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Recombinant human granulocyte-macrophage colony-stimulating factor accelerates engraftment kinetics after allogeneic bone marrow transplantation for childhood acute lymphoblastic leukemia

LUIS MADERO,* MIGUEL ANGEL DÍAZ,* JUAN JOSÉ ORTEGA,° TERESA OLIVE,° ANA MARTÍNEZ,# ISABEL BADELL,@ ARTURO MUÑOZ,§ PEDRO GÓMEZ^

**Hospital Niño Jesús, Madrid; °Hospital Vall d'Hebrón, Barcelona; #Hospital La Paz, Madrid; @Hospital de la Santa Creu i Sant Pau, Barcelona; §Hospital Ramón y Cajal, Madrid; ^Hospital Reina Sofía, Córdoba, Spain*

ABSTRACT

Background and Objective. The use of recombinant human granulocyte-macrophage stimulating factor (rhGM-CSF) has been shown to be well-tolerated and to reduce post-transplantation morbidity in adults undergoing HLA-identical allogeneic bone marrow transplantation (BMT). There is however, limited experience in children.

Design and Methods. We performed a prospective, comparative multicenter trial using rhGM-CSF after allogeneic BMT in children with acute lymphoblastic leukemia (ALL). The study comprised 24 patients with ALL who received rhGM-CSF and 22 patients with ALL who did not receive rhGM-CSF. There were no statistically significant differences in the demographic characteristics between the rhGM-CSF-treated and untreated groups. rhGM-CSF was given at a dose of 10 µg/kg/day infusion over 4 hours from day +1 until +28 or until the absolute neutrophil count (ANC) was ≥ 1×10^9/L. All patients received HLA-identical sibling marrow and cyclosporine alone for graft-versus-host disease (GvHD) prophylaxis. The number of cells infused was similar in both groups. A software program (Statview 4.0, Abacus Concept, Inc., Berkeley, CA, USA) was used for statistical analysis.

Results. The median of days to achieve ANC ≥ 0.5×10^9/L was shorter in the rhGM-CSF-treated patients (14 days vs 18.5 days; $p<0.0001$). Patients who received rhGM-CSF had a lower incidence of grade III-IV mucositis. The duration of hospital stay was significantly shorter in patients who received rhGM-CSF (31 days vs 45 days; $p < 0.005$). No differences in GvHD severity, relapse or survival were observed. At the dose and schedule used in the present study, rhGM-CSF was well-tolerated and no side effects were observed.

Interpretations and Conclusions. rhGM-CSF at a dose of 10 µg/kg/day in children with ALL undergoing allogeneic BMT is well tolerated, accelerates neutrophil and platelet engraftment, reduces the intensity and severity of mucositis and permits a more rapid discharge from hospital.

Key words: rhGM-CSF, allogeneic bone marrow transplantation, childhood, ALL

Correspondence: Luis Madero, M.D., Department of Pediatrics, Bone Marrow Transplant Unit, Hospital Niño Jesús, Menéndez Pelayo, 65, 28009- Madrid, Spain. Phone: international +34-1-5735200 – Fax: international +34-1-5744669.

Allogeneic bone marrow transplantation (BMT) from genotypically HLA-identical siblings is the treatment of choice for children with acute lymphoblastic leukemia (ALL) in second complete remission (CR).[1-3] Prolonged neutropenia, which occurs during transplantation continues to be a cause of morbidity and mortality in patients undergoing BMT.[4,5] There is evidence that the use of rhGM-CSF in autologous transplantation in patients with lymphocytic malignancies and solid tumors reduces the period of neutropenia.[6-8] There is less experience, however, with rhGM-CSF in patients undergoing allogeneic BMT. The phase I and phase II studies conducted to date suggest that benefits similar to those obtained in autologous BMT can be achieved without side effects or a higher incidence of rejection and graft-versus-host disease (GvHD).[9-12] These findings have been confirmed elsewhere in a phase III randomized, double-blind placebo controlled trial in adults with hematologic malignancies.[13,14] To our knowledge, no series comprised exclusively of pediatric patients treated with rhGM-CSF after allogeneic BMT has been reported. We performed a prospective, comparative multicenter trial using rhGM-CSF after allogeneic BMT in children with ALL.

Design and Methods

Patient selection

Male and female patients aged less than 16 years who were candidates for allogeneic BMT for ALL were eligible for the study. Forty-six patients who fulfilled the entry criteria were enrolled at six pediatric departments from September, 1994 to March, 1997. The main characteristics of the patients are shown in Table 1. There were no statistically significant differences in the demographic characteristics between the rhGM-CSF-treated and untreated groups.

Study design

This was a prospective, multicenter trial. Patients were assigned in each center to receive rhGM-CSF 10 µg/kg/day infusion over 4 hours or not.

Treatment with rhGM-CSF was started 24 hours after transplantation (day +1) and was continued until absolute neutrophil count (ACN) was greater than 1×10^9/L for three consecutive days.

Table 1. Characteristics of the patients treated with GM-CSF or not (control group).

	GM-CSF (n=24)	Controls (n=22)	p value
Age (years)			
Mean	7	7	NS
Range	3-16	1-15	
Sex			
Male	20	13	NS
Female	4	9	
Diagnosis			
ALL 1 CR	6	4	NS
ALL 2 CR	17	17	
ALL 3 CR	1	1	
Other			
Median infused marrow cells x10^9/kg	3.05	3	NS
Range	0.68-5.4	0.21-7.3	

Patients were examined during the two weeks following completion of treatment and after every month in order to evaluate the clinical course.

The study was approved by the Institutional Review Boards and Ethics Committees and parents' informed consent was obtained in all cases.

Bone marrow collection

Bone marrow was harvested from the HLA-identical donor using standard techniques on the day of transplantation (day 0). The median number of MNC administered was 3.0×10^6 (range $0.68\text{-}5.4\times10^6$) in the rhGM-CSF-treated group and 3.2×10^6 (range $0.21\text{-}7.3\times10^6$) in the untreated group.

Conditioning regimen for BMT

Fifteen patients of the rhGM-CSF-treated group and 12 patients of the untreated group were given intravenous cyclophosphamide 60 mg/kg on each of two successive days followed by 12 Gy of total body irradiation given in six fractions over three days (Cy-TBI).[15,16] Two patients of the rhGM-CSF-treated group and one patient of the untreated group received busulphan orally at a dose of 16 mg/kg over 4 days and intravenous cyclophosphamide 60 mg/kg on each of two successive days (Bu-Cy).[17] Two patients of the untreated group received busulphan orally at a dose of 16 mg/kg over 4 days, VP-16 40 mg/kg for one day and intravenous cyclophosphamide 60 mg/kg on each of two successive days.[18] One patient of the rhGM-CSF-treated group received busulphan orally at a dose of 16 mg/kg over 4 days (days –7 to –4) and melphalan 180 mg/m^2 on day –2 (Bu-Me).[19] Six patients of the rhGM-CSF-treated group and 7 patients of the untreated group received intravenous cyclophosphamide 60 mg/kg on each of two successive days, VP-16 40 mg/kg for one day and total body irradiation given in six fractions for three days.[20]

Supportive care

Bone marrow transplantation was performed in a BMT unit. A central venous catheter was placed in each patient. Patients were cared for in a room with laminar flow or reverse isolation by positive pressure. Prophylaxis for *Pneumocystis carinii* (cotrimoxazole 8 mg/kg/day from day –7 to 0 and then from +50 to +150 post-transplantation) and for herpes simplex virus (acyclovir 750 mg/m^2 from day –7 to +24) was employed. Non-absorbable antibiotics for gut decontamination were routinely administered. Transfusions were administered for a hematocrit of <25% and platelet count of $<20\times10^9$/L. All hemoderived transfusion products were irradiated to 1.5-2.5 Gy. Patients were started on i.v. broad spectrum antibiotics if their temperature was higher than 38°C and their neutrophil count was $< 1\times10^9$/L. If the patient continued to have fever and neutropenia on day 4-5 post-transplantation, amphotericin B (0.5-1 mg/kg/day) was added.

GvHD prophylaxis

Cyclosporin A alone was administered i.v. at a dose of 3 mg/kg from day –1 until oral intake was resumed. CyA was then given orally at a dose of 12.5 mg/kg until day 50, after which it was gradually tapered off and was discontinued by 6-8 months post-transplantation.[21]

Definitions

Neutrophil recovery was defined as the days to achieve ANC of $\geq 0.5\times10^9$/L for three consecutive days. Platelet recovery was defined as the time to achieve $\geq 20\times10^9$/L without requiring transfusion. Hospital stay was defined as days from day 0 to hospital discharge. Clinically documented infectious episodes were defined as the presence of symptoms and signs of infection. Fever was categorized as a clinically or microbiologically defined infection. Bloodstream infection was defined as one or more blood cultures positive for any organism. Interstitial pneumonitis was diagnosed if bilateral infiltrates on chest X-ray were associated with significant hypoxemia. Acute GVHD was diagnosed and graded according to the Seattle criteria.[22] The grading of regimen-related toxicity was classified according to criteria reported by Bearman *et al.*[23]

Statistical analysis

A software program (StatView 4.0, Abacus Concept Inc., Berkeley, CA, USA) was used for the statistical analysis. The data are expressed as median and range. The statistical significance was determined by Student's t-test. Results were considered significant if the p value was ≤ 0.05. Probabilities of achieving a neutrophil count of $\geq 0.5\times10^9$/L and a platelet count of $\geq 20\times10^9$/L and $\geq 50\times10^9$/L were calculated using the method of Kaplan and Meier and the comparison between them by the log-rank test. Disease free survival and the probability of relapse were calculated using the Kaplan Meier method.

Table 2. Hematologic and clinical values.

	GM-CSF (n=24)	*Controls (n=22)*	*p value*
Days to neutrophil count >0.5x10^9/L	14 9-24	18.5 11-29	<0.0001
Days to neutrophil count >1x10^9/L	16 18-28	24.5 13-40	<0.0001
Days to platelet >20x10^9/L	16 11-72	26 (14-120)	<0.04
Days to platelet >50x10^9/L	23 (17-120)	40 (18-150)	<0.02
Febrile days	6 0-21	8 (0-30)	NS
Parenteral antibiotics (days)	12 (6-41)	15 (0-49)	NS
Time in hospital (days)	31 (18-63)	45 (21-135)	0.005
Number of episodes of clinically documented infections	7	6	NS
Mucositis 0-I II-IV	18 6	5 17	0.003 0.003

Results

Hematopoietic recovery

All patients achieved an ANC of $\geq 0.5\times10^9$ cells/L within 28 days after marrow infusion. The patients who received rhGM-CSF reached myeloid engraftment before the patients who did not receive rhGM-CSF. The median of days to ACN $\geq 0.5\times10^9$ cells/L was 14 days for the rhGM-CSF-treated group versus 18.5 days for the untreated group, the difference being statistically significant ($p < 0.0001$).

rhGM-CSF significantly influenced platelet recovery. The medians of days to platelet count $\geq 20\times10^9$ platelets/L and $\geq 50\times10^9$ platelets/L without platelet infusion were 16 days and 23 days in the patients who received rhGM-CSF versus 26 days and 40 days in the patients who did not receive rhGM-CSF ($p < 0.04$ and $p < 0.02$, respectively) (Table 2). Figures 1 and 2 show the myeloid and platelet engraftment probabilities for both groups.

Fever and infection

The median duration of fever was 6 days in the patients who received rhGM-CSF versus 8 days in the patients who did not receive rhGM-CSF ($p < 0.2$).

Six patients of the untreated group and 7 patients of the rhGM-CSF-treated group developed clinically documented infection ($p < 0.2$) (Table 2).

The median number of days of i.v. antibiotics in the rhGM-CSF-treated patients was 12 (range 6-41) compared with 15 (range 0-49) days in the untreated group ($p < 0.2$) (Table 2).

GvHD

The incidence and severity of acute GvHD was not different between the two groups (Table 3).

Toxicity

The rhGM-CSF-treated patients had a lower incidence of severe mucositis (6 patients vs 17 patients of the untreated group, $p < 0.003$) (Table 2). There were no differences in non-hematologic toxicity (renal, hepatic, neurological, digestive and cardiac) in both groups.

Hospital stay

The median duration of hospital stay was shorter in patients who received rhGM-CSF than in patients who did not receive rhGM-CSF (31 days vs 45 days; $p < 0.003$).

Relapse

Three patients who received rhGM-CSF relapsed within the first 12 months post-transplantation. The probability of relapse in this group was 15.55±8.2%

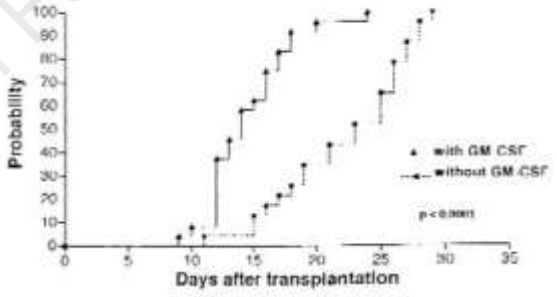

Figure 1. Kaplan-Meier probability of achieving $\geq 0.5\times10^9$/L neutrophils.

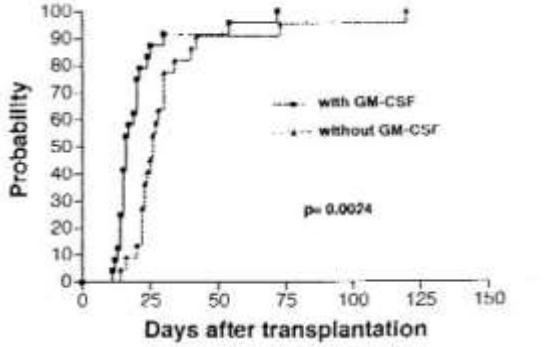

Figure 2. Kaplan-Meier probability of achieving $\geq 20\times10^9$/L platelets.

Table 3. Incidence of acute GvHD.

Grade	*GM-CSF (%) (n=24)*	*Control (%) (n=22)*	*p value*
0	7 (29.1)	8 (36.3)	NS
I	5 (20.8)	5 (22.7)	NS
II	6 (25)	6 (27.2)	NS
III	5 (20.8)	1 (4.5)	NS
IV	1 (4.1)	2 (9.0)	NS

at a median follow-up of 16.5 months. Five patients of the untreated group relapsed within the first 12 months post-transplantation, accounting for a probability of relapse of 25.13±9.8% at a median follow-up of 16.5 months (range 2-30). No differences were observed in the probability of relapse in both groups (log rank test $p < 0.47$).

Survival

Two patients of the rhGM-CSF-treated group developed grade III acute GVHD and interstitial pneumonia and died. Another patient of this group died of disseminated aspergillosis. One patient of the untreated group died of interstitial pneumonia and multiorgan failure. All patients of both groups who relapsed died.

The event free survival was 73% at a median follow-up of 16.5 months for the rhGM-CSF-treated patients. The event free survival was 69% at a median follow-up of 16.5 months for the untreated group. No significant differences were observed between both groups. No side-effects ascribable to rhGM-CSF were observed.

Discussion

Some clinical studies have shown the benefits of using rhGM-CSF following allogeneic BMT.[13,14] These studies have been performed chiefly in adults and there is limited experience in children. Unlike other studies when have evaluated patients with different malignancies who underwent BMT, our study was conducted exclusively in children with ALL. We utilized cyclosporine alone as prophylaxis for GvHD as advocated in order to avoid the effects of prophylactic methotrexate (MTX) in patients receiving rhGM-CSF.[24] However, a faster neutrophil recovery has also been reported recently in rhGM-CSF-treated patients who received prophylactic MTX for GvHD.[25]

Neutrophil recovery was significantly accelerated. The median number of days to achieve ANC $\geq 0.5 \times 10^9$ cells/L was lower in the rhGM-CSF-treated patients (14 vs 18.5 days, $p < 0.0001$). These observations are similar to the findings reported elsewhere.[13,24,25] The faster neutrophil recovery in our patients treated with rhGM-CSF resulted in fewer febrile days and a shorter duration of antibiotic use, although the differences were not significant ($p < 0.2$). Unlike the findings of the study by Nemunaitis *et al.*,[13] we did not find a decrease in the number of infectious episodes. We did, however, find that platelet recovery was influenced by the administration of rhGM-CSF. In our study the medians of days to platelet $\geq 20 \times 10^9$/L and $\geq 50 \times 10^9$/L were significantly shorter in patients who received rhGM-CSF. The foregoing finding has not been observed by other authors in patients undergoing allogeneic BMT,[13,14] but has been observed in patients with different malignancies who received high dose chemotherapy and autologous BMT.[26] This effect on platelet recovery may be due to the fact that rhGM-CSF is capable of stimulating megakaryocytic progenitor cell growth.[27]

Like other studies,[10,13] we found that the incidence and severity of GvHD did not increase. Growth factor administration has been reported to produce moderate side effects.[28] However, at the dose and schedule used in the present study, rhGM-CSF was well-tolerated and no side effects were observed, as we have also reported in a previous study on rhG-CSF.[29]

Although some authors have reported that the relapse rate is lower in patients undergoing allogeneic BMT who receive rhGM-CSF,[11] we did not observe a decrease in the relapse rate in our homogeneous group of patients. On the other hand, we have found a lower incidence of mucositis. This finding may be explained by an increase in the residual function of host macrophages.[13]

The duration of hospitalization was shorter for the rhGM-CSF-treated patients, which might be ascribable to the earlier neutrophil and platelet recovery. A shorter hospital stay implies an economic benefit, although cost reduction also depends on reducing the costs of supportive patient care. Although growth factor administration implies higher costs, to our knowledge no cost analysis concerning pediatric patients is available in the literature.

From the present data, we can conclude that rhGM-CSF at a dose of 10 µgm/kg/day in children with ALL undergoing allogeneic BMT is well-tolerated, accelerates neutrophil and platelet engraftment, reduces the intensity and severity of mucositis and permits earlier discharge from hospital.

Contributions and Acknowledgments

The study was designed and coordinated by LM. AM, JJO and AM collected the clinical and analytical data. IB, TO and PG were responsible for the data handling. MAD was responsible for the statistical analyses and references. LM wrote the manuscript which was submitted to the rest of the authors for their approval. The order of appearance of the names is based on the importance of each individual contribution, as previously established and accepted by all of the authors.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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