The Turkish Journal of Pediatrics 1996; 38: 491-495

SERUM ERYTHROPOIETIN AND GRANULOCYTE - MACROPHAGE - COLONY STIMULATING FACTOR LEVELS WITH MEGADOSE METHYLPREDNISOLONE TREATMENT*

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SUMMARY: Şaylı TR, Özsoylu Ş. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum erythropoietin and granulocyte-macrophage-colony stimulating factor levels with megadose methylprednisolone treatment. Turk J Pediatr 1996; 38: 491-495.

Serum erythropoietin (EPO) and granulocyte-macrophage-colony stimulating factor (GM-CSF) levels were determined in 12 children with acute and seven with chronic idiopathic thrombocytopenic purpura (ITP) prior to and during megadose methylprednisolone (MDMP) treatment by using enzyme-linked immunosorbant assay (ELISA) kits. It was shown that elevation of EPO and GM-CSF levels was correlated with an increase in hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), leukocyte (Leu), polymorphonuclear leukocyte (PMN) and monocyte (Mo) counts during MDMP treatment. *Key words: erythropoietin, granulocyte-macrophage-colony stimulating factor, megadose methylprednisolone.*

During the treatment of childhood acute and chronic idiopathic thrombocytopenic purpura (ITP) cases, we observed that megadose methylprednisolone (MDMP), besides correcting thrombocytopenia, elevated hemoglobin (Hb), packed cell volume (PCV), leukocyte (Leu), polymorphonuclear leukocyte (PMN) and monocyte (MO) counts[1-3]. Therefore, erythropoietin (EPO) and granulocyte-macrophage-colony stimulating factor (GM-CSF) levels were measured prior to and during MDMP treatment, and the corresponding Hb, PCV, red blood cell (RBC), PMN, Mo and lymphocyte (Lym) counts were determined in patients with ITP.

Material and Methods

Hemoglobin, PCV, Leu, PMN, Mo and Lym counts were obtained in 19 patients with ITP one and two weeks after MDMP treatment. Twelve of these children (10 girls 2 boys) had acute and seven had chronic (2 girls, 5 boys) ITP with age ranges of five to 13 ($\bar{X} \pm$ SD: 9.25 ± 2.73) and six to 14 (10.5 ± 2.99) years, respectively.

The diagnosis of ITP was based on platelet counts of less than 50×10^9/L, with excessive or normal numbers of megakaryocytes in the bone marrow. Platelets were enumerated by phase contrast microscopy[4]. Hepatosplenomegaly and lymphadenopathy were not detected, and there was no recent history of drug ingestion, including aspirin; no patients had received platelet or blood transfusions prior to the study. Underlying diseases were excluded as far as possible by lupus erythematosus (LE X 3) preparations, throat culture and antiglobulin test. Chronic ITP was considered when thrombocytopenia persisted longer than six months.

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Blood was obtained before 9:00 am. and prior to administration of MDMP. Serum EPO and GM-CSF levels were determined by enzyme-linked immunosorbant assay (ELISA) using Clinigen (Code No: ADC 060960) and Janssen Biochimica (Code No: 28.010.74) prior to the 3rd, 7th and 14th days of MDMP treatment (daily 30 mg/kg for three days, then 20 mg/kg for four days; 30 mg/kg and 20 mg/kg for one week each, administered before 9:00 am. orally, for acute and chronic ITP cases, respectively). The EPO and GM-CSF levels were calculated from the linear region of corresponding standard curves. Hb, PCV, RBC and Leu counts were obtained by using a coulter counter (Model S-plus VI); absolute values for polymorphonuclear leukocytes, monocytes and lymphocytes were calculated from total counts and simultaneous 100-cell differential counts on the 3rd, 7th and 14th days of the treatment and weekly for two more weeks after the treatment. For statistical evaluation, the Mann-Whitney U-test, Wlcoxon Rank test and regression correlation analysis were used.

Results

Erythropoietin (Fig. 1) and GM-CSF increased significantly ($p < 0.05$) after three days of MDMP administration. However, a hematologic response was first observed on the seventh day of treatment without being related to the platelet response. Elevation of GM-CSF was more marked than EPO on the third day of the treatment, which coincided with a significant elevation in Leu, PMN and Mo counts. GM-CSF elevation was not parallel to the platelet response in each case. PMN count changes were well correlated with leukocyte counts ($p < 0.05$). Lym counts did not change with Leu counts, and no decrease was observed with MDMP administ (Table I).

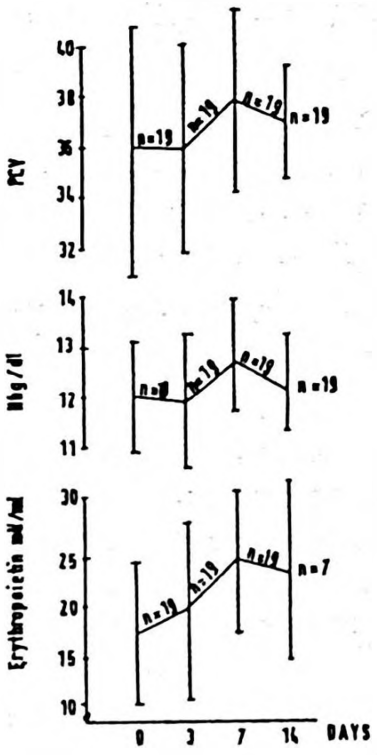

Fig. 1: Hb, PCV and serum EPO levels (mean ± SD) in patients prior to and during MDMP treatment.

Table I: EPO, Hb, PCV, RBC, GM-CSF, Leu, PMN, Mo, Lym Values Prior to, During and After MDMP Treatment in 19 Patients with ITP

	EPO (mU/mL)	Hb (g/dL)	PVC (%)	RBC (x 10^{12}/L)	GM-CSF (pg/mL)	Leu (x 10^9/L)	PMN (x 10^9/L)	Mo (x 10^9/L)	Lym (x 10^9/L)
Initial	17.21 ± 7.1	12.02 ± 1.07	36.07 ± 4.06	4.68 ± 0.5	7.62 ± 5.6***	6.98 ± 2.17	4.54 ± 2.13	0.18 ± 0.14	2.26 ± 0.84
	(8.8-30.4)	(10.3-13.62)	(28-44)	(4.02-5.58)	(5-25)	(5.00-14.30)	(1.70-10.30)	(0-0.57)	(1.05-4.86)
3rd day	19.59 ± 8.63	11.87 ± 1.44	36.02 ± 3.97	4.57 ± 0.58	13.25 ± 6.5***	11.17 ± 3.52	8.94 ± 3.18	0.32 ± 0.16	(1.91 ± 0.61)
	(12.2-36.4)	(9.8-14.2)	(31.4-43)	(3.36 ± 5.56)	(5.3-28)	(6.30-20.60)	(5.00-17.7)	(0.1-0.,82)	(1.0-3.36)
7th day	24.45 ± 7.27	12.77 ± 1.41	38.33 ± 3.44	4.91 ± 0.54	18.26 ± 6.9***	16.05 ± 4.33	12.96 ± 4.3	0.45 ± 0.20	2.64 ± 1.13
	(13.7-35.7)	(10.7-14.62)	(32-45)	(4.0-5.94)	(11-36)	(8.00-25.40)	(5.9-22.3)	(0.16-0.96)	(1.26-5.28)
14th day	23.53 ± 8.98***	12.21 ± 1.01	37.06 ± 3.29	4.90 ± 0.64**	19.1 ± 5.6*	14.61 ± 7.66	11.89 ± 6.95	(0.34 ± 0.24)	2.38 ± 1.37
	(10.2-36.0)	(11.0-13.48)	(32.8-44)	(4.02-5.67)	(12.6-26)	(5.20-29.0)	(2.7-26.1)	(0-0.10)	(1.1-6.8)
21st day	–	11.87 ± 1.32	35.79 ± 3.91	–	–	7.27 ± 2.02	5.13 ± 1.78	0.20 ± 0.11	1.94 ± 0.75
		(9.94-14.62)	(28-43)			(5.10-11.7)	(2.9-8.7)	(0-0.46)	(0.8-3.55)
28th day	–	12.32 ± 1.23	36.86 ± 3.8	–	–	7.05 ± 3.39	4.48 ± 3.2	0.16 ± 0.14	2.41 ± 0.68
		(10.9-14.9)	(32-45)			(5.00-19.3)	(2.1-17.1)	(0-0.48)	(1.4-4.28)

$\bar{X}$ ± SD ranges in parentheses.

* Only in 6.
** in 7.
*** in 12 patients.

EPO : Erythropoietin.
Hb : Hemoglobin.
Leu : Leukocyte.
MDMP: Megadose methylprednisolone.
PCV : Packed cell volume.
RBC : Red blood cell.
GM-CSF : Granulocyte-macrophage-colony stimulating factor.
ITP : Idiopathic thrombocytopenic purpura.
Lym : Lymphocyte.
Mo : Monocyte.
PMN : Polymorphonuclear leukocyte.

Discussion

Elevation of Hb and PCV during MDMP administration was observed[3]. The increase in the EPO level in this study could be an explanation for these observations, which strongly suggest bone marrow activation. Although chronic, large doses of EPO have been shown to cause thrombocytopenia in mice[5], this was not observed in this study at the detected EPO levels.

The in-vitro effects of GM-CSF on megakaryocyte progenitors have been well documented[6, 7]. Augmentation of GM-CSF levels in this study may be responsible for the elevation of platelet counts, but did not coincide in each patient. Prolongation of survival of human PMN by GM-CSF has been shown[8]. The observed increase in PMN and Leu counts may also be related to elevation of GM-CSF in our study, in addition to the well-known steroid effects[9, 10].

High-dose methylprednisolone has been shown to increase GM-CSF in children with leukemia[11]. In this study, oral MDMP administration in children with ITP resulted in elevation of EPO and GM-CSF. However, other cytokines could not be determined. A large body of data has indicated that platelet production is regulated by several humoral factors[12-16], and a megakaryocyte-colony-stimulating factor has been detected in the plasma and urine of severely thrombocytopenic individuals[13, 15, 16].

Elevation of EPO and GM-CSF levels during MDMP treatment may help to explain increases in Hb, PVC, RBC, Leu, PMN and Mo. Although other cytokines should also be evaluated to explain the increase in platelet counts, we believe that the increase of GM-CSF may also be a factor for improvement of thrombocytopenia with this treatment.

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