Effect of α-Interferon Therapy on Bone Marrow Fibrosis in Hairy Cell Leukemia


Iliac crest trephine biopsy specimens from 16 patients treated with recombinant α-interferon (α-IFN) for hairy cell leukemia (HCL) were examined for reticulin and collagen content. These data were compared with the hairy cell index (HCI), the proportion of hairy cells to the overall cellularity of the bone marrow. Specimens were studied immediately before α-IFN therapy, at 6-month intervals during, and in six patients 6 months after cessation of therapy. All patients presented with increased bone marrow fibrosis ranging from focally increased reticulin to a diffuse increase in both reticulin and collagen content. This fibrosis was observed to decrease during α-IFN therapy insomuch as the hairy cell population was diminished in the bone marrow in 13 patients. Regression analysis of HCI v bone marrow fibrosis showed a positive correlation ($r = .73$, $P < .02$). Six patients demonstrated a reduction in bone marrow reticulin and collagen to normal levels during α-IFN therapy. Two of six patients demonstrated increased bone marrow fibrosis and HCL 6 months after cessation of α-IFN therapy. Three of 16 patients exhibited no decrease in bone marrow reticulin content during therapy despite a decreased bone marrow hairy cell population.

MATERIALS AND METHODS

Patient population. Twenty patients with a diagnosis of HCL confirmed by examination of peripheral blood and bone marrow biopsy specimens were seen at Roswell Park Memorial Institute during the time period between April 1984 and November 1986 and initially included in the present study. Four of these patients were excluded from the study due either to insufficient material for study or inadequate follow-up. The remaining 16 patients ranged in age from 39 to 69 years (mean age, 54 years). Fourteen patients (88%) had previous splenectomy, and five patients (31%) had received previous chemotherapy including chlorambucil (Leukeran), prednisone, vincristine, bleomycin, and doxorubicin. All patients were treated with α-IFN (Intron A, Schering Corp, Kenilworth, NJ), 2 x $10^6$ U/m² subcutaneously three times weekly. Dosages were decreased to 75% on the initial dose for two patients. Nine patients completed 12 months' α-IFN therapy, and seven patients received 18 months' α-IFN therapy. All patients used in this study were advised of procedures and attendant risks in accordance with institutional guidelines and gave informed consent.

Bone marrow biopsies. A total of 61 bone marrow core biopsy specimens were obtained from the 16 study patients at time intervals including first, before IFN therapy; next, at 6-month intervals during therapy; and finally, in six patients 6 months after cessation of α-IFN therapy. All trephine biopsy samples were obtained from the posterior iliac crest by using an 11G (regular adult) Jamshidi needle. These specimens were initially fixed in 10% neutral formalin, embedded in paraffin, cut into sections 5 µm thick, and stained with hematoxylin-eosin for morphologic analysis, silver for reticulin analysis, and Masson trichrome for analysis of collagen fiber content. The reticulin content was evaluated by light microscopy, and the following grading system was used: grade 1, no increase in reticulin content—occasional fine and coarse individual fibers only or occasional fine and coarse individual fibers with foci of perivascular fiber network or reticulin associated with benign lymphoid follicles; grade 2, foci increase in reticulin content—focal increase in reticulin content away from the vessels and benign lymphoid follicles; grade 3, diffuse increase in reticulin content—diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain); and grade 4, diffuse increase in reticulin and collagen concentrations—diffuse, coarse fiber network with areas of collagenization (positive trichrome stain).

All bone marrow biopsy specimens were reviewed independently by two of the authors (P.M. and M.L.). The percentage of marrow hairy cells was estimated by visual inspection of the biopsy specimen. A hairy cell index (HCI) was calculated by multiplying the bone marrow cellularity by the percentage of hairy cells and dividing by...
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10,000. The strength of association (degree of linear relationship) between the HCI and bone marrow fibrosis was tested by using regression analysis and Student's t test.

RESULTS

Before α-IFN therapy, examination of the peripheral blood counts revealed that all but one patient presented with variable pancytopenia; the mean values for hemoglobin concentration, platelet count, and WBC count were 9.7 g/dL, 92 x 10^9/L, and 13.1 x 10^9/L, respectively. Four patients presented with elevated WBC counts (mean 31.3 x 10^9/L). After 6 months of α-IFN therapy, increases in hemoglobin and platelet levels to respective mean values of 13.0 g/dL and 237 x 10^9/L and decreased granulocyte counts to 4.1 x 10^9/L were observed. All patients responded to α-IFN treatment, with one patient demonstrating a complete response as defined by a decrease in bone marrow hairy cells to fewer than 5% and normalization of peripheral blood values and seven patients demonstrating a 50% decrease in pre-α-IFN hairy cell bone marrow infiltrate and normalization of peripheral blood values. The remaining eight patients demonstrated improvement in peripheral blood values alone.

Figure 1 demonstrates the typical appearance under light microscopy of bone marrow from an HCL patient before receiving α-IFN therapy (grade 3, diffuse increase in reticulin content). Mononuclear cells are distributed uniformly throughout the biopsy and are interspersed with reticulin fibers. In Fig 2, the bone marrow from the same patient after receiving 12 months of α-IFN therapy demonstrates a normalization of overall cellularity, a decreased proportion of hairy cells, and decreased amounts of reticulin fiber (grade 2, focal increase in reticulin content).

Table 1 summarizes the bone marrow analyses as to percent cellularity, percentage of hairy cells, and HCI during α-IFN therapy. Bone marrow cellularity was noted to decrease from an average 80% (±20%) before α-IFN therapy to 56% (±22%) after 6 months of therapy, with the percentage of hairy cells also decreasing from an average 77% (±12%) to 56% (±28%). Before α-IFN therapy, there was a diffuse uniform infiltration by mononuclear cells observed in all but one of the trephine biopsy specimens, with depletion of myeloid cells associated with the dominance of these lymphoid cells. Myeloid to lymphoid ratios ranged from 1:1.5 to 2.6:1 in these biopsy samples.

Table 2 summarizes bone marrow analyses by grade of fibrosis during α-IFN therapy. Before α-IFN therapy 56% of bone marrow biopsy specimens were evaluated as grade 3 (diffuse increase in reticulin content), 31% were grade 2 (focal increase in reticulin content) and 12% were grade 4 (diffuse increase in reticulin and collagen concentrations). No distortion of the architecture of trabecular bone was observed in any specimen. Bone marrow fibrosis was decreased by one grade in 12 patients during α-IFN therapy and remained unchanged in the remaining four patients. In the eight patients who demonstrated a complete or partial

Table 1. Bone Marrow Analyses—Percent Cellularity and HCI

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>No. of Specimens Examined</th>
<th>Percent Cellularity</th>
<th>Percentage of Hairy Cells</th>
<th>HCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of diagnosis</td>
<td>16</td>
<td>80 (20)</td>
<td>77 (12)</td>
<td>.62 (.19)</td>
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<tr>
<td>During α-IFN therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mo</td>
<td>16</td>
<td>56 (22)</td>
<td>56 (28)</td>
<td>.33 (.19)</td>
</tr>
<tr>
<td>12 mo</td>
<td>16</td>
<td>52 (26)</td>
<td>38 (26)</td>
<td>.19 (.14)</td>
</tr>
<tr>
<td>18 mo</td>
<td>7</td>
<td>46 (29)</td>
<td>63 (26)</td>
<td>.23 (.12)</td>
</tr>
<tr>
<td>6 mo after α-IFN therapy (24 mo)</td>
<td>6</td>
<td>54 (28)</td>
<td>52 (25)</td>
<td>.30 (.22)</td>
</tr>
</tbody>
</table>

HCI = bone marrow cellularity × percentage of hairy cells/10,000.
All values are expressed as means (± SD).
Table 2. Bone Marrow Analyses—Grade of Fibrosis

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>No. of Specimens Examined</th>
<th>Grade of Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Time of diagnosis</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>During α-IFN therapy</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>6 mo</td>
<td>16</td>
<td>4</td>
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<td>12 mo</td>
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<td>5</td>
</tr>
<tr>
<td>18 mo</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>6 mo after α-IFN therapy (24 mo)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>12</td>
</tr>
</tbody>
</table>

Bone marrow fibrosis grading system: grade 1, no increase in reticulin content; grade 2, focal increase in reticulin content; grade 3, diffuse increase in reticulin content; and grade 4, diffuse increase in reticulin and collagen concentrations.

response to α-IFN therapy, three demonstrated decreased bone marrow reticulin levels to a normal distribution. The remaining three patients who exhibited unchanged reticulin distribution in the bone marrow had shown only focal distribution of bone marrow reticulin (grade 2 fibrosis) before α-IFN therapy. A reduction in marrow reticulin and collagen to normal levels was observed in six patients. Two patients developed an increased percentage of hairy cells and marrow fibrosis 6 months after cessation of their α-IFN therapy. After 18 months of α-IFN therapy no bone marrow biopsy sample showed more than focal increases in reticulin distribution away from vessels (grade 2).

Figures 3 and 4 demonstrate the trends of the HCl and bone marrow fibrosis during α-IFN therapy. Regression analysis of the HCl v bone marrow fibrosis produced a Pearson's correlation coefficient of .73 (P < .02).

DISCUSSION

Previous reports of bone marrow changes in patients with HCL during α-IFN therapy include that of Bardawil et al,5 which showed that marrow reticulin was virtually unchanged in 12 HCL patients treated with α-IFN for 8 months despite a considerable reduction in the numbers of hairy cells noted in the marrow. Furthermore, they observed that the amounts of reticulin remained increased even in areas of active hematopoiesis. Naeim and Jacobs2 reported on 21 HCL patients treated with α-IFN for 6 months and observed that all patients showed increased bone marrow reticulin levels before therapy and that 89% of bone marrow aspirates were dry taps. Furthermore, they reported that the amount of reticulin remained increased after 6 months of α-IFN therapy with a persistence of dry taps (73%). Spiers et al3 observed in 37 HCL patients treated with pentostatin (2'-deoxycoformycin) the disappearance of bone marrow fibrosis after 9 to 27 weeks of treatment, thereby making it possible to aspirate bone marrow that had previously yielded only a dry tap. In the present study we observed decreased bone marrow fibrosis during α-IFN therapy as the hairy cell population in the bone marrow diminished. This study supports previous reports in that the amount of bone marrow reticulin remained increased after 6 months of α-IFN therapy. During the first 12 months of α-IFN therapy there was a gradual decrease in bone marrow fibrosis, and normalization of the bone marrow reticulin content was not observed until 18 months of α-IFN therapy. It is of interest that after 24 months (6 months not receiving α-IFN therapy) bone marrow fibrosis was observed to increase in patients who also demonstrated increased bone marrow hairy cells.

The etiology of the increased reticulin content in bone marrow that is associated with hematologic malignancies has not been elucidated. The source of reticulin and collagen fibers seen in HCL bone marrow has been observed by electron microscopy to be single fibroblastic cells with slender cytoplasmic processes that are randomly dispersed.
among and possibly induced by the hairy cells present. This distribution of fibroblasts with surrounding microfilaments and bundles of collagen fibers was not observed in patients with CLL or lymphomatous infiltrates. HCL patients also may demonstrate lymph node fibrosis similar to that seen in the bone marrow. It has been proposed that the increased bone marrow reticulin concentration in HCL is an integral part of the neoplastic process.

HCL is generally considered a B-lymphocyte neoplasm, and hairy cells exhibit a B phenotype (cells positive for surface immunoglobulins and Ia and Bl antigens). Moreover, hairy cells exhibit phagocytic capability, Fc receptors for IgG, and cytologic features of monocytes. In addition, the Tac antigen, which is generally expressed by T cells, has been reported on hairy cells. These observations indicate a possible defect in the pluripotent stem cell. Monocytes have been shown to release platelet-derived growth factor, which is both a chemotactic factor for fibroblasts and also stimulates fibroblasts to enter the G1 phase of the cell cycle. In addition, avian monocytic leukemia cells have been reported to release growth factors that induce the proliferation of fibroblasts. Whether hairy cells are capable of eliciting fibroblast growth factors would be of interest to investigate.

In summary, the findings of this study indicate that there is a positive correlation between the amount of hairy cell infiltration of the bone marrow and the presence of increased bone marrow fibrosis. Moreover, effective treatment with α-IFN resulting in a decrease in the percentage of hairy cells in the marrow results in dissolution of this abnormal marrow reticulin and collagen, thereby alleviating the problem of failed bone marrow aspirations (dry tap). Finally, observed increased bone marrow fibrosis is useful in heralding relapse and may therefore be a useful parameter to guide treatment of patients with HCL.

REFERENCES

Effect of alpha-interferon therapy on bone marrow fibrosis in hairy cell leukemia

M Laughlin, A Islam, M Barcos, P Meade, H Ozer, M Gavigan, E Henderson and T Han