Erythropoietin responsive progenitors in anaemia of prematurity

A J B Emmerson, N B Westwood, R A Rackham, C M M Stern, T C Pearson

Abstract

Circulating erythroid progenitors (BFU-E) in five anaemic preterm infants (haemoglobin<100 g/l) were about 2 and 4·4 times as abundant as in 10 preterm infants who were not anaemic and five healthy adults, respectively, and were significantly more responsive to low concentrations of recombinant human erythropoietin (rHuEpo) than those from healthy adults. These results encourage further studies in the use of rHuEpo for the treatment of the anaemia of prematurity.

There is interest in the use of recombinant human erythropoietin (rHuEpo) for the prevention and treatment of anaemia of prematurity, which is an hyporegenerative anaemia associated with low concentrations of erythropoietin.[1] The clinical efficacy of this hormone for the treatment of anaemic premature infants has not yet been shown, though one uncontrolled study suggests that it may increase the packed cell volume.[2]

Progenitors committed to the erythroid line can be recognised in cell culture systems. These are of two types: the primitive progenitor, the erythroid burst forming unit (BFU-E), which is found in the peripheral circulation, and the more mature progenitor, the erythroid colony forming unit (CFU-E), which is predominantly found in bone marrow. In this study we report the relative numbers of BFU-E progenitors in infants with the anaemia of prematurity, and their response to various doses of rHuEpo, compared with those of progenitors that are present in healthy adults and cord blood from term infants.

Patients and methods

Two millilitres of venous blood were drawn into preservative free heparin from each of 10 premature infants who were not anaemic and five who were anaemic (haemoglobin <100 g/l). Five cord blood samples from normal term infants and samples from 10 healthy adult volunteers were collected for comparison. The progenitors were separated by centrifugation over a Ficoll metrizoate density barrier (1077 g/l); 1×10^5 cells from the light density cell fraction were cultured for 14 days at 37°C under 5% carbon dioxide in air in a serum free culture medium with a 2·2% methyl cellulose base, and containing Iscove's modified Dulbecco's medium, bovine serum albumin, cholesterol, sodium pyruvate, transferrin, 1-glutamine, nucleosides, insulin, inorganic salts, and antibiotics.[3] This system had previously been validated in our laboratory for the growth of erythroid progenitors in blood samples from premature infants (submitted for publication).

Six doses of rHuEpo from 0–2IU were added to the culture dishes containing a total volume of 1 ml, and all cultures were done in duplicate. The BFU-E were counted through an Olympus CK2 tissue culture microscope and the number of circulating erythroid progenitors was derived from the dishes containing 2IU/ml of rHuEpo. The significance of differences was assessed by Wilcoxon's rank sum test. Ethical approval was given by the local ethics committee.

The baseline measurements for the premature infants are shown in table 1. All infants were well and were not being ventilated. No blood transfusions had been given within a week of the blood samples being taken.

Results

The results show that in the five infants with anaemia of prematurity there were 4·4 times the concentration of circulating erythroid progenitors found in normal healthy adults (p=0·01, table 2). There are twice the number of circulating BFU-E progenitors in anaemic infants (35·5) than in infants who were not anaemic (17·5) although this is not significant.

In addition, neonatal progenitors respond at least as well to rHuEpo as those from adult or cord blood (figure). At the low concentrations of 0·1 and 0·5 IU/ml of rHuEpo there is a significant difference between the responsiveness of progenitors from anaemic preterm infants and those from healthy adults (p=0·05).

Table 1 Characteristics of the preterm infants studied. Values are expressed as mean (SD)

	Anaemic infants (n=5)	*Infants not anaemic (n=10)*
Gestational age (weeks)	31·1 (2·2)	29·7 (3·9)
Age at time of study (days)	35·4 (15·6)	27·2 (17·7)
Birth weight (g)	1670 (450)	1440 (450)
Haemoglobin concentration (g/l)	88 (9)	143 (33)

Table 2 Median (upper and lower quartile) numbers of circulating erythroid progenitors (BFU-E)/10^5 mononuclear cells at 2IU/ml recombinant erythropoietin

Subjects	*Median (quartiles)*
Anaemic infants (n=5)	35·5 (17·4–52·4)*
Infants not anaemic (n=10)	17·5 (8·5–21·9)
Cord blood from term infants (n=5)	10·5 (8·5–11·0)
Healthy adults (n=10)	8·0 (2·0–17·0)

*p=0·01 compared with healthy adults.

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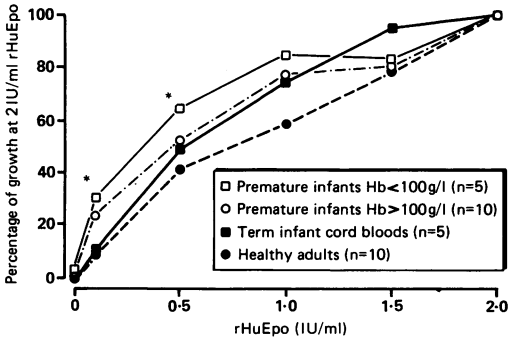

*Recombinant human erythropoietin (rHuEpo) dose response curves for circulating erythroid progenitors (BFU-E) from peripheral blood samples from anaemic preterm infants with haemoglobin (Hb) concentrations of <100 g/l and non-anaemic preterm infants, cord blood from term infants, and healthy adult volunteers; *p=0·05 for difference between the results for preterm infants and healthy adults.*

There is no significant difference at concentrations of rHuEpo of 1 IU/ml and above.

Discussion

The most important factor that affects the growth and development of the erythroid line is erythropoietin. Since the characterisation of rHuEpo by Jabobs *et al*,[4] and its commercial production, many therapeutic roles have been suggested for it. The high incidence of anaemia of prematurity, which has considerable morbidity, and the reported low concentrations of endogenous erythropoietin,[1] imply that this hormone may be a possible alternative treatment for the anaemia of prematurity.

The current treatment of transfusion with adult blood is unsatisfactory as there are many complications, including the risk of transmission of infective agents (especially cytomegalovirus), difficulties of venous access, and the further suppression of natural erythropoiesis.[5] An alternative treatment that would reduce or prevent the requirement for transfusion would be welcomed.

Rhondeau *et al* have reported dose response curves for the erythroid progenitor that is mainly found in bone marrow, CFU-E, and has shown that the CFU-E from the marrow of premature infants are as responsive to rHuEpo as those from cord blood from term infants and from healthy adult marrow. The present studies show both a significant increase in the numbers of circulating erythroid progenitors in anaemic premature infants, and an increased responsiveness of these progenitors to rHuEpo compared with adult BFU-E. These findings lend weight to the suggestion that there may be a role for the clinical use of rHuEpo in the prevention or treatment of anaemia of prematurity.

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Notes