

A trial of recombinant human granulocyte colony stimulating factor for the treatment of very low birthweight infants with presumed sepsis and neutropenia

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Abstract

Objectives—The primary objective was to investigate the safety of recombinant human granulocyte colony stimulating factor (rhG-CSF) for the treatment of very low birthweight infants (VLBW) with sepsis and relative neutropenia, specifically with regard to worsening of respiratory distress and thrombocytopenia and all cause mortality. Secondary objectives were to evaluate duration of ventilation, intensive care, and antibiotic use as markers of efficacy.

Design—Neonates (≤ 28 days) in intensive care, with birth weights of 500–1500 g, absolute neutrophil count (ANC) of $\leq 5 \times 10^9/l$, and clinical evidence of sepsis, were randomly assigned to receive either rhG-CSF (10 $\mu\text{g}/\text{kg}/\text{day}$) administered intravenously ($n = 13$), or placebo ($n = 15$) for a maximum of 14 days, in addition to standard treatment and antibiotics. All adverse events, oxygenation index, incidence of thrombocytopenia, all cause mortality, duration of ventilation, intensive care and antibiotic treatment, and ANC recovery were compared between the two groups.

Results—Adverse events and oxygenation index were not increased by, and thrombocytopenia was not attributable to, treatment with rhG-CSF. At 6 and 12 months postmenstrual age, there were significantly fewer deaths in the group receiving rhG-CSF (1/13 *v* 7/15; $p \leq 0.038$). There was a non-significant trend towards a reduction in duration of ventilation, intensive care, and antibiotic use in the rhG-CSF group. There was a significantly more rapid increase in ANC in the rhG-CSF treated babies ($p < 0.001$).

Conclusions—In a small randomised placebo controlled trial in a highly selected group of neonates, adjuvant treatment with rhG-CSF increased ANC rapidly, and no treatment related adverse events were identified. Mortality at 6 and 12 months postmenstrual age was significantly lower in the treatment group. A large trial investigating efficacy in a similar group of neonates is warranted.

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Keywords: neutropenia; sepsis; very low birthweight infants; recombinant human granulocyte colony stimulating factor; antibiotic use; intensive care

In spite of significant advances in neonatal intensive care and the development of broad spectrum antibiotics, sepsis remains a leading cause of morbidity and mortality especially when associated with neutropenia.¹⁻⁵ Neutropenia has been variably defined in neonates,⁶⁻⁸ but even relative neutropenia ($< 5 \times 10^9/l$) is often indicative of infection⁹⁻¹⁰ and is associated with a worse outcome.¹⁻³ This is probably the consequence of failing to mount an appropriate neutrophil response to infection. The highest risk group for sepsis and its sequelae are very low birthweight infants (< 1500 g),¹¹ in whom infection results in prolonged ventilation and hospital stay and increased risk of chronic lung disease of prematurity, intraventricular haemorrhage, and death.¹²⁻¹⁴

Antibiotic treatment alone is unreliable in an immune compromised host, and long term or recurrent use facilitates antibiotic resistance.¹⁵ Efforts have therefore been directed at means of enhancing neonatal host mechanisms, deficiencies in which are thought to contribute to increased susceptibility to infection.¹⁶ Haemopoietic growth factors have been investigated for their capacity to augment neonatal neutrophil number and function.¹⁶⁻¹⁷

Recombinant human granulocyte colony stimulating factor (rhG-CSF) is a lineage specific growth factor which acts on neonatal haemopoietic progenitor cells in vivo to increase numbers of granulocyte colony forming units.¹⁸ rhG-CSF has been shown to qualitatively and quantitatively enhance neonatal neutrophils in clinical studies of both non-neutropenic¹⁹ and neutropenic babies²⁰⁻²³ in all but one study.²⁴

No study has yet investigated the potential clinical benefit of rhG-CSF treatment in neonates who are both infected and neutropenic (or relatively neutropenic) in a prospective randomised controlled study. However, before the initiation of such a study, which would necessarily involve many babies, safety issues need to be addressed. A theoretical problem that may arise from the use of rhG-CSF is the development of "sepsis syndrome" from overstimulation of the proinflammatory cascades.²⁵ This could lead to worsening of respiratory distress syndrome and chronic lung disease.²⁶⁻²⁸

rhG-CSF treatment of neutropenic and septic neonates has also resulted in the development of thrombocytopenia, albeit in a small uncontrolled pilot study.²⁰

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The primary objectives of our study were to evaluate the safety of rhG-CSF use, with specific reference to acute respiratory side effects, platelet count, and mortality.

As clinical efficacy in this population has been difficult to define, the study secondarily aimed to explore the feasibility of using surrogate markers of morbidity (duration of mechanical ventilation, intensive care, and antimicrobial use) with the intention of using these as short term end points in a study of efficacy if appropriate.

Methods

DESIGN

This was a prospective randomised double blind placebo controlled study in four centres, designed to test the hypotheses that (a) treatment related side effects would be seen in 20% or fewer of neonates receiving rhG-CSF, and (b) the number of serious adverse events observed in neonates receiving rhG-CSF would be no more than 20% greater than the number observed in the control group. As a marker of effect of rhG-CSF, the absolute neutrophil count (ANC) was expected to recover to $> 5 \times 10^9/l$ within four days of the start of rhG-CSF treatment in 90% of the neonates. The overall mortality in both placebo and treatment groups was expected to be in the range 15–35%. Safety was evaluated by documenting all adverse events and serious adverse events, oxygenation index, and trends in laboratory values including serial platelet counts. An adverse event was defined as being any new undesirable medical experience or change of an existing condition that occurred during or after treatment, whether or not considered to be drug related. The range of adverse events included many often encountered in any sick premature infant—for example, apnoea, abdominal distension, anaemia, jaundice, hyponatraemia, and hypoglycaemia or hyperglycaemia. A serious adverse event was defined as being any experience that suggested a significant hazard and included any event that was fatal, or life threatening, required prolonged hospital stay, was permanently disabling, or was a suspected overdose. Oxygenation index was defined as being “worse” if there was a $> 20\%$ increase in oxygenation index over pretreatment values. If a baby was having pretreatment with continuous positive airways pressure, the oxygenation index was said to increase if ventilation was required after treatment with the study medication. Thrombocytopenia was defined as being a reduction in platelet count to $> 25\%$ below baseline or below the lower limit of the reference range of each neonatal unit.

Thrombocytopenia $< 50 \times 10^9/l$, intraventricular haemorrhage $>$ grade II, deterioration in oxygen index in the first three days of enrollment in the study, splenomegaly, necrotising enterocolitis, rebound neutropenia, and any allergic symptoms were also specifically documented and investigated. Survival at 6 and 12 months postmenstrual age was recorded.

Preliminary markers of efficacy were explored by documenting duration of ventilation,

together with level 1 neonatal intensive care stay (British Association of Perinatal Medicine category 1²⁹), and duration of antibiotic treatment. Neonates were recruited from the neonatal intensive care units at St George's Hospital, London (n = 12), St Mary's Hospital, Manchester (n = 12), The Royal Maternity Hospital, Belfast (n = 3), and The Queen Mother's Hospital, Glasgow (n = 1).

PATIENT ELIGIBILITY

Patients were enrolled if they fulfilled all the following criteria: postnatal age ≤ 28 days with a minimum gestational age of 25 completed weeks; birth weight ≤ 1500 g but ≥ 500 g; requiring intensive care (as defined by the British Association of Perinatal Medicine²⁹); with clinical signs of sepsis; ANC $\leq 5 \times 10^9/l$; requiring treatment with intravenous antibiotics; written informed parental consent had been provided. An ANC lower limit of $5 \times 10^9/l$ was chosen because failure to mount a neutrophil response to infection is associated with a higher risk of mortality.^{1–3} Neonates were ineligible if they had a known lethal genetic disorder, a family history of haemopoietic disorders, Down's syndrome, previously received a granulocyte transfusion or prior treatment with cytokines, had received or were receiving any other investigational drug or had any known sensitivity to products derived from *Escherichia coli*.

CLINICAL SEPSIS

This was based on the following in the absence of an alternative explanation within 24 hours of randomisation: either fever (axillary/central temperature of $\geq 38^\circ\text{C}$ on one occasion or $\geq 37.5^\circ\text{C}$ on two occasions separated by at least one hour) or two or more of the following in the absence of an alternative explanation: poor perfusion; persisting metabolic acidosis (base excess ≥ -8 mmol/l over four hours in spite of corrective measures); increasing ventilation or supplemental oxygen to maintain adequate gas exchange over a minimum of four hours; $\geq 25\%$ reduction in platelet count from baseline or lower limit of normal; persisting glucose imbalance ($\leq 2.2/\geq 10$ mmol/l for four hours in spite of corrective measures); abdominal signs (abdominal distension, blood in stool, or bilious aspirates). Assessment of infection status at time of study entry was made by day 4 after study entry as microbiological results and other diagnostic data became available. Infection was confirmed if at least one sign of suspected sepsis was accompanied by a positive blood culture (within 48 hours if *Staphylococcus epidermidis*), positive cerebrospinal fluid culture, or growth of pathogenic organisms from any other site of infection. Necrotising enterocolitis (confirmed by at least two of the following clinical features: abdominal distension, blood in stool, or bilious aspirates together with pneumatosis intestinalis on abdominal radiograph and histopathological evidence at surgery or autopsy where available) was included in the “confirmed infection” category. Probable infection was defined as signs of infection as described in the

inclusion criteria but without microbiological confirmation. This included chest radiographic changes consistent with pneumonia, with increased C reactive protein and pathogenic organisms in the endotracheal secretions. Doubtful infection was defined as signs of infection at entry that were not supported by subsequent results and observations. Recurrences of sepsis within the initial 42 day study period were also defined as above.

EXPERIMENTAL DESIGN

Neonates who fulfilled all the inclusion/exclusion criteria and for whom written informed consent was obtained from the parent or guardian were randomised to receive either active rhG-CSF (Filgrastim; Amgen Inc, Thousand Oaks, California, USA) or placebo.

Randomisation and treatment procedure

Treatment was allocated according to a predetermined randomisation generated by Amgen and held in each hospital pharmacy. Randomisation was stratified by centre and age at onset of infection/sepsis (< 72 hours or ≥ 72 hours). Each infant was assigned to either the active or placebo arm of the trial by a predetermined schedule held by the hospital pharmacy. Placebo and rhG-CSF were both clear colourless solutions so that administration was blind. Study medication (10 µg/kg rhG-CSF or an equal volume of placebo) was dispensed and delivered to the neonatal unit for intravenous administration to the patient over 30 minutes as soon as possible thereafter. Study medication was continued daily for a maximum of 14 consecutive days or until discontinuation of antibiotics. This was because sepsis was a recruitment criteria, and it was presumed that if antibiotics were stopped it was because sepsis was no longer considered by the attending neonatologist to be the cause of the symptoms. Study medication was withheld if ANC ≥ 20 × 10⁹/l, and was restarted if it fell to ≤ 5 × 10⁹/l within the 14 day dosing period, but not beyond this date.

Antibiotics were prescribed according to local policy. Investigators could prescribe any concomitant medications deemed necessary to provide adequate supportive care, but all were recorded on case report forms.

Evaluations

All basic details and information on perinatal and maternal obstetric history, neonatal examination, vital signs, ventilation status (including oxygenation index), radiology, biochemical and haematological laboratory investigations (including full blood count and blood film, coagulation profile, C reactive protein, blood glucose, serum urea and electrolytes, creatinine, liver enzymes, and bilirubin), and category of neonatal care were recorded on the case report form before the first administration of the study medication and for 42 days thereafter. Clinical, laboratory, and radiological reassessments were obtained during the 42 days after recruitment and on the day of discharge from the neonatal unit. Specifically, full blood counts were obtained daily during

the 14 day administration period, for the first three days immediately thereafter, and at least twice a week until day 42. The occurrence of any relapses or new episodes of infection throughout the study period was also documented.

At 6 and 12 months corrected age, infants underwent full physical examination and assessment of wellbeing, which included detailing any hospital admissions for respiratory tract infections or other respiratory and non-respiratory complications in the interim period. Full blood count, blood film, and blood chemistry profiles were also obtained. Cause and date of death were recorded for any non-survivors. Adverse events were monitored continuously throughout the study period, and any serious ones were reported within 24 hours of discovery to the sponsoring company (Amgen) and the local research ethics committees. Treatment related side effects had to be documented before unblinding. Safety aspects were monitored by the safety monitoring group, which comprised two independent neonatal consultants, a statistician, and a representative of Amgen Ltd.

STATISTICAL ANALYSIS

All analyses were based on intention to treat principles. Study groups were unblinded only after all data had been collected and the database "locked". The statistical hypothesis was tested against a two sided alternative at the 5% level of significance. The treatment groups were compared with respect to mortality using Fisher's exact test. Kaplan-Meier plots were produced for the duration of mechanical ventilation, antibiotic treatment, and hospital stay. These values and the time to ANC recovery were compared between the treatment groups using the log rank test.

ETHICS

This study was approved by each hospital's local research ethics committee.

Results

Twenty eight babies were recruited and randomised to receive either rhG-CSF (n = 13) or placebo (n = 15). Table 1 summarises the basic details of this population. There were no significant differences, although median birth weight in the placebo and treatment groups were just below and just above 1000 g respectively. Similarly there were no significant differences in maternal and peripartum characteristics between the two groups. No infant received any granulocyte transfusion or other cytokines.

INFECTION STATUS

Of the babies receiving rhG-CSF, infection was confirmed in six, was probable in five, and doubtful in two (table 1). Confirmed infections were group B *streptococcus* (n = 2), *E coli* (n = 1), *Enterobacter cloacae* (n = 1), *Enterococcus* and *Staphylococcus aureus* (n = 1), and necrotising enterocolitis (n = 1). This compared with eight babies with confirmed infection in the group receiving placebo, six with probable infection, and one with doubtful

Table 1 Characteristics of study infants by treatment group

Variable	rhG-CSF (n=13)	Placebo (n=15)
Gestation (weeks)	28 (25–33)	28 (25–32)
Birth weight (g)	1030 (679–1380)	980 (650–1350)
Sex		
Males	3	4
Females	10	11
Age at recruitment (hours)	86 (39–670)	124 (29–652)
Confirmed infection	6	8
Culture negative infection	5	6
Doubtful infection	2	1

Where applicable, results are presented as median (range). rhG-CSF, recombinant human granulocyte colony stimulating factor.

infection. Confirmed infections were *S epidermidis* (n = 3), group B *streptococcus* (n = 1), and necrotising enterocolitis (n = 4); one of the latter also had *E coli* bacteraemia and another concurrent *S epidermidis*.

ADVERSE EVENTS

All babies suffered at least one of 122 documented adverse events according to criteria in the study protocol. Treatment related adverse events (peripheral oedema, bradycardia, anaemia, thrombocytopenia, and hyponatraemia) occurred in five placebo treated infants and two infants receiving rhG-CSF. Seven babies in the placebo group developed thrombocytopenia compared with four in the rhG-CSF group. There were no statistically significant differences. Similarly the oxygenation index was not increased by rhG-CSF (it worsened in 4/13 infants given rhG-CSF compared with 10/15 receiving placebo). The difference in mortality in the initial 42 day study period was not significant (table 2). However, there were three further deaths in the control group by 6 months of age (two as a result of worsening chronic lung disease and one as a result of hepatic and renal failure in a baby who had suffered from necrotising enterocolitis). The difference in mortality at 6 months was significant ($p \leq 0.038$; table 2) and this result remained unchanged at 12 months postmenstrual age.

The ANC recovered significantly more quickly in the rhG-CSF treated infants (table 2), suggesting that it had a biological effect.

SECONDARY END POINTS

Babies treated with rhG-CSF spent fewer days on mechanical ventilation, fewer days in intensive care, and fewer days receiving antibiotics during the initial 42 day study period (table 2). However, these results did not reach statistical significance. At 6 and 12 months postmenstrual age, there were no differences in growth

Table 2 Time to absolute neutrophil count (ANC) recovery, duration of antibiotic use, mechanical ventilation, and intensive care, and mortality

	rhG-CSF (n=13)	Placebo (n=15)	p Value
Time to ANC recovery to $>5 \times 10^9/l$ (days)	2 (1–3)	5 (4–7)	<0.001
Duration of antibiotics (days)	8 (6–16)	16 (13–35)	0.14
Duration of mechanical ventilation (days)	5 (3–13)	12 (6–34)	0.23
Duration of intensive care (days)	8 (3–17)	12 (8–34)	
Deaths during 42 day study period	1	4	0.33
Total deaths at 6 and 12 months postmenstrual age	1	7	0.038

Where applicable, results are presented as median (interquartile range). rhG-CSF, recombinant human granulocyte colony stimulating factor.

measurements or laboratory variables in surviving babies.

Discussion

The primary objective of this study was to evaluate the safety of rhG-CSF as adjunctive treatment in VLBW infants with moderate neutropenia and sepsis. Treatment with rhG-CSF resulted in a significantly more rapid recovery of the ANC. The reduction in mortality at 6 months should be interpreted with caution as this study did not have adequate power to investigate efficacy. No treatment related side effects were detected. Specifically there was no evidence of rhG-CSF related thrombocytopenia as previously reported in our uncontrolled pilot study.²⁰ Theoretical concerns that respiratory distress syndrome and chronic lung disease of prematurity may be exacerbated by rhG-CSF were also not substantiated. It is physiologically feasible that lung disease is not exacerbated because rhG-CSF downregulates activity of the proinflammatory cytokines, tumour necrosis factor α and interleukin 1β ,^{30,31} which have been implicated in the pathogenesis and severity of respiratory distress syndrome and chronic lung disease.^{26–28}

Similarly, clinical consequences of overactivation of the systemic inflammatory response to infection with rhG-CSF were not detected. Specifically the requirement for intensive care and mortality were lower in babies receiving active treatment. This is consistent with rhG-CSF having anti-inflammatory effects during sepsis.³⁰

In the previously published treatment intervention studies,^{19–21,23,24} rhG-CSF induced a significant increase in ANC in all but one study.²⁴ The latter was in a heterogeneous gestational age population of 20 neonates, only six of whom had culture proven sepsis. The studies varied with respect to design and patient population but no adverse events were reported, other than thrombocytopenia in our pilot study. Only one other case-control study of rhG-CSF, in septic neutropenic low birthweight infants, has shown a potential treatment benefit.²¹ No previous randomised placebo controlled study has specifically addressed safety or clinical efficacy in VLBW neonates who are both neutropenic (or relatively neutropenic) and who also have evidence of infection.

In summary, this was a small study in a highly selected “at-risk” population of infected and relatively neutropenic VLBW neonates. No treatment related adverse events were encountered, specifically no worsening of lung disease or thrombocytopenia. Mortality by 6 months postmenstrual age was significantly lower in the rhG-CSF treated group, and remained so at 12 months corrected age. The surrogate markers of efficacy—duration of mechanical ventilation, intensive care, and antibiotic use—may be appropriate short term end points in a similar population of neonates. In the long term, survival and neurodevelopmental status at school age are the more important outcome measures. We conclude that a trial of efficacy in a similar population is now warranted, ideally with international

Key messages

- rhG-CSF increases the absolute neutrophil count in very low birthweight infants with neutropenia and sepsis
- Thrombocytopenia is not an effect of rhG-CSF treatment
- Potential beneficial effects on long term survival require further investigation

collaboration between those with a specific interest in neonatal infection. To achieve a 90% chance of detecting a difference in mortality of 20% (10% in the treatment group and 30% in the placebo group) as being significant at the 5% level, about 100 babies per group would be needed.

Conflict of interest: the investigators would like to thank Amgen Inc, Thousand Oaks, California, USA, who funded the study. All information regarding the study is owned by the company. A B R and the study collaborators have been free to publish the results of the study as they see fit. The investigators are extremely grateful to the Amgen UK team for their support and advice.

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