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THE EFFECT OF HIGH – DOSE METHYLPREDNISOLONE ON CD34 – POSITIVE BONE MARROW CELLS IN CHILDREN WITH ACUTE MYELOBLASTIC LEUKEMIA*

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SUMMARY: Tuncer AM, Hiçsönmez G, Gümrük F, Albayrak D, Duru F, Güzel E, Şaylı T. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). The effect of high-dose methylprednisolone on CD34-positive bone marrow cells in children with acute myeloblastic leukemia. Turk J Pediatr 1995; 37: 345-349.

The expression of CD34 antigen on the surface of bone marrow (BM) cells during remission induction was studied in 20 patients with CD34-negative acute myeloblastic leukemia (AML). The patients were given high-dose methylprednisolone (HDMP) alone for one week, after which time mitoxantrone and low-dose Ara-C were added.

BM cells from all patients were studied one, two and four weeks after initiation of treatment to evaluate CD34 antigen expression using a three-step peroxidase anti-peroxidase staining technique. The mean percentage of CD34-positive BM cells was 5.3% at presentation, increasing to 15.6% in the first week, 12.9% in the second week and 21.7% in the fourth week of therapy. During the same period the mean percentages of the initial BM blasts decreased from 64% to 22%, 7% and 2% in the first, second and fourth weeks of therapy, respectively. The increase in the CD34-positive BM cells one week after HDMP treatment alone suggests that HDMP directly or indirectly stimulates CD34-positive hematopoietic progenitor cells while decreasing BM blasts in patients with AML. *Key words: high-dose methylprednisolone, acute myeloblastic leukemia, CD34.*

All hematopoietic progenitor cells express the CD34 antigen, expression of which decreases during maturation of progenitor cells[1-3]. CD34 membrane glycoprotein expression is restricted to about one to five percent of normal bone marrow (BM) cells. Stimulation of CD34-positive cells in BM and acceleration of the recovery of peripheral blood leukocytes in leukopenic children with acute lymphoblastic leukemia (ALL) by high-dose methylprednisolone (HDMP) treatment have previously been reported by the current authors[4, 5].

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This study was undertaken to evaluate the effect of HDMP and its combination with cytotoxic chemotherapy on the expression of CD34 antigen-positive cells in the BM of CD34-negative acute myeloblastic leukemia (AML) patients during induction therapy.

Material and Methods

Patients: From September 1990 to April 1991, the expression of CD34 antigen of BM cells from 20 patients with CD34 negative AML (one with M1, 12 with M2, three with M3, three with M4, one with M5) was studied. The patients' ages ranged from five to 15 years. Diagnosis was made by morphology according to the French-American-British (FAB) classification, cytochemistry and cell surface marker analysis. Only CD34-negative cases were chosen in order to avoid any confusion of residual leukemic cells in bone marrow in remission. In this study less than 15 percent positivity was considered to be CD34-negative[6].

All patients received HDMP (30 mg/kg/day per orally) alone in the first week. Then mitoxantrone (0.3 mg/kg/week intravenous infusion) and low-dose Ara-C (0.3 mg/kg/twice daily subcutaneously) were combined with HDMP (20 mg/kg/day per orally). This combination was given for four weeks.

Immunoperoxidase Staining: Cells were obtained from heparinized BM aspirates at diagnosis and one, two and four weeks after the initiation of therapy. Mononuclear cells were separated by Ficoll-Hypaque^{R} (density 1.077 g/ml) gradient centrifugation. Air-dried cytospin slides were acetone-fixed and incubated with anti-CD34 monoclonal antibody (MoAb) (Anti HPCA-1, Sera Lab, England) as a primary antibody (diluted 1:10 in Tris-NaCl buffer containing 0.1% BSA, pH 7.4) and washed with tris-NaCl wash buffer[7]. They were incubated with peroxidase-conjugated rabbit anti-mouse IgG (p161, DAKO, Copenhagen, Denmark) and peroxidase-conjugated goat anti-rabbit IgG (Medao, Hamburg, Germany) followed by the addition of the substrate, 3 amino-9 ethylcarbazole (AEC, 30% Merck, Frankfurt, Germany). Hydrogen peroxide was added to the AEC solution to block endogenous peroxidase activity. Two hundred cells were counted to assess the antibody reactivity with CD34 antigen.

Statistical Analysis: Statistical analysis was performed by variance analysis and student's t test.

Results

CD34 reactivities, found to be 5.3 percent at diagnosis, increased to 15.6 percent, 12.9 percent and 21.7 percent one, two and four weeks after the initiation of treatment, respectively (Fig. 1). During the same period the mean percentages of BM blasts decreased from 64 percent to 22, 7 and 2 percent in the first,

second and fourth week of therapy, respectively (Fig. 1). There was significant difference in the percentages of CD34-positive cells at diagnosis and in the first week of treatment ($p < 0.05$). Although there was no significant difference between the first and second week results, the difference in the percentages of CD34-positive cells obtained in the second and fourth weeks of therapy was statistically significant ($p < 0.05$).

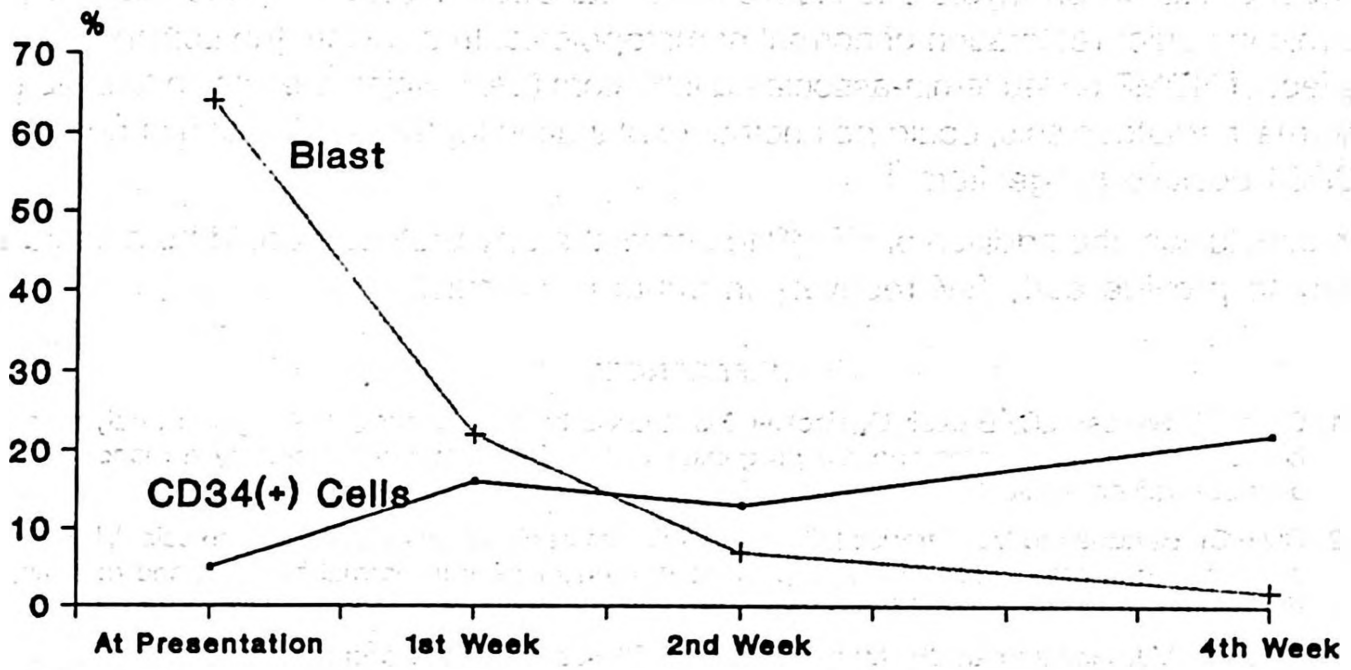

Fig. 1: The percentages of CD34 + cells and blasts in bone marrow.

Discussion

Our data has shown that the proliferation of $CD34^+$ progenitor cells is induced by HDMP treatment in AML during remission induction therapy. In our previous study we demonstrated that BM CD34-positive cells significantly increased during induction therapy in CD34-negative ALL patients who received HDMP treatment. In this study the increase in CD34-positive cells was found significantly higher than that obtained in patients who received conventional dose (2 mg/kg prednisolone) steroid therapy[4].

In the present study, in CD34-negative AML patients who received only HDMP treatment during the first week of therapy, the number of CD34 + cells increased, while BM blasts decreased significantly. The increase in the number of CD34-positive cells indicates the stimulation of normal hematopoietic progenitors.

The acceleration of peripheral blood leukocyte recovery in leukopenic patients with ALL by HDMP treatment was reported previously by Hiçsönmez et al.[5] This result might also be explained by the stimulation effect of HDMP on the proliferation of bone marrow $CD34^+$ hematopoietic cells. Similarly, enhanced

$CD34^+$ cells by intravenous administration of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) has been shown by Sienna et al.[8] The proliferation of hematopoietic progenitors could also be explained by the stimulatory effect of HDMP on the endogenous production of some growth factors. Stimulation of endogenous production of GM-CSF in patients with ALL and AML has been reported by Tuncer et al.[9] Moreover, the in vivo differentiation effect of HDMP on myeloid leukemia cells has been shown[10-12]. This may play a role in earlier restoration of normal hematopoiesis. In addition, the suppressive effect of HDMP on leukemia-associated inhibitors (LAI), which are known to inhibit normal hematopoiesis, could be another explanation for the proliferation of normal CD34-positive progenitors[13].

In conclusion, the addition of HDMP to chemotherapy protocols could be a useful way to provide early BM recovery in acute leukemias.

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