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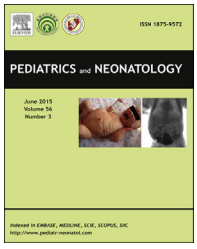

ORIGINAL ARTICLE

A Randomized Case-Controlled Study of Recombinant Human Granulocyte Colony Stimulating Factor for the Treatment of Sepsis in Preterm Neutropenic Infants

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colony stimulating factor;
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Background: To investigate the efficacy and safety of recombinant human granulocyte colony-stimulating factor, recombinant human granulocyte-macrophage colony-stimulating factor (rhG-CSF) to treat sepsis in neutropenic preterm infants.
Methods: Fifty-six neutropenic preterm infants with suspected or culture-proven sepsis hospitalized in Zeynep Kamil Maternity and Children's Educational and Training Hospital, Kozyatağı/Istanbul, Turkey between January 2008 and January 2010 were enrolled. Patients were randomized either to receive rhG-CSF plus empirical antibiotics (Group I) or empirical antibiotics alone (Group II). Clinical features were recorded. Daily complete blood count was performed until neutropenia subsided. Data were analyzed using SPSS version 11.5.
Results: Thirty-three infants received rhG-CSF plus antibiotic treatment and 23 infants received antibiotic treatment. No drug-related adverse event was recorded. Absolute neutrophil count values were significantly higher on the 2nd study day and 3rd study day in Group I. Short-term mortality did not differ between the groups.
Conclusion: Treatment with rhG-CSF resulted in a more rapid recovery of ANC in neutropenic preterm infants. However, no reduction in short-term mortality was documented.

1. Introduction

Sepsis remains a leading cause of morbidity and mortality in the newborn population despite modern treatment and preventive strategies.[1] Neonatal sepsis, unless it leads to a

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mortality, prolongs duration of hospitalization, increases the risk of long-term neurocognitive dysfunction, and contributes to chronic lung disease.[2–4] Bacterial sepsis is seen in 0.1–1% of term newborns.[5] It is almost 50 times more common in extremely low birth weight infants, mostly due to immaturity of humoral and phagocytic defense systems.[6,7]

It is believed that relative immaturity of the preterm immune system makes these babies especially prone to lethal infections.[8–10] Neutrophils play a pivotal role in defense against bacterial infections.[11] Neutropenia is frequent in overwhelming sepsis and it is an ominous sign of poor prognosis.[12] Neutropenia is a persisting reduction in neutrophil counts. It is more precisely defined as an absolute neutrophil count (ANC) $< 1.0 \times 10^9$/L.[13] The unique susceptibility of neonates to sepsis-associated neutropenia is caused by a smaller neutrophil storage pool, a reduced capacity for neutrophil mobilization from bone marrow reserves, and a slower regeneration of neutrophils in the bone marrow.[14] In addition to quantitative aspects, qualitative features of neutrophils are also of vital importance.[15] Even when peripheral neutrophil counts are normal in premature newborns, the organisms that cause bacterial infections are similar to those seen in older children and adults with profound neutropenia, which is suggestive of a functional neutropenia.[16]

Recombinant human granulocyte colony stimulating factor (rhG-CSF) is a hematopoietic colony stimulating factor that has been shown to specifically promote granulocyte development and maturation by stimulating myeloid progenitor proliferation.[17–19] Systematic reviews conducted to document the efficacy and safety of rhG-CSF administration concluded that the agent was safe, but the evidence was insufficient to support routine use either for treatment or for prophylaxis of neonatal sepsis.[20–22] Although the level of evidence is far from adequate, hematopoietic growth factors have been frequently used in neonatal intensive care units for treatment of sepsis in the presence of neutropenia.[22]

We aimed to investigate the efficacy and safety of rhG-CSF administration to treat sepsis in our neutropenic preterm population.

2. Methods

Neutropenic preterm infants with culture-proven or suspected sepsis who were hospitalized in Zeynep Kamil Maternity and Children's Educational and Training Hospital, Kozyatağı/Istanbul, Turkey between January 2008 and January 2010 were enrolled in the study. Newborns with congenital malformations, intrauterine infections, and hydrops fetalis were regarded as ineligible for the study.

Diagnosis of sepsis was established when the following were present within 24 hours of randomization: either fever (axillary temperature of $\geq 38^\circ$C on 1 occasion or $\geq 37.5^\circ$C on 2 occasions separated by at least 1 hour) or two or more of the following in the absence of an alternative explanation: poor perfusion; persisting metabolic acidosis (base excess ≥ -8mM over 4 hours in spite of corrective measures); increasing ventilation or supplemental oxygen requirement; ≥ 25 % reduction in platelet count from baseline or lower limit of normal; persisting glucose imbalance; and abdominal signs (abdominal distension, blood in stool, or bilious aspirates). Neutropenia was defined as ANC $< 1.0 \times 10^9$/L. When systemic signs and symptoms reminiscent of neonatal sepsis were present, a complete blood count (CBC), C-reactive protein, and blood smear were performed and blood cultures (urine and cerebrospinal fluid cultures in late sepsis) were obtained. If a microorganism was isolated from the blood culture, the diagnosis was culture-proven sepsis. Otherwise, diagnosis was stated as suspected sepsis. Empirical antibiotic treatment was started as soon as the culture specimens were obtained.

Participants of our study population were randomized either to receive rhG-CSF plus empirical antibiotics (Group I) or to receive empirical antibiotics alone (Group II). All infants received supportive treatment, such as inotropes and mechanical ventilation, when clinically indicated. rhG-CSF (Neupogen) was administered daily at a dose of 10 μg/kg/day in 5% dextrose (final concentration, 15 mg/mL) until ANC reached $> 1.0 \times 10^9$/L. At the beginning of the research, case numbers of Groups I and II were 33 and 31, respectively. However, eight infants in Group II received rhG-CSF later due to intractable neutropenia ($< 0.5 \times 10^9$/L for ≥ 3 days) and deteriorating clinical status; therefore, they were excluded from the study.

Blood samples were obtained via venipuncture or an umbilical catheter. Daily CBC and blood smears were performed until neutropenia subsided. ANC was calculated by direct examination of the blood smears under light microscope. When possible, 100 leukocytes were counted by the same senior resident (D.A.) who was blinded to the study and the ratio of neutrophils/total leukocyte count was documented. For each infant the day at which ANC was detected to be $< 1.0 \times 10^9$/L was regarded as the 1st day of the study and the day at which ANC was detected $> 1.0 \times 10^9$/L was accepted as the final day of the study (recovery of neutropenia). Hemoglobin levels, white blood cell and platelet counts, band/total and band/neutrophil ratios, daily ANC, and duration of neutropenia were recorded.

Clinical and demographic characteristics such as gender, gestational age, birth weight, mode of delivery, presence of premature rupture of membranes and preeclampsia in the mother, presence of symptoms of sepsis in the baby, positive blood cultures, concurrent morbidities, and mortality were also recorded.

The primary outcome of our study was sepsis related short-term mortality that occurred during hospitalization. Secondary outcomes were correction of preexisting neutropenia, rhG-CSF-related toxicity, and adverse effects attributable to rhG-CSF therapy.

The study was approved by the local ethics committee and written informed consent was obtained from the parents. For the statistical analyses, SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used. To compare the differences between groups, Student t test was used when continuous variables were distributed normally. Mann–Whitney U test was the statistical test of choice when the distribution of continuous variables was nonhomogeneous. Categorical data were analyzed using χ^2 test or Fischer's exact test. A p value < 0.05 was considered significant.

3. Results

Fifty-six neutropenic preterm infants with sepsis were randomized. Thirty-three infants received rhG-CSF (1–4 days; median, 2 days) plus conventional medical treatment and 23 infants received conventional medical treatment alone. Clinical and demographic features demonstrated no significant difference except for birth weight, which was lower in Group I (Table 1). Twenty patients (35.7 %) were diagnosed as culture-proven sepsis and others ($n = 36$, 64.3 %) were diagnosed as suspected sepsis.

Thirteen (23.2 %) infants had radiographic evidence of pneumonia, three (5.4 %) infants had necrotizing enterocolitis, 13 (23.2 %) infants suffered from intraventricular hemorrhage, seven (12.5 %) infants were diagnosed as patent ductus arteriosus, 39 (69.6 %) infants had respiratory distress syndrome, seven (12.5 %) infants had meningitis, and six (10.7 %) infants developed bronchopulmonary dysplasia (BPD). Follow-up period of patients ranged from 2 days to 7 days. Forty (71.4 %) infants survived to discharge. Sixteen infants (28.6 %) died. No side effect that can be attributed to rhG-CSF was observed.

Six patients, four in Group I and two in Group II, died before their neutropenia subsided. Therefore, they were excluded from the calculation of the day of recovery. All were culture-proven sepsis. Duration of recovery was significantly longer in Group II (3 ± 1.5 days and 6 ± 0.7 days, respectively; $p < 0.001$). ANC values were significantly higher on the 2nd study day and 3rd study day in Group I compared to Group II, whereas there was no difference in immature/mature cell ratios (Table 1).

In Group I, duration of recovery was significantly longer in patients with culture-proven sepsis when compared with patients with suspected sepsis (Table 2). Similarly, in Group II duration of recovery was also longer in patients with culture-proven sepsis (Table 2); however, this finding lacked statistical significance.

Table 1 Clinical and demographic features of the patients.

	Group I ($n = 33$)	Group II ($n = 23$)	p
Gestational age (wk)	28.9 ± 2	29.7 ± 2	NS
Birth weight (g)	1001 ± 240	1118 ± 173	0.04
Gender			
Female	10 (30)	6 (26)	NS
Mode of delivery			
Cesarean delivery	29 (87.8)	19 (82.6)	NS
SGA	8 (24.2)	4 (17.4)	NS
PPROM	11 (33.3)	7 (30.4)	NS
Culture-proven sepsis	11 (34.3)	9 (39.2)	NS
Outcome			
Mortality	10 (30.3)	6 (26.1)	NS

Data are presented as n (%) or mean ± SD.
PPROM, preterm premature rupture of membranes; SGA, small for gestational age.

When patients with positive blood cultures were analyzed separately it was documented that duration of recovery was significantly shorter in infants who received rhG-CSF when compared with infants in Group II ($p = 0.005$). Similarly, analyzing culture-negative patients (suspected sepsis), we demonstrated that rhG-CSF administration shortened the duration of recovery also in these infants ($p = 0.002$; Table 3). That is, rhG-CSF shortened duration of neutropenia both in infants with culture proven sepsis and in those with suspected sepsis.

In Group I, 10 (30.3 %) infants died during hospitalization. Seventy percent of these infants died after their neutropenia had subsided and in 30% neutropenia did not recover at all. Similarly, six infants (26.6%) in Group II died of morbidities including stage IV necrotizing enterocolitis, BPD, aspiration pneumonia and pneumothorax. Three of six infants died after their neutropenia had subsided and, in the remaining three, neutropenia did not recover at all. Short-term mortality rate did not differ significantly between groups. Finally, none of the infants had altered liver function tests (data not shown); and the number of thrombocytopenic patients is given in Table 3.

4. Discussion

In the present study, we demonstrated that treatment with rhG-CSF results in a significantly more rapid recovery of the ANC in neutropenic preterm infants with sepsis. However, no reduction in short-term mortality was documented. Theoretical concerns that rhG-CSF worsens respiratory distress syndrome and BPD by overactivating systemic inflammatory response exist in the literature.[23] Nonetheless, treatment-related side effects (such as thrombocytopenia) and toxic effects attributable to rhG-CSF were not detected in our study population.

In accordance with previously published studies,[24–26] our study documented that rhG-CSF induced a significant increase in ANC in neonates with systemic infections. Conversely, Schibler et al[27] failed to demonstrate a significant increase in ANC after rhG-CSF infusion. Their study was performed on 20 neonates with heterogeneous gestational ages and only six of the study participants had culture-proven sepsis. These studies varied with respect to design and patient population but no adverse events were reported. Only one case–control study investigating the efficacy of rhG-CSF infusion to low birthweight infants with neutropenia and sepsis has shown a reduction of short-term mortality.[28] However, our study failed to document a short-term survival advantage in the study group who received rhG-CSF compared to the control group.

In *Cochrane Systematic Review*, Carr and Modi[22] published a meta-analysis of seven studies to determine the safety and efficacy of rhG-CSF to reduce mortality in the treatment of suspected or proven systemic infections. They concluded that there was no evidence to support the addition of G-CSF or granulocyte–macrophage-CSF to antibiotic therapy in preterm infants with suspected systemic infection to reduce the immediate mortality. In addition, no significant survival advantage was seen at 14 days from the start of therapy [typical risk ratio (RR) 0.71 (95% confidence interval, CI, 0.38, 1.33); typical risk

Table 2 Comparison of duration of recovery of neutropenia in Groups I and II.

	Group I ($n = 30$)	Group II ($n = 20$)	p
Duration of recovery of neutropenia of infants with culture (+) sepsis (d)	3.1 ± 0.6	4.8 ± 1.3	0.005
Duration of recovery of neutropenia of infants with suspected sepsis (d)	2.4 ± 0.6	3.7 ± 1.5	0.002
p	0.01	0.09	

Data are presented as mean ± SD.
SD, standard deviation.

Table 3 Comparison of peripheral blood neutrophil counts, thrombocyte counts, and band/total leukocyte ratios between groups.

	Group I ($n = 33$)	Group II ($n = 23$)	p
Day of recovery of neutropenia ($n = 30/20$)	2.6 ± 0.7	4.3 ± 1.5	< 0.001
Number of thrombocytopenic infants*			
1st day ($n = 33/23$)	17 (51.5)	10 (43.5)	0.38
2nd day ($n = 33/23$)	18 (54.5)	13 (56.5)	0.55
3rd day ($n = 19/21$)	10 (52.6)	12 (57.1)	0.51
ANC			
1st day ($n = 33/23$)	556 ± 158.6	508 ± 164.8	0.275
2nd day ($n = 33/23$)	1034 ± 583.7	702 ± 535.2	< 0.001
3rd day ($n = 19/21$)	1150 ± 351.8	755 ± 309.2	< 0.001
Immature/mature			
1st day ($n = 33/23$)	0.155 ± 0.117	0.170 ± 0.102	0.438
2nd day ($n = 33/23$)	0.158 ± 0.120	0.176 ± 0.182	0.701
3rd day ($n = 19/21$)	0.157 ± 0.108	0.103 ± 0.077	0.057

Data are presented as n (%) or mean ± SD.
ANC, absolute neutrophil count; SD, standard deviation.
* Platelet count $< 100 \times 10^9$/L was defined as thrombocytopenia.

difference (RD) −0.05 (95% CI, −0.14, 0.04)]. However, all seven studies had small sample size, the largest recruiting only 60 infants. The subgroup analysis of 97 infants from three treatment studies who, in addition to systemic infection, had clinically significant neutropenia ($< 1.7 \times 10^9$/L) at trial entry, does show a significant reduction in mortality by Day 14 [RR, 0.34 (95% CI, 0.12, 0.92); RD, −0.18 (95% CI, −0.33, −0.03); number needed to treat, 6 (95% CI, 3, 33)]. However, our study could not document a decrease in short-term mortality in patients who received rhG-CSF, although they were even more neutropenic ($< 1 \times 10^9$ /L) at trial entry.

In some studies, rhG-CSF was administered for 5–10 days.[21,24,26] However, in our study neutropenia recovered on 2.2 ± 1.4 days in Group I and rhG-CSF infusions were halted as soon as ANC rose above 1×10^9 /L. Instead of administering rhG-CSF for constant periods, it may be more reasonable to perform daily CBC and terminate rhG-CSF infusions as soon as ANC is over 1×10^9 /L. Therefore, potential toxicity and adverse effects may be minimized.

Duration of recovery of neutropenia in infants with positive blood cultures was longer compared with infants who have negative blood cultures. This finding may be attributed to a smaller neutrophil storage pool, a reduced capacity for neutrophil mobilization from bone marrow reserves, and a slower regeneration of neutrophils in the preterm bone marrow.[14]

The limitations of this study are that it is not placebo-controlled and it has a relatively small sample size. The long-term survival and neurodevelopmental outcomes are important outcome measures that are lacking in this study. These outcome measures should be investigated in randomized placebo-controlled studies recruiting larger samples of neutropenic preterm infants with clinical sepsis in order to settle the controversy surrounding routine use of rhG-CSF for the treatment of neutropenia associated with preterm systemic infections.

Conflicts of interest

The authors declare no conflict of interest.

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