

Comparison of Two Regimens of RhG-CSF in Neutropenic Neonatal Septicemia: A Randomized Clinical Trial

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Abstract- Considering the 50% mortality rate of neonatal septicemia associated with neutropenia and increasing resistance to antibiotics, simultaneous antibiotic therapy strategies are becoming more important. However, few studies have been performed to evaluate effectiveness of RhG-CSF in the treatment of neutropenia in neonates. This randomized clinical trial was performed on 40 neutropenic neonates with septicemia who were hospitalized in Vali-e-Asr and Mirza Koochak Khan Hospitals (Tehran, Iran). The neonates were randomly divided into two equal groups RhG-CSF was administered as a subcutaneous single dose of 10µg/kg/s.c. to neonates in group A and as 10µg/kg/s.c./day once daily for 3 days to neonates in group B. CBC and differential count was checked 6, 24 and 48 hours after the last dose. There was no significant difference in mean birth weight, gender, age, and risk factors between two groups. Neutropenia was improved 48 hours after the last dose, whilst there was no significant statistical difference between two groups ($P>0.05$). The final outcome including death, duration of hospitalization and duration of antibiotics therapy after RhG-CSF administration did not differ between two groups ($P>0.05$). The results of this study showed that administration of a single dose of RhG-CSF (10µg/kg) was effective in treating neonatal septicemic neutropenia.

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Introduction

Septicemia is one of the most important causes of neonatal mortality throughout the world (1). Quantitative and qualitative deficiencies in the phagocytic system play a major role in increasing the risk of infection in newborns. The migration of neutrophils is poor at the time of birth in both full-term and pre-term infants and other functions of neutrophils such as adhesion, chemotaxis and morphologic changes are also reduced, which result in delayed response to infection (1). The total neutrophil reserve of the newborns is around 20%-30% of the adults' and there is a higher incidence of neutropenia during the course of an infection (1). Neutropenia is defined as an absolute neutrophil count less than 1500/mm³ in the peripheral blood (2). Total neutrophil count is abnormal in about sixty percent of infants with sepsis. However, a variety of clinical

conditions can affect total neutrophil count. Causes of neonatal neutropenia include asphyxia, meconium aspiration, lupus, drugs, etc (3,4). If sepsis is associated with neutropenia, mortality rate increases (3). RhG-CSF and Granulocyte- Macrophage Colony- Stimulating Factor (RhG-CSF) are cytokines which play important roles in the production, differentiation, and function of phagocytes (3). The first report on the administration of RhG-CSF in newborns was presented by Robert *et al.* in which resistant neutropenia occurred in a premature infant with sepsis and a history of maternal pre-eclampsia. Both sepsis and neutropenia showed improvement after 10 µg/kg RhG-CSF administration (5). In several studies performed in the NICU ward of Stony Brook University Hospital in New York, some neonates with neutropenia were given 10 µg/kg/day RhG-CSF intravenously for a maximum of three days and neutropenia improved 72 hours after treatment (6-8).

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In a study performed by Carr *et al.* in 2003, the effects of RhG-CSF administration (reduced mortality rate and increased rate of survival of premature newborns with sepsis) were evident 14 days after treatment (9). In a study performed by Ahmad *et al.* in 2004, phagocyte function improved after RhG-CSF administration even though absolute neutrophil count did not reach the count of the subjects in the control group (10µg/kg). In spite of the vast number of studies which have been performed on adults and children, but studies on newborns are scanty and the clinical use of cytokines in the prevention of neonatal infection is still under investigation (1). Regarding the uncertainty about the dose of RhG-CSF, its high cost, possible complications and the possible effectiveness of the single dose therapy, we decided to compare the effect of the single dose (10) with once-daily dose for 3 days (6,7).

Materials and Methods

In this pilot randomized clinical trial, all newborns with septicemic neutropenia (Neutrophils<1500 /mm³) that were born between July 2005 and July 2006 in Vali-e-Asr and Mirza Koochak Khan Hospitals were included. The newborns were randomly divided into two equal groups of 20 using a randomization table in each hospital. RhG-CSF (Pooyeshdarou-Iran, filgrastim) was administered as a subcutaneous single dose of 10 µg/kg to neonates in group A (interventional therapy group) and as 10 µg/kg/day once daily for 3 days to neonates in group B (conventional therapy group). According to the reference books and the Ethic Committee of Tehran University of Medical Sciences, we were not allowed to have a placebo group (12,13). The coulter counter and manual methods were used to determine CBC and differential counts 6, 24, and 48 hours after the last dose. Demographic data and laboratory test results were recorded in a specific questionnaire and the subjects were followed until either hospital discharge or death. The criterion for diagnosing septicemia included positive cultures obtained from sterile body fluids (inclusion criterion). Those infants who had neutropenia for causes other than septicemia such as infants of

mothers with systemic lupus erythematosus, history of some drugs, infants with intraventricular hemorrhage, asphyxia and maternal preeclampsia were excluded. The main outcome of this study was increase in the number of leukocytes and neutrophils. The study was approved by the Medical Ethics Committee of Tehran University of Medical Sciences according to the Helsinki Declaration. Study conditions were similar in both hospitals in order to prevent bias. Data was processed with SPSS version 15 and analyzed using statistical and analytic tests (Chi-square test, *t* student test) *P*<0.05 was considered significant.

Results

In this study which was conducted simultaneously in two NICU wards in Vali-e-Asr And Mirza Koochak Khan Hospitals in Tehran in a period of one year (July 2005-July 2006), a total of 40 newborns with septicemic neutropenia were studied. The smallest newborn was 26 weeks gestational age and 650 g and the biggest was 38 weeks gestational age and 3800 g. Mean body weight of the newborns in our study was 1408g and mean gestational age was 30.88 weeks. On the whole, eight (20%) newborns died during the course of our study but none developed complications during RhG-CSF administration or thereafter. In group A, 12 (60%) and 8 (40%) newborns were male and female while in group B, 11 (55%) and 8 (40%) were male and female, respectively. The two groups did not differ significantly regarding gender (*P*=0.92). Analysis showed that there was no significant difference between the two groups regarding birth weight (*P*=0.79) and current age (*P*=0.34). Table 1 shows the increasing trend in the neutrophil count at different times after the last dose of RhG-CSF until 48 hours. This increase was not significant between the two groups (*P*<0.71), but neutrophil count showed a significant rise in both groups after treatment (*P*<0.0001 in group A and *P*<0.01 in group B). Table 2 shows the final outcome of the neonates in both groups; however, the difference was not significant (*P*<0.63).

Table 1. Neutrophil count in the newborns under study prior and after RhG-CSF administration based on group

Time of testing	Group	Once daily (mean ± SD)	Once daily for 3 days (mean ± SD)	Total (mean ± SD)	P-value inside groups		P between groups
					Thrice	Single dose	
Prior to therapy		1209±238	1001±409	1104±345	-	-	0/06
6 hours after administration		3086±1560	3616±2871	3359±2306	0/007	0/000	0/4
24 hours after ad		5106±719	5095±818	5100±3555	0/000	0/000	0/9
48 hours after ad		9256±1140	11793±1415	10557±8785	0/000	0/01	0/5

*T- test for one group

**T- test for two independent groups.

Table 2. Final outcome in the neonates under study based on group

Dose	Final viability status	Living (%) N.	Death (%) N.	Total (%) N.
Once daily		(85) 17	(15) 3	(100) 20
Once daily for 3 days		(75) 15	(25) 5	(100) 20
Total		(80) 32	(20) 8	(100) 40

χ^2 , $P=0.695$

Discussion

The current study showed that single dose and triple dose of RhG-CSF were equally effective in neutropenic newborns with septicemia when conditions were similar. It seems that there was an equal response to treatment with a significant rise in neutropenia in subjects in both groups. The ideal scientific studies would have included a placebo-control group for efficacy assessment. However, since life-threatening septicemia in newborns needs immediate treatment, the use of placebo in this severe medical condition was considered unethical by the investigators and Ethic Committee of Tehran University of Medical Sciences (12,13). The results derived from this study showed that RhG-CSF exerted its effect after the administration of the first dose and therefore there is no need for 3 doses and this finding is in agreement with report of Cairo *et al.* (14). Also, comparison might have been easier if survival and death rates had been compared between the two groups 14 days after drug administration (7). Also, as seen in a similar study, response to varying doses of RhG-CSF does not depend on gestational age or gender (7). Our study showed that the outcome (death and duration of hospitalization) did not differ whether G-CSF was administered in a single dose or in 3 doses. This is in agreement with the results of the study performed by Gillian *et al.* in which RhG-CSF was administered at a dose higher than 5 μ /kg, which showed a good response in both peripheral and bone marrow neutrophil counts (15). As compared to previous studies, this study was performed on a larger number of newborns and it was made unbiased with the other group. Results showed that the administration of single dose RhG-CSF (10 μ g/kg) could result in an effective and satisfactory rise in peripheral blood neutrophil level and the results did not differ significantly with newborns who received a triple-dose therapy. Finally, our results in confirmation of other studies showed that the use of single-dose or triple-dose G-CSF does not produce any form of complications but rather increases the number of

neutrophils and reduces the mortality rate in neonates with sepsis (16).

The small sample size was our major study limitation, but our facilities did not allow us to assemble a bigger sample in this pilot study. In conclusion, considering the efficacy of the single dose of RhG-CSF and its high cost, we recommend that its use should be limited to a single subcutaneous dose of 10 μ g/kg. However, this study is considered to be a pilot study and we hope to perform it on a larger scale addressing various causes of neutropenia.

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