the leukocyte counts in Cases 2 and 5 fell to 10,000 and 6,500 cells/mm\textsuperscript{3}, respectively and that in Case 5 the serum B\textsubscript{12} dropped to 100\(\mu g/ml\). (figs. 1 and 2).

DISCUSSION

The results presented in this paper clearly indicate that a reduction in leukocyte count following 6-MP or Myleran therapy in chronic myelocytic leukemia patients is followed by a gradual reduction in serum vitamin B\textsubscript{12} concentrations. The decrease in serum B\textsubscript{12} in patients with chronic myelocytic leukemia treated with x-irradiation is also reported\textsuperscript{3} not to parallel the reduction in leukocytes but often to occur weeks after the maximum drop in leukocytes takes place. Since the decrease in serum vitamin B\textsubscript{12} following therapy is gradual and follows the drop in leukocytes, at the time therapy is terminated the total leukocytes may fall to around 10,000 cells/mm\textsuperscript{3} while the serum B\textsubscript{12} is significantly higher than the average values reported\textsuperscript{1,3,5} for normals (Cases 1–3, table 1).

Preliminary studies on the nature of the vitamin B\textsubscript{12}-binding proteins of the serum have been conducted. After fractionation of the serum proteins by paper electrophoresis, the vitamin B\textsubscript{12} bound to the individual fractions has been liberated and measured microbiologically.\textsuperscript{6} The results of this study indicate that in chronic myelocytic leukemia patients, the \(a_1\)-globulin fractions contain several times the normal amount of bound vitamin B\textsubscript{12}, and that following 6-MP or Myleran therapy a reduction in total leukocyte count is followed by a lowering of the amount of B\textsubscript{12} bound to the \(a_1\)-globulin fraction. These data are in accord with the findings of Heinrich and Erdman-oehecker\textsuperscript{7} who noted a decrease in B\textsubscript{12} bound to the \(a_1\)-globulin fractions of chronic myelocytic leukemia cases following x-ray therapy. The source of the \(a_1\)-globulin fraction which binds vitamin B\textsubscript{12} is obscure. In this connec-
tion, it is interesting to note that an increase in serum $B_{12}$ values is also reported in viral hepatitis, metastatic liver disease and occasionally in cirrhosis of the liver.\textsuperscript{8,9}

**SUMMARY**

Serum vitamin $B_{12}$ determinations and the total leukocyte counts were made at regular intervals in chronic myelocytic leukemia patients before, during and after therapy with 6-MP or Myleran. The results indicate that a reduction in total leukocytes following therapy is followed by a gradual lowering of serum vitamin $B_{12}$. The levels of serum vitamin $B_{12}$ at the completion of therapy may be related to the duration of remission.

**SUMMARIO IN INTERLINGUA**

Deterrninationes del nivello seral de vitamina $B_{12}$ e numerationes del leucocytos total esseva effectuate a intervallos regular in patientes con chronic leucemia myelocytic ante, durante, e post therapia con 6-mercaptopurina o Myleran. Le resultatos indica que un reduction del total de leucocytos que occurre post le therapia es sequite per un reduction gradual del nivello seral de vitamina $B_{12}$. Le nivelloseral de vitamina $B_{12}$ al fin curso therapeutic es possibilemente relationate al durata del remission.

**REFERENCES**

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