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USE OF GRANULOCYTE COLONY-STIMULATING FACTOR FOR TREATMENT OF APLASTIC ANEMIA

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ABSTRACT

Over the last ten years, recombinant human granulocyte colony-stimulating factor (rh G-CSF) has been widely used in the treatment of aplastic anemia (AA). It has been shown to facilitate the recovery of neutrophil count and useful for complicated bacterial or fungal infections. However, recent randomized clinical trials showed that the addition of rh G-CSF to immunosuppressive therapy had no clinical benefit for the prophylaxis of severe infections. These results suggested that rh G-CSF should be used for the treatment of infectious complications, not for the prophylaxis of infections in patients with AA.

Key Words: aplastic anemia, granulocyte colony-stimulating factor

I. INTRODUCTION

Acquired aplastic anemia (AA) encompasses a group of stem cell diseases characterized by peripheral blood pancytopenia and hypocellullar bone marrow. Bone marrow transplantation (BMT) and immunosuppressive (IS) therapy with antilymphocyte globulin (ALG) or cyclosporine (CyA) are the main therapeutic modalities currently used in patients with AA.

Hematopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of hematopoietic progenitor cells and the function of mature blood cells. To date, seven hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF)[1], granulocyte-macrophage colony-stimulating factor (GM-CSF)[2], interleukin-3 (IL-3)[3], interleukin-1 (IL-1)[4], interleukin-6 (IL-6)[5], erythropoietin (EPO)[6] and stem cell factor (SCF)[7], have been used against AA to increase the production of mature blood cells from the remaining small pool of precursor cells (Table 1). G-CSF has been the most extensively evaluated clinically. In clinical trials using recombinant human G-CSF (rh G-CSF), a transient increase of neutrophil count was induced, and it is useful for the treatment and prophylaxis of complicated bacterial or fungal infections in the majority of patients with AA. This review will summarize the current state of rh G-CSF treatment for AA.

II. ENDOGENOUS PLASMA G-CSF CONCENTRATIONS IN PATIENTS WITH AA

Several early studies showed a profound deficit, in the primitive progenitor cells[8] and elevated circulating level of hematopoietic growth factors in patients with AA[9-14] (Table 2). We

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Table 1. Clinical Trials of Hematopoietic Growth Factors in Aplastic Anemia

Investigator[Ref]	Cytokines	No. of Patients	Hematopoietic Response		
			Neutrophils	Platelets	Red Blood Cells
Kojima[1]	G-CSF	20	12	0	0
Vadhan-Raj[2]	GM-CSF	10	9	0	0
Ganser[3]	IL-3	9	5	1	0
Walsh[4]	IL-1	4	0	0	0
Schrezenmeier[5]	IL-6	6	0	0	0
Urabe[6]	Epo	27	0	0	3
Kurzrock[7]	SCF ± G-CSF	39	16	5	7

G-CSF: granulocyte colony-stimulating factor SCF: stem cell factor
GM-CSF: granulocyte-macrophage colony-stimulating factor
IL-3: interleukin-3 IL-1: interleukin-1
IL-6: interleukin-6 Epo: erythropoietin

Table 2. Circulating Levels of Hematopoietic Growth Factors in Aplastic Anemia

Hematopoietic Growth Factor[Ref]	Median (Range)	Normal Range
Epo[9]	2,700 (100~20,000) mIU/mL	10~25 mIU/mL
G-CSF[10]	40 (10~100) pg/mL	4~10 pg/mL
TPO[11]	25 (5~60) fmol/mL	0.5~1.5 fmol/mL
SCF[12]	1,100 (700~2,000) pg/mL	800~1,500 pg/mL
GM-CSF[13]	50 (<16~500) pg/mL	<16 pg/mL
Flt-3 L[14]	2,600 (1,000~5,900) pg/mL	0~150 pg/mL

TPO; thrombopoietin; Flt-3 L; Flt-3 ligand

measured endogenous plasma G-CSF levels in patients with AA by chemiluminescent immunoassay[10]. The levels were less than 10 pg/mℓ in normal controls. In patients with AA, however, they were significantly elevated from 10 to 100 pg/mℓ. Moreover, the plasma G-CSF concentrations increased remarkably and ranged from 100 to 1,000 pg/mℓ in AA patients with signs of bacterial or fungal infections.

Bone marrow stromal cells are known to produce G-CSF. Our study showed that the ability of stromal cells to release G-CSF is either normal or elevated in patients with AA[15]. Thus, the rationale for treating the cytopenia of AA patients with G-CSF is not based on findings of G-CSF deficiency but on the expectation that impaired proliferation and differentiation of hematopoietic stem cells may be overcome by pharmacological doses of G-CSF. In fact, our study showed that plasma G-CSF levels are much higher than the physiological levels after administration of rh G-CSF.

III. PHASE I/II TRIAL OF rh G-CSF FOR AA

Twenty patients with AA ranging in age from 1 to 17 years entered a phase I/II clinical trial of rh G-CSF, which was administered at a dose of 400 μg/m^2 per day by 30-minute intravenous infusion for 2 weeks[1]. This increased the neutrophil counts (2.7 to 28.0-fold) in 12 of the 20 patients. Nine patients also showed at least a two-fold increase in the peripheral monocytes. There was no significant change in either the lymphocyte or eosinophil count. The number of circulating red blood cells, platelets, and the patient's transfusion requirements were unaffected by the treatment. Differential counts of bone marrow (BM) aspirates showed an increase in the myeloid/erythroid ratio. However, neither BM cellularity nor the number of committed myeloid progenitors changed significantly. The response was transient, and the neutrophil count returned to baseline within 2 to 10 days of discontinuing treatment. No severe toxicity attributable to rh G-CSF was observed.

In general, patients with very severe hypoplasia who received conventional doses of rh G-CSF failed to show any increase in neutrophil counts. Because the increases in neutrophils were dose-dependent in the earlier studies, we administered high-dose rh G-CSF to 10 patients with very severe AA who had fewer than 0.1 × 10^9/ℓ neutrophils in subsequent trial[16]. Doses of rh G-CSF ranging from 400 to 2,000 μg/m^2 per day were administered as 30-minute intravenous infusions daily for 4 weeks. In 6 of 10 patients, the doses increased the neutrophil count by 10-fold to greater than 60-fold. Bacterial or fungal infections that were present at study entry resolved in all responders. Three of 4 non-responders died of infection, whereas 1 non-responder received a bone marrow transplant from a HLA-mismatched donor and is alive. The patients tolerated the therapy well at doses up to 2,000 μg/m^2 per day.

IV. THERAPY COMBINING IMMUNOSUPPRESSIVE AGENTS AND rh G-CSF

Patients with severe and refractory AA who can not be offered BMT and who do not respond to IS therapy present a difficult therapeutic problem. We tried combined therapy with rh G-CSF and CyA in five patients with very severe AA who had not responded to ALG[17]. In our trial of high-dose rh G-CSF, it was administered subcutaneously three times a week during maintenance phase. CyA therapy was started when the number of neutrophils exceeded 1 × 10^9/L. In 4 of 5 patients, there was an increase in the number of reticulocytes and platelets after 2 to 3 months of maintenance therapy, and these patients became transfusion-independent. These results suggest that long-term administration of rh G-CSF and CyA may induce a trilineage recovery in some patients with severe AA.

Because patients with severe AA are exposed to early mortality from infectious complications following ALG treatment, the addition of rh G-CSF may lower the risk of early mortality and improve the chance for a complete response. The Severe AA Working Party of the European Group for Blood and Marrow Transplantation (EBMT) conducted a pilot study that included ALG, CyA, methylprednisolone, and rh G-CSF in 40 patients with severe AA[18]. Thirty-three of 40 patients had trilineage hematologic recovery and became transfusion-independent. The actuarial survival rate was 92% with a median follow-up period of longer than 1 year. These very encouraging results suggest that the addition of rh G-CSF to ALG and CyA is associated with a low risk of mortality, and offers a good chance of a hematologic response.

Based on the encouraging result of an European pilot study, we conducted a multi-center trial in 111 patients with newly diagnosed AA, comparing treatment with ALG, CyA, and Danazol (DAN) with or without rh G-CSF[19]. This study addressed the issues of whether the addition of rh G-CSF to IS therapy could affect the incidence of infections, rate of complete or

partial response, and survival rate. All patients with neutrophil counts < 0.2×10^9/L received rh G-CSF (group A), and other patients were randomized to receive ALG, CyA, DAN (group B) and rh G-CSF, or ALG, CyA and DAN (group C). Analysis was based on the intention to treat principle, and non-responders to IS therapy were censored as surviving at the time of BMT. The total number of infections was identical in groups B and C. A trilineage response was observed in 76% of group A, 55% of group B, and 75% of Group C. The actuarial 5-year survival rate of the each group was 87%, 97% and 100%, respectively. Four of 111 patients developed myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) within 2 years from the start of therapy. Our result showed that the addition of rh G-CSF to IS therapy had no clinical benefit in AA patients with neutrophil counts > 0.2×10^9/L.

At the same period, another European group also conducted a multi-center randomized trial aimed at evaluating the effect of rh G-CSF in combination with ALG and CyA in patients with newly diagnosed AA[20]. The result showed that there was no difference between both groups in the rate of a trilineage response and 2-year survival rate. It was concluded that rh G-CSF therapy did not modify long-term hematopoietic recovery and survival in patients with severe AA.

V. EVOLUTION OF AA TO MDS/AML

Recently, several studies have suggested that long-term survivors of AA treated with IS therapy are prone to develop MDS and AML[21)22)]. We reported three children with severe AA in whom MDS with monosomy 7 developed during and following treatment with rh G-CSF[23)]. MDS later evolved into AML in all of them. Because G-CSF has biologic effects resulting in stimulation of leukemic clones, long-term administration may facilitate progression into MDS and AML in patients with AA.

We retrospectively analyzed the incidence of MDS/AML in 167 children with severe AA, who were registered with the AA Committee of the Japanese Society of Pediatric Hematology[24)]. Among them, eleven patients developed MDS/AML. The actuarial incidence of MDS/AML was 16 ± 6% at 7 years. All eleven children were treated with rh G-CSF and immunosuppressive agents. If the estimate was restricted to the 62 children who received both IS therapy and rh G-CSF, the incidence increased to 47 ± 17%. Most of the children who developed MDS/AML had been treated with rh G-CSF for period of over 1 year. Cytogenetic analysis of bone marrow (BM) cells at the time of diagnosis of MDS/AML revealed monosomy 7 in 10 patients and trisomy 21 in 1 patient. Previous BM cytogenetic studies showed a normal karyotype in 5 children but they could not be performed in the other 6 children because of low mitotic rates at the time of AA diagnosis. It is unclear whether these patients already had a MDS clone at the time of AA diagnosis or acquired it after treatment for AA. Ongoing controlled clinical trials will reveal whether therapeutic modalities affect the development of MDS/AML in patients with AA.

CONCLUSION

rh G-CSF facilitates the recovery of neutrophils and is useful to treat bacterial or fungal infections in AA patients. When the infections subside, rh G-CSF should be discontinued. For prophylaxis of severe infections, its use should be restricted to patients with neutrophil counts less than 0.2×10^9/L.

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