The effect of short-course high-dose methylprednisolone on peripheral blood CD34+ progenitor cells of children with acute leukemia during remission induction therapy

Bahattin Tunç[1], Ahmet F. Öner[2], Gönül Hiçsönmez[3]

[1]Department of Pediatrics, Süleyman Demirel University Faculty of Medicine, Isparta, [2]Department of Pediatrics, Yüzüncü Yıl University Faculty of Medicine, Van and [3]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Tunç B, Öner AF, Hiçsönmez G. The effect of short-course high-dose methylprednisolone on peripheral blood CD34+ progenitor cells of children with acute leukemia during remission induction therapy. Turk J Pediatr 2002; 44: 1-4.

This study was undertaken to determine the effect of short-course high-dose methylprednisolone (HDMP) treatment on peripheral blood (PB) CD34+ progenitor cells during remission induction treatment in 11 children with newly diagnosed acute leukemia (7 with ALL, 4 with AML) whose bone marrow (BM) cells expressed fewer than 5% CD34 at the time of diagnosis. All children who had no infection were given HDMP as a single daily oral dose of 30 mg/kg for the first four days of induction therapy. The number of CD34+ progenitor cells were determined by flow cytometry before and after four days of HDMP treatment. While the number of PB blast cells significantly decreased after only a four-day course of HDMP treatment, the number of PB CD34+ progenitor cells increased in all patients. In addition, after four days of HDMP treatment polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) increased significantly ($p < 0.05$). We suggest that the potential beneficial effects of HDMP in the induction treatment of acute leukemia may occur partly by the stimulation of PB CD34+ hematopoietic progenitor cells in a short period of time.

Key words: high-dose methylprednisolone, children, acute leukemia, CD34+ progenitor cells.

Although all hematopoietic progenitor cells in the bone marrow (BM) express the CD34 antigen, its expression decreases during the maturation of progenitor cells[1]. Only small number of CD34+ hematopoietic progenitor cells are found in peripheral blood (PB)[2]. These cells have been shown to increase during the period of hematopoietic recovery following myelosuppressive chemotherapy[3]. Treatment with recombinant hematopoietic growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) can increase PB CD34+ progenitor cells[4]. In addition, high-dose methylprednisolone (HDMP) combined chemotherapy has been shown to increase the number of BM CD34+ hematopoietic progenitor cells in children with acute lymphoblastic leukemia (ALL) during remission induction therapy. However, the increase in CD34+ cells was not significant in patients who received a conventional dose of steroid instead of HDMP[5]. Furthermore, short-course HDMP treatment has been shown to increase the circulating CD34+ hematopoietic progenitor cells during maintenance therapy in children with ALL and acute myeloblastic leukemia (AML) who had chemotherapy-induced neutropenia[6,7].

In the present study, we have evaluated the effect of short-course HDMP treatment of PB CD34+ cells during remission induction treatment to elucidate whether it could also increase the PB CD34+ progenitor cells in children with ALL and AML.

Material and Methods

Eleven children with newly diagnosed acute leukemia (7 with ALL, 4 with AML) were enrolled in this study. There were six boys and five girls with a median age of 5.6 years (range 3-14 years). None of them had infection.

Diagnosis was made by morphology according to the French-American-British (FAB) classification, by cytochemistry, and by cell surface marker analysis. With informed consent, methylprednisolone sodium succinate (Prednol-L 30 mg/kg/day) as a single agent was administered orally once a day to all children for the first four days of induction therapy. No other agent was given during that time. Treatment was then continued with HDMP containing chemotherapy regimens according to our institutional ALL and AML protocols.

Complete blood counts were performed using an automatic analyzer (STKS Coulter), and manual differential counts were performed on Wright-stained PB smears. Surface marker analysis was performed using a flow cytometry (FAC Scan, Becton Dickinson, San Jose, CA, USA) with a panel of monoclonal antibodies[8]. $CD34^+$ progenitor cells were measured in anticoagulated peripheral venous blood samples before and after four days of HDMP treatment. The patients were excluded if their BM blast cells expressed > 5% $CD34^+$ progenitor cells. The absolute number of circulating progenitor cells per mm^3 was calculated by multiplying the percentage of $CD34^+$ cells by the absolute number of mononuclear cells. The final results were expressed as mean ± SD. In addition, changes in the number of BM blasts, polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) were determined four days after HDMP treatment.

Statistical comparison was performed using the Wilcoxon test.

Results

Changes in the absolute numbers of PB $CD34^+$ cells before and after four days of HDMP treatment are shown in Table I. After four days of HDMP treatment, the number of PB $CD34^+$ progenitor cells increased significantly ($p < 0.05$) in children whose BM cells expressed < 5% $CD34^+$ progenitor cells. In addition, the absolute numbers of PB blast cells significantly decreased in all children with AML ($12.8 \pm 9.9 \times 10^9$/L vs $2.3 \pm 8.9 \times 10^9$/L) and ALL ($23.7 \pm 9.4 \times 10^9$/L vs $2.5 \pm 1.18 \times 10^9$/L) (Fig. 1). As also seen in Figure 1, significant increases were also observed in the number of PMN cells and monocytes in children with ALL and AML, four days after HDMP treatment.

Table I. Changes in Absolute Numbers of Peripheral Blood $CD34^+$ Progenitor Cells After 4 Days of HDMP Treatment in Children with Both ALL and AML whose Bone Marrow Cells Expressed < 5% $CD34^+$ Progenitor Cells

	Day 0	Day 4	p
PB $CD34^+$ cells/mm^3 n = 11	402±228	916±491	<0.05

PB: peripheral blood.

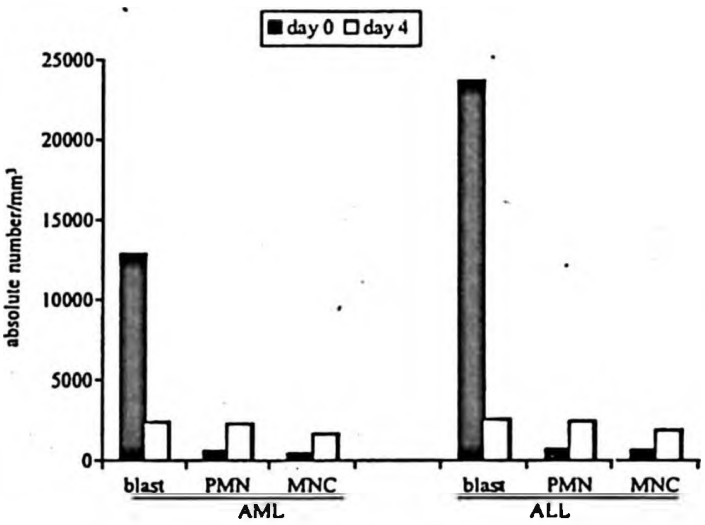

Fig. 1. Mean value of the absolute numbers of peripheral blood blast cells, PMN and MNC in children with AML (n = 4) and ALL (n = 7 before and after HDMP treatment. PMN: polymorphonuclear leukocytes; MNC: mononuclear cells; AML: acute myeloblastic leukemia; ALL: acute lymphoblastic leukemia.

Discussion

Although conventional doses of corticosteroids have long been used successfully for the treatment of ALL, more favorable results have been obtained by administration of higher doses of corticosteroid in children with ALL[9-13]. Administration of short-course (3 to 5 days) HDMP accelerates leukocyte recovery in leukopenic children with ALL and AML[14]. In addition, remarkable antileukemic effects of HDMP therapy through induction of terminal differentiation and apoptosis of myeloid leukemic cells have been shown in children with AML[15,16].

In the present study, we demonstrated that while PB leukemic cells decreased, PMN and MNC increased significantly, and PB $CD34^+$ progenitor cells increased in children with ALL and AML who were treated with short-course HDMP during the early phase of remission induction therapy. Although the mechanism of action of HDMP on PB hematopoietic

progenitor cells is not clear, the increase in the number of progenitor cells expressing CD34 antigen could be related to the stimulatory effect of HDMP on the production of some endogenous cytokines[17,18]. In addition, it has been reported that corticosteroids increased the levels of G-CSF and GM-CSF in normal subjects and in children with aplastic anemia and myelodysplastic syndrome[19,20]. Interestingly, the inhibitory effect of steroids on production of the leukemia-associated inhibitor (LAI) from human myeloid leukemic cells, which can have a myelosuppressive effect on normal progenitor cells, has been shown in vitro[21]. The inhibitory effects of high-dose steroid treatment on LAI could be another explanation for the stimulation of PB CD34+ progenitor cells.

Successful results with HDMP administration have also been reported in several hematologic diseases such as aplastic anemia[22], congenital hypoplastic anemia[23], and myelofibrosis[24]. The effect of HDMP in all these diseases might be related to its stimulatory effect on CD34+ hematopoietic progenitor cells.

In the present study, we have shown that administration of short-course HDMP treatment alone increases not only the number of PB CD34+ cells but also the PB PMN and MNC counts significantly during an early phase of remission induction therapy (Fig. 1). These results indicate that in addition to the remarkable antileukemic effects of HDMP in children with AML and ALL, short-course administration of HDMP can mobilize the CD34+ hematopoietic progenitor cells into PB and rapidly accelerate the hematopoietic recovery. Therefore, short-course HDMP treatment is recommended as an initial treatment of ALL and AML patients. Whether addition of HDMP alone or combined with chemotherapy is beneficial for the outcome remains to be determined. Its effects on hematopoietic recovery in children with other malignancies who developed myelosuppression should also be evaluated in further studies.

REFERENCES

1. Abrowitz MJ, Gockerman JP, Moore JO, et al. Clinicopathologic and cytogenic features of CD34 (My 10)/positive acute nonlymphocytic leukemia. Am J Clin Pathol 1989; 91: 265-270.
2. Bender JG, Unverzagt KL, Walker DE, et al. Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. Blood 1991; 77: 2591-2596.
3. To LB, Shepperd KM, Haylock DN, et al. Single high doses of cyclophosphamide enable the collection of high numbers of hemopoietic stem cells from the peripheral blood. Exp Hematol 1990; 18: 442-447.
4. Baumann I, Testa NG, Lange C, et al. Haemopoietic cells mobilised into the circulation by lenograstim as alternative to bone marrow for allogeneic transplants. Lancet 1993; 341: 369.
5. Tuncer AM, Hiçsönmez G, Gümrük F, et al. The effect of high-dose methylprednisolone combined chemotherapy on CD34-positive cells in acute lymphoblastic leukemia. Hematol Pathol 1994; 8: 169-175.
6. Çetin M, Hiçsönmez G, Tuncer AM, Kansu E, Canpınar H. The effect of short-course high-dose corticosteroid therapy on peripheral blood CD34+ progenitor cells in children with acute leukemia. Exp Hematol 1996; 24: 1191-1194.
7. Özbek N, Yetgin S, Tuncer AM. Effect of high-dose methylprednisolone and G-CSF treatments on lymphocyte subtypes in neutropenic children with acute lymphoblastic leukemia: a pilot study. Pediatr Hematol Oncol 1998; 15: 539-544.
8. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. Working Group on Flow Cytometry and Image Analysis. Leukemia 1996; 10: 877-895.
9. Shanbrom E, Miller S. Critical evaluation of massive steroid therapy of acute leukemia. N Engl J Med 1962; 266: 1354-1358.
10. Hiçsönmez G, Özsoylu S, Onat N. Zamani VP, Gümrük F, Tuncer AM. High-dose methylprednisolone in resistant and relapsed children with acute lymphoblastic leukemia. Med Pediatr Oncol 1994; 22: 68-69.
11. Hiçsönmez G, Gümrük F, Zamani PV, et al. High-dose methylprednisolone for children with acute lymphoblastic leukemia and unfavorable presenting features. Eur J Haematol 1997; 58: 26-31.
12. Yetgin S, Gürgey A, Tuncer AM, et al. A comparison of the effect of high-dose methylprednisolone with conventional-dose prednisolone in acute lymphoblastic leukemia children with randomization. Leuk Res 1998; 22: 485-493.
13. Rylalls MR, Pinkerton CR, Meller ST, Talbot D, McElwain TJ. High-dose methylprednisolone sodiumsuccinate as a single agent in relapsed childhood acute lymphoblastic leukemia. Med Pediatr Oncol 1992; 20: 119-123.
14. Hiçsönmez G, Onat N, Albayrak D, Yetgin S, Özsoylu S. Acceleration of leukocyte recovery by administration of short-course high-dose methylprednisolone in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol 1991; 8: 193-197.
15. Hiçsönmez G, Tuncer AM, Toksoy HB, Yenicesu I, Çetin M. Differentiation of leukemic cells induced by short-course high-dose methylprednisolone in children with different subtypes of acute myeloblastic leukemia. Leuk Lymphoma 1999; 33: 573-580.
16. Hiçsönmez G, Erdemli E, Tekelioglu M, et al. Morpohologic evidence of apoptosis in children with acute myeloblastic leukemia treated by high-dose methylprednisolone. Leuk Lymphoma 1996; 22: 91-96.

17. Tuncer AM, Hiçsönmez G, Ertürk G, Gümrük F, Albayrak D, Oğuz H. The effect of high-dose methylprednisolone treatment on GM-CSF level in children with acute leukemia: a pilot study. Leuk Res 1992; 16: 615-619.

18. Tuncer AM, Hiçsönmez G, Gümrük F, et al. Serum TNF-alfa, gamma-INF, G-CSF and GM-CSF levels in neutropenic children with acute leukemia treated with short-course, high-dose methylprednisolone. Leuk Res 1996; 20: 265-269.

19. Nissen C, Moser Y, Speck B, Burgin M, Bendy H. Dexamethasone enhances "CSA" release and depresses "BPA" release. Br J Haematol 1983; 53: 301-310.

20. Bagby GC, Gabourel JD, Linman JW. Glucocorticoid therapy in the preleukemic syndrome (hemopoietic dysplasia): identification of responsive patients using in-vitro techniques. Ann Intern Med 1980; 92: 55-58.

21. Olofsson T, Sallerfors B. Modulation of the production of leukemia associated inhibitor (LAI) and its interaction with granulocyte-macrophage colony-forming cells. Exp Hematol 1987; 5: 1163-1167.

22. Özsoylu S, Coşkun T, Minassazi S. High dose intravenous glucocorticoid in the treatment of childhood acquired aplastic anaemia. Scand J Haematol 1984; 33: 309-316.

23. Özsoylu S. High-dose intravenous corticosteroid treatment for patients with Diamond-Blackfan syndrome resistant or refractory to conventional treatment. Am J Pediatr Hematol Oncol 1988; 10: 217-223.

24. Özsoylu S. High dose intravenous methylprednisolone for idiopathic myelofibrosis. Br J Haematol 1988; 70: 388.