The role of haemopoietic colony-stimulating factors in the management of newborn infection

When neonatal sepsis or necrotising enterocolitis is accompanied by neutropenia, mortality is high. The haemopoietic growth factors rhG-CSF (recombinant human granulocyte colony stimulating factor) and rhGM-CSF (recombinant human granulocyte macrophage-colony stimulating factor) have been investigated for their ability to enhance neutrophil numbers and function. Evidence to date does not support the routine use of rhG-CSF or rhGM-CSF for either prophylaxis or treatment of newborn sepsis, but use in preterm, neutropenic and septic babies <1500g may be of benefit and could be considered in extreme situations, after consultation with parents.

Characteristics of neonatal neutrophils

Polymorphonuclear neutrophils (PMN) are key components of the innate immune system, provide first line immune defence mechanisms against pathogenic challenge, and are both quantitatively and qualitatively deficient in neonates. Neutrophil deficiencies contribute significantly to the increased susceptibility of neonates to serious and overwhelming bacterial and fungal infections.

Although circulating absolute neutrophil counts (ANC) may not differ greatly from those in older children and adults, the release of marrow reserves in response to systemic infection can be markedly impaired1 as a result of depletion of the neutrophil storage pool, held in reserve within the bone marrow1,2. Severe neonatal sepsis may thus result in neutropenia, often with fatal consequences1,3-5.

Normal neonatal neutrophil counts

Normal ranges for neonatal ANC are different from those of infants and children, and there are a variety of published ranges. The most widely used reference ranges for normal neonatal neutrophil counts are derived from those published by Manroe6, and revised by Schelonka et al7, and Mouzinho et al8, and give normal values in the first 24 hours of life ranging between 1.8 to 14.4 x 10^9/L. In infants and older children the range is 5.0-19.5 x 10^9/L.

The ANC in babies born small for gestational age (SGA), are lower than those born at weights appropriate for gestational age (AGA), and may take up to two weeks of age to normalise9. Similarly, infants born to mothers with pregnancy-induced hypertension (PIH), are more frequently neutropenic10 and may be at higher risk of infection11. Such infants are also more likely to be thrombocytopenic11.

An ANC of 5.0 x 10^9/L or less in the presence of infection or necrotising enterocolitis (NEC) represents relative “neutropenia”, based broadly on the reference ranges of Manroe and Schelonka et al and the fact that there is a high mortality in neonates who fail to mount a neutrophil response to infection4,5. Even for older children, an ANC of ≤ 4.0 x 10^9/L, in the presence of profound sepsis, eg meningococcal sepsis and Falciparum malaria, is associated with a higher mortality11.

Qualitative defects in neonatal neutrophils

The most significant neutrophil functional impairments are related to defective adherence and chemotaxis4, and are even more pronounced in the very premature neonate. Several studies have demonstrated that phagocytosis and microbiocidal activity of neonatal neutrophils are similar to adults, though may be reduced in the...
presence of high bacterial inocula and by limited concentrations of opsonins and by limited concentrations of opsonins.

Haemopoietic colony stimulating factors

Haemopoietic colony stimulating factors, also variably termed 'haemopoietic cytokines' or 'haemopoietic growth factors', control the production, proliferation, differentiation, maturation, survival and functional activation of haemopoietic cells of all lineages. Individual cytokines may be lineage-specific, eg granulocyte colony-stimulating factor (G-CSF) and erythropoietin; or may regulate cells from a number of different lineages, eg granulocyte-macrophage colony stimulating factor (GM-CSF).

Some cell types may require the simultaneous action of a number of cytokines in order to effect the desired response. Multipotential stem cells have the capacity to undergo either self-renewal, or to differentiate into either lymphoid or myeloid primitive progenitor cells, under the controlling influence of factors such as stem cell factor.

Granulocyte colony-stimulating factor (G-CSF)

G-CSF is a secreted glycoprotein that is a major extracellular regulator of haemopoiesis, and of the innate immune system. G-CSF is known to be relatively specific in stimulating the growth of neutrophil progenitor cells in vitro, and also influences the survival, proliferation and differentiation of all cells in the neutrophil lineage from haemopoietic stem cells to mature neutrophils.

Mature adult neutrophils usually undergo apoptosis within 24 hours of leaving the bone marrow. G-CSF is not only essential for the recruitment of fresh neutrophils but also delays apoptosis of mature neutrophils, so prolonging survival. There is also a suggestion that G-CSF may inhibit apoptosis in neuronal cells, thereby attenuating hypoxic-ischaemic brain injury.

G-CSF is produced by endothelial cells, fibroblasts and macrophages in virtually all organs and is stimulated by inflammatory cytokines derived from activated monocytes, such as TNFα, interleukin-1 (IL-1) and interleukin-6 (IL-6), hence the mechanism for the neutrophilia observed in patients with infection or inflammation. G-CSF is a regulator of both innate and adaptive immune responses, specifically enhancing a variety of functions of mature neutrophils including phagocytic capacity, generation of superoxide anions and bacterial killing and influencing T-cell function and dendritic cell activation.

Part of the potential benefit of rhG-CSF, (though not of rhGM-CSF, – see below), has been related to the 'down-regulation' of pro-inflammatory cytokines such as TNFα and IL-6. High levels of TNFα and IL-6 in neonates are associated with an increased risk of chronic lung disease of prematurity, and poor neurodevelopmental outcome. Babies who suffer from NEC are also at increased risk of poor neurodevelopmental outcome. Theoretically rhG-CSF may provide benefit by down-regulation of such cytokines.

Endogenous G-CSF

When compared to healthy term neonatal controls, plasma G-CSF concentrations are significantly higher in neonates with sepsis and/or NEC, and those born to mothers with chorioamnionitis. Although in one study high G-CSF concentrations in the presence of sepsis, were sufficiently marked to suggest that G-CSF may be used as a biological marker of sepsis, these findings have not been consistent.

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF acts on multipotential, erythroid and eosinophilic haemopoietic progenitor cells, and has activities overlapping with, but distinct from, IL-3. Thus GM-CSF, in addition to early actions, stimulates the production of eosinophils, basophils, monocytes and dendritic cells, as well as neutrophils. In vitro study results suggest that rhG-CSF is more likely than rhGM-CSF to enhance neutrophil counts alone.

RhGM-CSF has been shown to have effects related to the secondary induction of pro-inflammatory cytokines such as TNFα and IL-6. Monocytes and macrophages, stimulated by rhGM-CSF, are a principle cellular source of TNFα. In a rat model of neonatal sepsis, rhGM-CSF-treated rats had a higher mortality and died more rapidly than controls. This was thought to be related to the stimulation of an intense pro-inflammatory response.

There is less concern that such pro-inflammatory activation would occur with rhG-CSF therapy in the light of studies demonstrating its inflammatory modulating effects.

Potential therapeutic use of rhG-CSF and rhGM-CSF in neonates

In adults and older children, rhG-CSF and rhGM-CSF were initially trialled for the prevention of chemotherapy-induced neutropenia and its complications, specifically infection, which is a particular risk when the neutrophil count falls below 0.5 x 10^9/L and persists for longer than 10-14 days. RhG-CSF has also become established for the management of congenital and cyclic neutropenia. Prior to the advent of treatment with rhG-CSF, such conditions were usually fatal in childhood as a result of infection.

RhG-CSF in the neonate

In initial animal models, recombinant human rhG-CSF increased the circulating neutrophil number, neutrophil storage pool and bone marrow progenitor pool, and improved survival from group B streptococcus infection when administered in combination with antibiotics.

Roberts et al, were the first to report a successful outcome following administration of rhG-CSF to a septic, preterm baby in 1991.

In a case control study of rhG-CSF in septic neonatal low birthweight infants (LBW), survival was significantly greater in the rhG-CSF group (13/14 vs 5/11). There was a suggestion of potential specific benefit for babies with NEC, which has been related to the potential for rhG-CSF to down-regulate inflammation, yet improve the quantitative and qualitative neutrophil response. The authors concluded that a randomised trial was necessary to validate the beneficial effects and also to determine whether any significant side effects of therapy exist.

In a randomised controlled trial in 35 septic, moderately neutropenic LBW infants receiving rhG-CSF for severe sepsis, a reduction in the duration of neutropenia and mortality was observed.
infants, rhG-CSF induced neutrophilia and enhanced neutrophil function, but did not affect mortality.

A randomised controlled multicentre UK study recruited only very low birth-weight (VLBW) neonates (<1500g), who were both moderately neutropenic (ANC <3.0 x 10^9/L), and who had clinical evidence of infection. This was a small study in a highly selected 'at-risk' population of infected and relatively neutropenic VLBW neonates. Adverse events and oxygenation index were not increased by rhG-CSF-treatment. There was a significantly more rapid rise in ANC in the rhG-CSF-treated babies (p<0.001). At six and 12 months post-menstrual age there were significantly fewer deaths in the rhG-CSF group (1/13 vs 7/15; p=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.

Treatment of neonates, as well as adults, with rhG-CSF is now known to produce a measurable rise in ANC, with a peak response between 12 hours to 10 days depending upon duration of treatment. RhG-CSF therapy may have a role in neonates who are both neutropenic and septic, especially those of lower birthweights, but the UK study was inconclusive. Similar conclusions were reached in the meta-analysis by Bernstein et al. The authors identified five studies, in which rhG-CSF was administered to human neonates with either proven or suspected septicaemia, and in which a control group was involved, (either placebo recipients or historic controls), and included the UK study. The metaanalysis, when compared with the Cochrane review, illustrates how different methods of analysis, can lead to different interpretations of any treatment benefit. In the Bernstein metaanalysis, the results from 73 rhG-CSF recipients and 82 control subjects were pooled. The rhG-CSF recipients had a lower mortality than control subjects (odds ratio (OR) of 0.17; CI 0.03-0.07; p<0.05). Exclusion of non-randomised, controlled studies increased the OR for death to a non-significant level (0.43; CI 0.14-1.23; p=0.13). Including only babies <2kg, reduced the OR (0.32; CI 0.11-0.83; p<0.02); as did including babies with neutropenia (OR 0.20; CI 0.06-0.56; p<0.001).

The authors of the Cochrane review of rhG-CSF and rhGM-CSF for treating or preventing neonatal infections, analysed the available studies by pooling the studies whereby rhG-CSF or rhGM-CSF were given as treatment, in conjunction with antibiotics, for suspected, or microbiologically proven systemic infection. Survival analysis was at 14 days from the start of treatment. Subgroup analysis of 97 infants from three treatment studies, who in addition to systemic infection had clinically significant neutropenia, demonstrated a significant reduction in mortality by day 14 (RR 0.34; CI 0.12, 0.92). The authors concluded that there is no evidence to support the introduction of either rhG-CSF or rhGM-CSF for the treatment or prophylaxis of infection. This may be overstating the lack of evidence for the use of rhG-CSF from large randomised controlled studies in babies who have both sepsis and/or NEC, and who are neutropenic. (The lack of evidence for benefit not being the same as evidence of no benefit).

It remains the case that no single study has demonstrated a statistically convincing improved clinical outcome for septic neonates or those with NEC, treated with rhG-CSF as an adjuvant, but few studies have concentrated on recruiting those neonates at highest risk of sepsis and its complications, namely the smallest and most immature, and those with any degree of neutropenia. None have been sufficiently large.

There are no published studies of the prophylactic administration of rhG-CSF in human neonates, although animal models suggest that both rhG-CSF and rhGM-CSF are more effective when administered before bacterial inoculation.

Potential adverse effects of rhG-CSF
Adverse side effects following chronic administration of rhG-CSF to patients with congenital neutropenia include mild splenomegaly, thrombocytopenia, osteoporosis and, more concerning, acute myeloid leukaemia (AML) in 10-15% of patients.

Rh-G-CSF and risk of malignant transformation
Since the introduction of rhG-CSF, patients with congenital neutropenia live longer, but in approximately 10-20%, a malignant myeloid disorder develops. RhG-CSF has arguably been associated with this increase in risk of acute myelogenous leukaemia. One theory is that the administration of rhG-CSF to patients with congenital neutropenia, may select for cells with a mutation in the G-CSF receptor that enhances the proliferation of myeloid cells.

An alternative suggestion is that as leukaemia development is related to a poor response to rhG-CSF, patients who are more severely affected by congenital neutropenia (reflected by being poor rhG-CSF responders), have an intrinsic predisposition to the development of myelodysplasia or acute myelogenous leukaemia.

Studies of patients treated for breast cancer with cyclophosphamide and doxorubicin who have also received rhG-CSF have also reported an increased risk of myelodysplasia or acute myelogenous leukaemia, but distinguishing the contribution of intensified chemotherapy (which rhG-CSF facilitates) from that of rhG-CSF itself has been much debated.

There are no published data regarding long-term outcomes following short-term use of rhG-CSF in neonatal sepsis, although in a two-year follow-up study, there were no discernible haematological or immunological adverse effects. While the precise contribution of rhG-CSF to malignant transformation remains unclear, it is vital that the leukaemogenic hazards of rhG-CSF should always be weighed against its potential therapeutic benefits.

RhGM-CSF in the neonate
In clinical studies rhGM-CSF given on an equal-mass basis did not increase neutrophil levels as much as rhG-CSF, and resulted in a monocytosis and eosinophilia. Concerns regarding activation of pro-inflammatory cytokines, while largely theoretical, have limited use of rhGM-CSF as an interventional treatment for neonates with established sepsis, but there has been one small randomised trial of rhGM-CSF treatment of 60 babies with sepsis and neutropenia, in which mortality was decreased in the rhGM-CSF group. In a comparison of rhG-CSF, rhGM-CSF and placebo given as adjuvant treatments to septic preterm infants, the ANC in the rh-GCSF treated group increased more rapidly and to a greater degree than in the rhGM-CSF or placebo groups. There was no difference in morbidity or mortality between the groups, but the numbers were small.
Adverse effects relating to inflammatory cytokine up-regulation following adjuvant rhGM-CSF treatment of neonates remain theoretical.

In the PROGRAMS trial\(^8\), Carr et al assessed whether or not rhGM-CSF administered as prophylaxis to extremely preterm, small for gestational age, neonates who were at high risk of neutropenia, would reduce sepsis, mortality and morbidity. In an initial pilot study, the authors had observed sepsis-free survival at 14 days from trial entry in six of 14 (43\%) control infants and nine of 11 (82\%) receiving rhGM-CSF. The PROGRAMS trial subsequently demonstrated that while early postnatal rhGM-CSF corrects neutropenia, sepsis rates, survival and short-term outcomes are not improved\(^9\). The PROGRAMS trial results illustrate the great importance of studies which are large enough to be sufficiently powered to yield statistically meaningful results: having observed a potential benefit for prophylactic rhGM-CSF in a small pilot study, there was no benefit from rhGM-CSF prophylaxis in a large randomised controlled study.

Conclusions

There remains substantial uncertainty about the benefits of rh-GCSF as adjuvant treatment in neutropenic (however neutropenia may be defined), preterm babies whose birthweight is less than 2kg, with established sepsis or NEC. Current evidence does not support the routine use of either rhG-CSF or rhGM-CSF in such situations.

RhG-CSF therapy is unlikely to benefit more than a few infants, as there are relatively few who become both septic and neutropenic. Immune host defence requires a complex interaction and partnership between innate and adaptive immune systems, and simply altering one element may be insufficient to truly make a difference to the other elements. A risk:benefit analysis should be applied to each individual’s case, in the evaluation of whether or not rhG-CSF could benefit outcome. While there may be a benefit in a small population of persistently neutropenic, septic babies, or those with NEC, the long-term risk of malignant transformation, though small in this population, remains a matter of debate, and must be considered in any evaluation.

Increasingly, it has become necessary to develop local guidelines for treatments, in accordance with governance and clinician regulation. As rhG-CSF is not licensed for use in neonates and there is no evidence to support routine use, it seems likely that such considerations may limit its use. Further large randomised controlled trials with long-term follow-up programmes would be ideal in order to obtain more definitive answers. However funding for such trials of treatments which would only be used in a small number of patients has a low priority. As a result, it may not be possible to obtain definitive evidence to support or refute the benefit of rhG-CSF as an intervention in neonatal neutropenic sepsis. The PROGRAMS study demonstrated conclusively that there is no place for prophylactic rhGM-CSF treatment.

References

30. Kocherlakota R., La Gamma E.F. Human granulocyte

Short listed entries announced

Entries for the Innovating for Life Awards, hosted by Infant journal and the British Journal of Midwifery and sponsored by Cow and Gate, closed on the 20th January 2012. We received eight entries in the neonatal section (one was subsequently withdrawn due to technical reasons) and all entries were of very high standard.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lead applicant name</th>
<th>Job title</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bedside assessment, stabilisation and initial cardiorespiratory support</td>
<td>Amanda Burleigh</td>
<td>Midwife</td>
<td>St James’s University Hospital, Leeds Teaching Hospitals</td>
</tr>
<tr>
<td>2 Network video-conferencing facility intervention</td>
<td>John Madar</td>
<td>Clinical Lead, Neonatologist</td>
<td>Derriford Hospital, Plymouth</td>
</tr>
<tr>
<td>3 An intervention to reduce nosocomial infection</td>
<td>Kylie Hart</td>
<td>Advanced Neonatal Nurse Practitioner</td>
<td>University Hospital of Wales, Cardiff</td>
</tr>
<tr>
<td>4 Difficult airway management protocol, equipment set and training module</td>
<td>Dr Lauren Johansen</td>
<td>Specialist Registrar in Paediatrics</td>
<td>Birmingham Heartlands Hospital Neonatal Unit</td>
</tr>
<tr>
<td>5 Improving outcomes for babies requiring physiotherapy</td>
<td>Lynsey Clarke</td>
<td>Network Practice Educator</td>
<td>Staffordshire, Shropshire &amp; Black Country Newborn &amp; Maternity Network, University Hospital of North Staffordshire Trust</td>
</tr>
<tr>
<td>6 Parent sensitivity training</td>
<td>Dr Maggie Brierton</td>
<td>Clinical Psychologist</td>
<td>Lynebank Hospital, Dunfermline</td>
</tr>
<tr>
<td>7 Accurate weighing of babies within the incubator on integral scales</td>
<td>Dr Ula El-Kafrawy</td>
<td>Consultant Neonatologist</td>
<td>Royal Bolton Foundation Trust</td>
</tr>
</tbody>
</table>

I would like to thank all the teams who took the time and effort to enter the awards. Two entries were shortlisted for the neonatal award: Entry 4 – Difficult Airway Management and Entry 7 – Integral Scales.

The winning team, which wins £10,000, will be announced at a lunchtime reception at The Royal Society in London on 2nd March 2012. Full details and photos will be published in the May issue of Infant.

Christine Bishop, Publisher