Correlation of Granulocyte Mobilization with Etiocholanolone and the Subsequent Development of Myelosuppression in Patients with Acute Leukemia Receiving Therapy

By Herman A. Godwin, Theodore S. Zimmerman, Harry R. Kimball, Sheldon M. Wolff and Seymour Perry

Despite the improved results in the treatment of acute leukemia, severe myelosuppression is a frequent complication and leads to increased morbidity and mortality due to infections. Therefore, measurement of the functional status of the bone marrow prior to therapy should be beneficial in predicting the development of toxicity. Bacterial endotoxin which mobilizes granulocytes from marrow reserve stores has been employed by several investigators to assess marrow function in various neoplastic disorders especially during chemotherapy. Recently the granulocyte response to etiocholanolone, a naturally occurring pyrogenic steroid metabolite causing granulocytosis in man, has been shown to be similar to that obtained with endotoxin. Furthermore, among a group of solid tumor patients, an abnormal granulocyte response to etiocholanolone was associated with the subsequent development of increased myelosuppression following intensive antitumor therapy. Etiocholanolone has several advantages compared with endotoxin. For example, it can be administered by the intramuscular route; it has a known chemical structure; it is nonantigenic; and, its administration has not been associated with hypotension.

In this report, the numbers of granulocytes mobilized in response to etiocholanolone in patients with acute leukemia immediately prior to separate courses of therapy is related to the subsequent development of myelosuppression. The results indicate that etiocholanolone is helpful in the assessment of granulocyte reserves prior to treatment in acute leukemia.

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GRANULOCYTE MOBILIZATION

MATERIALS AND METHODS

The study group, composed of 36 patients (23 males and 13 females) ranging in age from 3 to 67 years, was treated on the Acute Leukemia Service of the National Cancer Institute. Twenty-six individuals were diagnosed as having acute lymphocytic leukemia, 7 acute myelocytic leukemia, 3 undifferentiated acute leukemia, and 1 blastic phase of chronic myelocytic leukemia. Seventy-nine separate studies were performed. Three newly diagnosed cases were studied prior to any treatment. The remaining 76 studies were conducted before reinduction therapy or before monthly maintenance therapy. All tests were performed 1–3 days prior to the institution of a course of treatment. In 47 instances, combination chemotherapy with prednisone, vincristine, 6-mercaptopurine, and methotrexate was given. Twenty-four studies were followed by 4 daily infusions of cytosine arabinoside, 5 with a weekly infusion of vincristine and daily oral prednisone, and 3 with fractionated doses of total body irradiation.

Etiocholanolone* prepared at a concentration of 10 mg./ml. in propylene glycol was administered intramuscularly to patients in a dose of 0.1 mg./kg. at midnight. Blood samples were obtained for white blood cell and differential counts prior to injection and at 9, 12, 15, and 18 hours after etiocholanolone. Sterile, pyrogen-free needles and syringes were used throughout. With two exceptions tests were performed only in patients with baseline temperatures of 38.5°C or less. All patients were at bed rest except for bathroom privileges during the period of study. Temperature was obtained before etiocholanolone injection and every 2 hours for 22 hours. Blood pressure, pulse, and respiration were observed every 4 hours for the same time period. Any unusual signs or symptoms were noted and recorded. White blood cells were counted in duplicate in an electronic counter* after saponin lysis of red blood cells, and the average was recorded as the white blood cell count. Differential counts of 100 cells on a Wright-Giemsa stained blood smear were performed. Total granulocyte counts included mature and band neutrophilic granulocytes, eosinophils, and basophils. Granulocyte counts prior to injection of etiocholanolone were designated as baseline. The maximal change of granulocytes per mm.³ from baseline was recorded as the granulocyte increment (ΔG). In a large series of studies in normals, it was established that a ΔG of 2600 granulocytes/mm.³ constituted a positive test response, and any smaller granulocyte increment was considered negative. Bone marrow remission was defined as the presence of 5 percent or less blast cells while greater than 5 percent blast cells constituted relapse. Since it has been shown previously that etiocholanolone does not affect platelet counts, these were not done during the test in the present studies.

Toxicity associated with a course of treatment was assessed by examination of the patient’s clinical record for the immediate post-therapy period up to 14 days following therapy and without knowledge of the response to etiocholanolone. This evaluation was made at the maximum of hematologic depression. A circulating white cell count of 4000/mm.³, granulocyte count of 1500/mm.³, and platelet level of 100,000/mm.³ were considered to be the lower limits of normal. Toxicity was graded as follows:

| Mild Toxicity | WBC 2500–4000/mm.³ and/or granulocytes 1000–1500/mm.³ and/or platelets 50,000–100,000/mm.³ |
| Moderate Toxicity | WBC 1000–2500/mm.³ and/or granulocytes 500–1000/mm.³ and/or platelets 20,000–50,000/mm.³ |
| Severe Toxicity | WBC <1000/mm.³ and/or granulocytes <500/mm.³ and/or platelets <20,000/mm.³ |

If myelosuppression existed at the time of baseline studies, toxicity was not attributed to subsequent therapy unless a more severe degree of depression ensued. Otherwise, if the extent of myelosuppression were unchanged, no toxicity was assigned. Other manifestations

*Merck, Sharp, and Dohme, West Point, Pennsylvania. Kindly supplied by the Cancer Chemotherapy National Service Center.
*Coulter Counter, Model B, Hialeah, Florida.
Table 1.—Relationship of Hematologic Status to Etiocholanolone Test Response

<table>
<thead>
<tr>
<th></th>
<th>Etiocholanolone Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>A. Baseline White Blood Cell Count (per mm.(^3))</td>
<td></td>
</tr>
<tr>
<td>7500 or greater</td>
<td>5</td>
</tr>
<tr>
<td>5000 – 7499</td>
<td>7</td>
</tr>
<tr>
<td>4000 – 4999</td>
<td>10</td>
</tr>
<tr>
<td>2500 – 3999</td>
<td>6</td>
</tr>
<tr>
<td>1000 – 2499</td>
<td>0</td>
</tr>
<tr>
<td>&lt;1000</td>
<td>0</td>
</tr>
<tr>
<td>B. Baseline Granulocyte Count (per mm.(^3))</td>
<td></td>
</tr>
<tr>
<td>3000 or greater</td>
<td>12</td>
</tr>
<tr>
<td>1500 – 2999</td>
<td>14</td>
</tr>
<tr>
<td>1000 – 1499</td>
<td>2</td>
</tr>
<tr>
<td>500 – 999</td>
<td>0</td>
</tr>
<tr>
<td>&lt;500</td>
<td>0</td>
</tr>
<tr>
<td>C. Bone Marrow Status</td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>15</td>
</tr>
<tr>
<td>Relapse</td>
<td>13</td>
</tr>
</tbody>
</table>

Results

Test Response

Among the 79 studies, 28 tests were positive and 51 were negative. Table 1 shows that with baseline peripheral white cell counts 4000/mm.\(^3\) or greater, slightly more than one-half the studies yielded positive results. No individual with an initial white cell count less than 2500/mm.\(^3\) had a normal granulocyte increment. At baseline granulocyte levels above 1500/mm.\(^3\), approximately 60 percent had a normal 8G whereas an initial granulocyte count less than 1000/mm.\(^3\) was invariably associated with a negative response (Table 1).

Fifteen of 35 studies in patients with morphologic bone marrow remission were positive, while 13 of 44 patients with marrow relapse still had a normal test response (Table 1). Three patients studied prior to the institution of any therapy had negative tests indicating an inadequate marrow granulocyte reserve. Increasing bone marrow involvement by the leukemic process made a positive test result less likely.

Among patients with normal baseline white cell and granulocyte counts, normal granulocyte mobilization was only slightly more likely than was an abnormal granulocyte increment (Table 2). There was no correlation with bone marrow morphologic status. However, when either the initial white cell count or the granulocyte level was abnormal, a positive test response could still occur. On the other hand, when both the peripheral white cell and granulocyte counts were depressed below normal, a positive test result did not occur (Table 2).
Table 2.—Test Responses as Related to Combined Baseline White Cell Count, Granulocyte Level, and Bone Marrow Status

<table>
<thead>
<tr>
<th>WBC (per mm.$^3$)</th>
<th>Granulocytes (per mm.$^3$)</th>
<th>Bone Marrow Status</th>
<th>Etiocholanolone Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4000</td>
<td>&gt;1500 Remission</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>&gt;4000</td>
<td>&gt;1500 Relapse</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>&gt;4000</td>
<td>&lt;1500 Remission</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>&gt;4000</td>
<td>&lt;1500 Relapse</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&gt;1500 Remission</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&gt;1500 Relapse</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&lt;1500 Remission</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&lt;1500 Relapse</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3.—Incidence of Hematologic Toxicity After Therapy as Related to Etiocholanolone Test Response and Baseline Hematologic Values

<table>
<thead>
<tr>
<th>Baseline Status</th>
<th>Etiocholanolone Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive*</td>
</tr>
<tr>
<td>White Count</td>
<td></td>
</tr>
<tr>
<td>&gt;4000/mm.$^3$</td>
<td>7/22 (31.8%)</td>
</tr>
<tr>
<td>&lt;4000/mm.$^3$</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>Granulocyte Count</td>
<td></td>
</tr>
<tr>
<td>&gt;1500/mm.$^3$</td>
<td>9/26 (34.6%)</td>
</tr>
<tr>
<td>&lt;1500/mm.$^3$</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Bone Marrow Status</td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>5/15 (33.3%)</td>
</tr>
<tr>
<td>Relapse</td>
<td>5/13 (38.5%)</td>
</tr>
</tbody>
</table>

*Incidence of hematologic toxicity after therapy in patients demonstrating a positive etiocholanolone test response.
†Incidence of hematologic toxicity after therapy in patients demonstrating a negative etiocholanolone test response.

Hematologic Toxicity

Incidence. In 10 of 28 (35.7 percent) studies with a positive etiocholanolone test, there was some manifestation of peripheral hematologic toxicity following therapy (white blood cell, granulocyte, and platelet depressions either singly or in various combinations). In only one instance was there a selective depression of platelet levels. In contrast, 38 of 51 (74.5 percent) abnormal etiocholanolone test responses were followed by the development of toxicity after treatment (p < 0.001*).

When baseline white cell counts, granulocyte counts, or bone marrow status were compared with the response to etiocholanolone to determine if these might predict myelosuppression following therapy, it can be seen that a lesser incidence of toxicity always occurred among those cases with a positive study (Table 3). For example, among patients with normal baseline white cell counts, the incidence of toxicity among those with an abnormal test response was much more common (80.9 percent vs. 31.8 percent, Table 3).

Among individuals with both normal baseline white cell and granulocyte...
Table 4.—Incidence of Toxicity as Related to Combined Baseline White Cell and Granulocyte Counts

<table>
<thead>
<tr>
<th>WBC (per mm.³)</th>
<th>Granulocytes (per mm.³)</th>
<th>Etiocholanolone Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4000</td>
<td>&gt;1500</td>
<td>6/20¹</td>
</tr>
<tr>
<td>&gt;4000</td>
<td>&lt;1500</td>
<td>1/2</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&gt;1500</td>
<td>3/6</td>
</tr>
<tr>
<td>&lt;4000</td>
<td>&lt;1500</td>
<td>0/0</td>
</tr>
</tbody>
</table>

¹Numerator: number of patients developing toxicity to therapy. Denominator: number of patients with positive or negative responses to etiocholanolone.

Table 5.—Severity of Toxicity as Related to Etiocholanolone Test Response

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>All Patients</th>
<th>Etiocholanolone Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>None</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Mild</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td>22</td>
</tr>
</tbody>
</table>

Total Studies 28 51 20 15

counts, the incidence of toxicity was almost 2½ times greater in the group with negative etiocholanolone tests (p<0.02) (Table 4). The small number of studies with depression of either the white cell or granulocyte level alone did not permit any conclusions related to test response. Those studies where both baseline white cell and granulocyte counts were depressed had a very high incidence of toxicity (19 of 26 cases, 73.1 percent). As noted earlier no tests in this group were positive (Table 4).

Severity of toxicity. In the group with positive etiocholanolone tests toxicity was usually mild (Table 5). By comparison, toxicity in the group with inadequate granulocyte reserve tests was more often moderate or severe (Table 5).

Table 5 demonstrates that among 20 patients with normal white cell and granulocyte counts and a positive test result, only two instances of moderate or severe toxicity occurred. In the patient group with normal baseline peripheral counts but an abnormal δG, six of 15 developed moderate or severe toxicity. These differences in toxicity are significant (p<0.05).

Although there were two instances of severe toxicity occurring in persons with positive test results, the granulocyte increments with etiocholanolone were borderline (2600/mm.³ and 3000/mm.³ respectively). Figure 1 illustrates the low incidence of toxicity at granulocyte increments above 2600/mm.³. No severe toxicity occurred in patients at a δG above 3000/mm.³.

Severe complications in addition to hematologic depression developed in 7 patients during the 14 day post-therapy period. Complications included pharyngitis, septicemia, leg abscess, felon, hepatitis (drug-induced), gastrointestinal bleeding and hematuria (both due to thrombocytopenia). In six of these individuals, the etiocholanolone test response prior to therapy was abnormal.
Fig. 1.—The incidence of drug induced toxicity (in percent) in relationship to the granulocyte response to etiocholanolone. An increment of 2600 granulocytes per mm$^3$ and above is normal, and it is in this group of studies that the least toxicity occurred. Definition of degrees of toxicity is in the text.

**Complications with Etiocholanolone**

There were no significant problems encountered with the use of etiocholanolone. Pain at the site of injection, myalgia, and headache usually occurred but were minor and transient. The average temperature rise was 1.1 C. (range 0 C.–3 C.). On two occasions, etiocholanolone was administered to a patient with temperature above 38.5 C. with no accentuation of the fever.

**DISCUSSION**

Granulocyte mobilization in response to etiocholanolone was studied in a group of patients with acute leukemia prior to the administration of therapy in an attempt to determine if a correlation existed between test response and the subsequent development of hematologic toxicity following treatment. Etiocholanolone proved to be safe for use and there were no significant side effects. Studies were conducted in newly diagnosed cases prior to therapy, during periods of complete and partial remission, and in relapse. Only three individuals were tested prior to receiving any treatment and each had a negative
response. This substantiates previous observations with endotoxin demonstrating poor granulocyte mobilization in untreated acute leukemia. Among treated patients with normal peripheral white cell, granulocyte, and platelet counts and remission bone marrows, a considerable number had abnormal test responses. Adequate granulocyte reserves were increasingly less frequent as baseline peripheral blood counts were depressed and the bone marrow was more extensively involved with the leukemic process. No patient had a normal granulocyte increment when both the peripheral white blood cell and granulocyte counts were below normal, regardless of marrow status, making performance of the test seem unnecessary in this group.

Myelosuppressive toxicity following treatment was significantly more frequent among individuals with inadequate granulocyte mobilization to etiocholanolone. With a single exception, platelet count depression was accompanied by a concomitant fall in white cell and/or granulocyte counts. In patients having normal peripheral blood counts at the time of study, regardless of bone marrow status, there was a markedly increased incidence of toxicity in those with an abnormal test response.

The severity of toxicity was greater among persons with abnormal granulocyte increments suggesting that the dose of cytotoxic agents should be adjusted according to the etiocholanolone test result. This was true for the group having normal baseline white cell, granulocyte, and platelet levels as well as for patients with depressed baseline counts. Two patients having normal granulocyte increments did develop severe toxicity, but as noted each had only marginally normal granulocyte mobilization. No severe myelosuppression occurred when the granulocyte increment was greater than 3000/mm³. Severe miscellaneous toxicities associated with myelosuppression were present in less than 10 percent of cases. Only one of these involved a patient having had a positive test result prior to therapy.

While a normal response to etiocholanolone does not preclude the subsequent development of toxicity with therapy, the marked differences in incidence and severity of complications between patients responding and those not responding to the agent suggest that this test can provide a safe and valuable guide in the therapy for acute leukemia.

**Summary**

1. Etiocholanolone was employed in the assessment of bone marrow granulocyte reserves in a group of patients with acute leukemia prior to the administration of therapy.

2. Normal levels of circulating white blood cells and granulocytes and/or remission bone marrow status were often associated with abnormal test responses.

3. Individuals having a positive test response experienced significantly less hematologic toxicity following therapy than did those patients having a negative response.

4. Etiocholanolone proved to be a safe agent without significant side effects.
5. This test can be helpful in the prediction of toxicity following antileukemic therapy.

SUMMARIO IN INTERLINGUA

1. Etiocholanolona esseva utilisate in le evaluation del reservas granulocytic del medulla ossee in un gruppo de patientes con leucemia acute ante le administration de ullle agente therapeutic.
2. Normal numerationes leucocytic in le circulation e/o un stato de remission quanto al medulla ossee esseva frequentemente associate con responsas anormal in le tests.
3. Subjectos con positive responsas in le tests experientiava significativamente minus toxicitate hematologic post le therapia que le subjectos in qui ille responsas esseva negative.
4. Etiocholanolona se provava un salve agente non associate con significative effectos lateral.
5. Iste test pote esser utile in le prediction de toxicitate post therapia antileucemic.

ACKNOWLEDGMENT

The authors wish to thank Dr. Edward S. Henderson, Head, Acute Leukemia Service, National Cancer Institute, for allowing us to study these patients. We are indebted to Mrs. Pamela Woodruff, Miss Betty Walsh, Mrs. Susan Cornell, and Mrs. Cynthia O’Connor for technical assistance.

REFERENCES


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