

Iron supplementation enhances response to high doses of recombinant human erythropoietin in preterm infants

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Abstract

Aims—To determine whether iron supplementation would enhance erythropoiesis in preterm infants treated with high doses of human recombinant erythropoietin (r-HuEPO).

Methods—Sixty three preterm infants were randomly allocated at birth to one of three groups to receive: r-HuEPO alone, 1200 IU/kg/week (EPO); or r-HuEPO and iron, 1200 IU/kg/week of r-HuEPO plus 20 mg/kg/week of intravenous iron (EPO+iron); or to serve as controls. All three groups received blood transfusions according to uniform guidelines.

Results—Infants in the EPO+iron group needed fewer transfusions than controls—mean (95% CI) 1.0 (0.28–1.18) vs 2.9 (1.84–3.88) and received lower volumes of blood—mean (95% CI) 16.7 (4.9–28.6) vs 44.4 (29.0–59.7) ml/kg. The EPO group also needed lower volumes of blood than the controls—mean (95% CI) 20.1 (6.2–34.2) vs 44.4 (29.0–59.7) ml/kg, but the same number of transfusions, 1.3 (0.54–2.06) vs 2.9 (1.84–3.88). Reticulocyte and haematocrit values from postnatal weeks 5 to 8 were higher in the EPO+iron than in the EPO group, and both groups had higher values than the controls. Mean (SEM) plasma ferritin was lower in the EPO group—65 (55) µg/l than in the EPO+iron group 780 (182) µg/l, and 561 (228) µg/l in the control infants.

Conclusions—Early administration of high doses of r-HuEPO with iron supplements significantly reduced the need for blood transfusion. Intravenous iron (20 mg/kg/week in conjunction with r-HuEPO yielded a higher reticulocyte count and haematocrit concentration after the fourth week of life than r-HuEPO alone. Infants treated with r-HuEPO alone showed signs of reduced iron stores.

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Small preterm infants receive multiple red blood cell transfusions, mainly because of blood loss from repeated blood sampling and anaemia of prematurity.^{1,2} Inadequate erythropoietin production and abnormally functioning red cell progenitors, are major causes of neonatal anaemia.³⁻⁸ r-HuEPO treatment stimulates erythropoiesis and reduces the need

for blood transfusions in preterm infants.⁹⁻²¹

Studies of r-HuEPO treatment show that this hormone stimulates reticulocyte response in a dose dependent manner, but there are few data on the role of supplemental iron.²⁰ Different iron supplements are given in all randomised published r-HuEPO trials, starting at different postnatal ages.⁹⁻²⁰ In our previous study¹³ we administered high doses of intravenous r-HuEPO and 20 mg/kg of intravenous iron once a week from day 2 of life until discharge. Control preterm infants were not given either r-HuEPO or iron. In the present study we hypothesised that treating preterm infants with high doses of r-HuEPO would produce a marginal iron deficiency, so that iron supplementation would enhance erythropoiesis.

Low birthweight preterm infants were randomly allocated to receive r-HuEPO alone, r-HuEPO plus iron, or to serve as controls: to confirm in a larger series of infants the marked reduction in blood transfusions found in our previous study¹³; to see whether similar results could be achieved without iron supplementation; and to evaluate if the infants treated with high doses of r-HuEPO alone would develop signs of reduced iron stores.

Methods

Sixty three consecutive preterm infants, admitted to our neonatal intensive care unit after parental consent had been obtained, were studied. Inclusion criteria were: birthweight ≤1750 g and gestational age ≤32 weeks. Infants with disabilities, congenital heart defects or congenital infections, severe perinatal asphyxia (Apgar score at 5 minutes <5) and other diseases not strictly related to prematurity, were excluded. Infants meeting the inclusion criteria were randomly allocated to receive from the second day to the eighth week of life (or hospital discharge): (i) 400 IU rHuEPO/kg three times a week (Eprex, Cilag, Italy) + 20 mg/kg/week of intravenous iron (Ferrum, Hausmann SA San Gallo, Switzerland) (EPO+iron, n=22); (ii) 400 IU of rHuEPO/kg three times a week (EPO, n=20); or (iii) to serve as controls (n=21). rHuEPO was administered intravenously if the patient had an intravenous line and then continued subcutaneously at the same dose. Iron was administered once a week as a continuous intravenous infusion over 3 hours under the direct supervision of one of the investigators (RD). All infants were fed the same preterm formula and they received about 80 µg/kg of folic acid and 25 IU/day of vitamin E during the study period

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Table 1 Clinical data at birth and during study period

| | p Value | EPO (n=20) | EPO+iron (n=22) |
|--|-------------------------|-------------------------|-------------------------|
| Birthweight (kg) | 1.33 (0.28) (1.19–1.45) | 1.27 (0.28) (1.14–1.41) | 1.32 (0.28) (1.19–1.44) |
| Gestational age (weeks) | 29.8 (2.2) (28.8–30.8) | 29.3 (2.0) (28.4–30.4) | 29.7 (1.6) (28.9–30.3) |
| Standard deviation score of birthweight | -1.1 (0.7) (-1.4–0.7) | -0.9 (0.8) (-2.3–0.5) | -0.9 (0.7) (-1.4–0.7) |
| Apgar score at 5 mins | 6.9 (1.3) (6.6–7.4) | 7.0 (1.5) (6.6–7.4) | 7.1 (1.2) (6.2–7.3) |
| Systolic blood pressure* (mm Hg) | 70.3 (10.2) (68.2–72.1) | 70.2 (7.8) (68.8–72.3) | 72.3 (8.7) (68.8–72.1) |
| Diastolic blood pressure* (mm Hg) | 41.2 (6.3) (40.4–42.9) | 42.4 (5.6) (42.0–44.5) | 42.8 (4.9) (41.8–43.8) |
| Days on ventilator | 12 (14) (5.0–18.6) | 13 (18) (3.7–18.1) | 9 (13) (3.7–17.4) |
| Days of oxygen | 18 (19) (9.3–26.9) | 16 (19) (5.5–22.7) | 11 (14) (3.3–19.5) |
| Days of IV | 19 (14) (12.6–21.0) | 18 (15) (11.8–19.3) | 17 (12) (11.1–20.4) |
| Days on antibiotics | 8.3 (7.4) (4.9–11.6) | 8.3 (7.9) (4.5–12.1) | 8.0 (4.6) (5.3–11.7) |
| Days in hospital | 56 (30) (42.5–70.0) | 58 (39) (38.0–63.6) | 45 (23) (34.8–68.2) |
| Days to regain birthweight | 13 (6) (10.0–16.1) | 14 (6) (10.9–16.4) | 17 (6) (13.9–19.5) |
| Weight gain from birth to 8 weeks (g/kg/day) | 9.5 (2.9) (7.9–11.0) | 10.1 (1.8) (10.9–16.4) | 8.7 (3.1) (7.3–10.2) |

Data are give as group mean (SD) and (95% CI).
 *mean values (SD) (95% CI) of the averaged weekly weight gains of each patient during the study.
 There are no significant differences among groups.

as is customary in our intensive care unit. No oral iron supplements were given during the study period and the preterm formula contained 0.6 mg of iron per 100 ml ready to feed. Clinical data are reported in table 1.

R-HuEPO and iron were administered to the patients by one investigator (RD); the attending physician, who judged the need for blood

transfusions, was blinded to the infant's treatment group. Infants received transfusions of packed cells (10 ml/kg) during the first week of life if their peripheral haematocrit (heel stick) was below 42% or 36%, depending on whether the patient was receiving supplemental oxygen. After the first week of life, indications for transfusion were haematocrit below 36% for oxygen dependent patients and 32% if in room air. Haematocrit concentrations for red blood cell transfusions for blood obtained from venepuncture or arterial samples were 4% lower than the above mentioned values (38% and 32% for oxygen dependent and non-oxygen dependent patients in the first week and 32% and 28% thereafter). All infants received dedicated units of red blood cells.

Blood samples were obtained from the patients weekly for determination of haemoglobin, haematocrit, red cell indices (mean cellular volume (MCV), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC)), white cells with differential counts, thrombocytes, and reticulocyte count. Plasma ferritin concentrations were determined every two weeks from week 4, using a fluorimetric enzyme immunoassay (Baxter Diagnostics Inc. Deerfield, ILL, USA). Routine biochemical determinations including urea, creatinine, uric acid, cholesterol, triglycerides, total proteins, albumin, bilirubin and iron were also obtained weekly. Blood samples were taken before iron administration. The following data were recorded during the treatment period: heart rate; respiratory rate (hourly); blood pressure (twice a day); evidence of apnoeas, brady/tachycardias; transfusion requirements; blood volume sampled, volume of packed red cells transfused; weight gain; energy intake (daily), as described before.¹³

Results are expressed as mean and standard deviation (SD) or mean and standard error (SE), as appropriate. Statistical comparison between groups analysis was accomplished using ANOVA, and the χ^2 test. To avoid skew distributions, such as the number of blood transfusions or number of days in hospital, the logarithms of the original data values were used for analysis. For the haematological picture, the mean of each subject was calculated and the means were analysed as if raw data.

Table 2 Incidence of the common diseases of prematurity

| | Controls (n=21) | EPO (n=20) | EPO+iron (n=22) |
|-------------------------------|-----------------|------------|-----------------|
| Respiratory distress syndrome | 13 (61%) | 10 (50%) | 11 (50%) |
| Bronchopulmonary dysplasia | 7 (33%) | 6 (30%) | 5 (22%) |
| Patent ductus arteriosus | 9 (43%) | 11 (55%) | 7 (32%) |
| Intracranial hemorrhage | 3 (14%) | 2 (10%) | 2 (9%) |
| Sepsis | 2 (10%) | 3 (15%) | 0 (0%) |
| Retinopathy of prematurity | 1 (5%) | 2 (10%) | 2 (9%) |

There are no significant differences among groups.

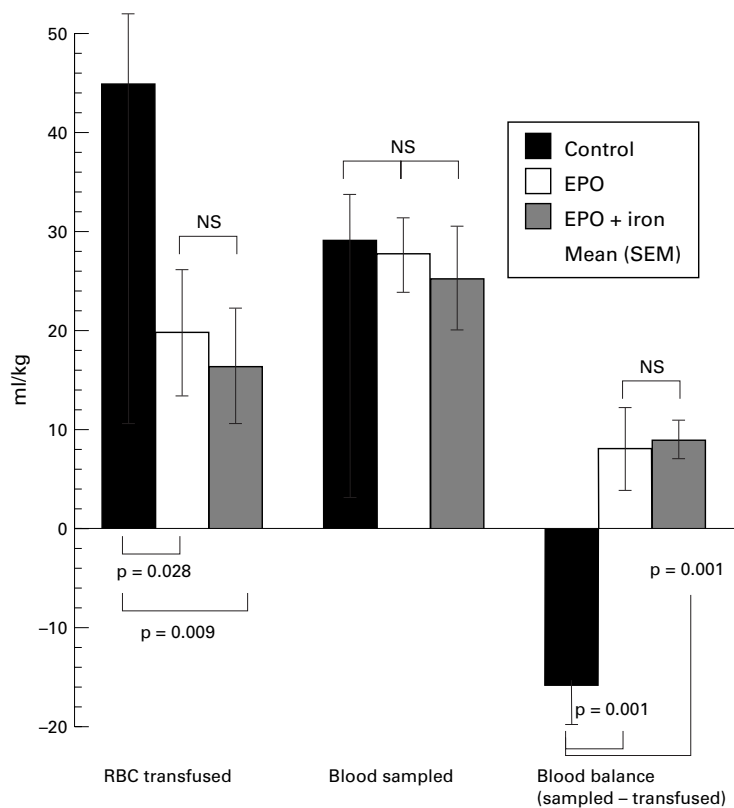


Figure 1 Blood balance in preterm infants treated with EPO+ iron, EPO alone, and in controls. Left hand columns represent volume of red blood cells transfused, central columns indicate volume of blood sampled for laboratory testing, and right hand columns show difference between blood sampled and transfused.

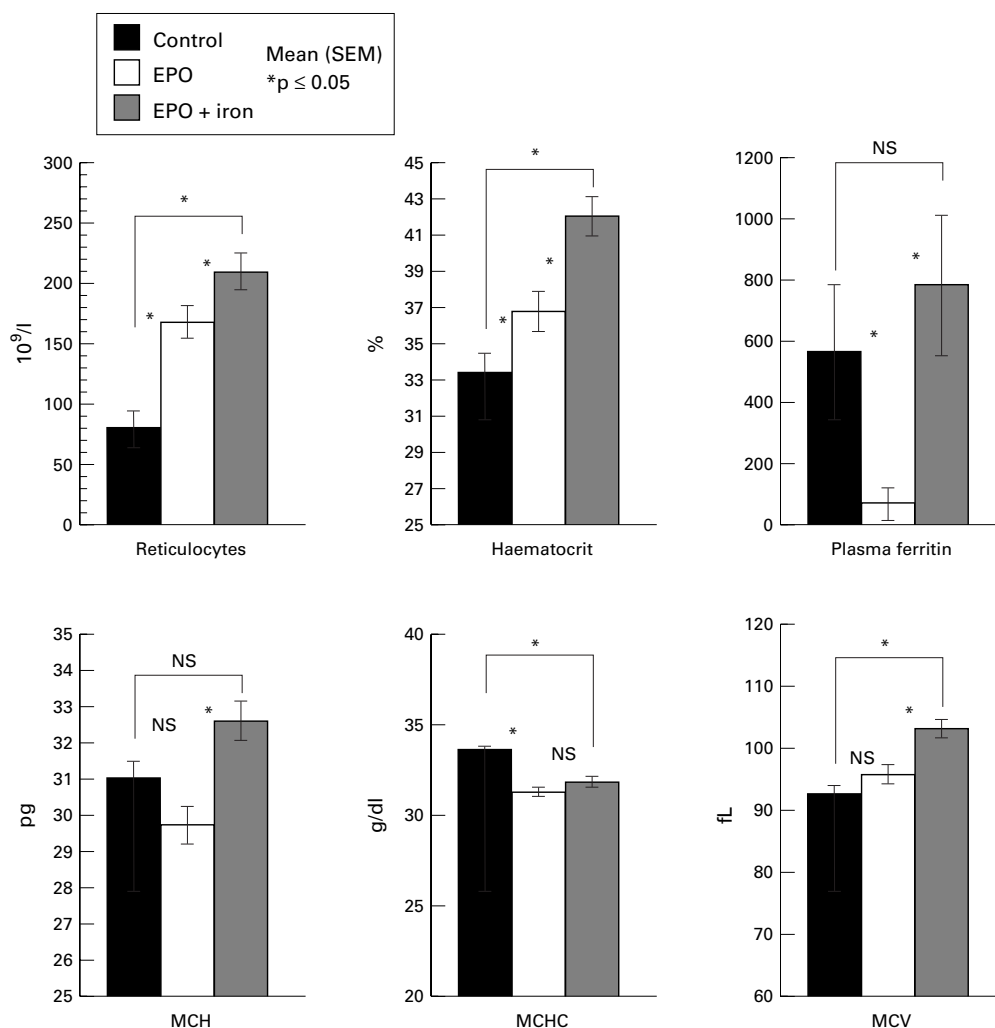


Figure 2 Reticulocytes, haematocrit, plasma ferritin, mean cellular haemoglobin (MCH) mean cellular haemoglobin concentration (MCHC), and mean cellular volume (MCV) in three groups of preterm infants during the second month of life: EPO + iron ($n = 10$); EPO alone ($n = 11$), and controls ($n = 10$). * $p < 0.005$.

Results

Clinical characteristics at birth and during the hospital stay are shown in table 1. The diseases of prematurity were remarkably similar among the study groups (table 2). Heart rate, respiratory rate, number of episodes of apnoea, and other clinical variables were no different. In spite of similar clinical characteristics and severity of the illnesses, the infants who were assigned to the EPO and EPO+iron groups required lower volumes of blood despite similar losses from phlebotomies (fig 1). The mean number of blood transfusions was 2.9 (95% CI 1.84–3.88), 1.3 (0.54–2.06), and 1.0 (0.28–1.18) in the control, EPO, and EPO+iron groups, respectively (control *vs* EPO, $p=0.065$ and control *vs* EPO+iron, $p=0.035$). Figure 2 shows the mean values of selected haematological variables and plasma ferritin from study weeks 5 to 8.

Reticulocytes were increased in the treated groups compared with the controls. The EPO+iron group had significantly higher reticulocytes than the EPO group ($p=0.047$). No significant differences were found during the first 4 weeks of life (data not shown). Infants treated with EPO+iron also had signifi-

cantly higher haematocrit, haemoglobin, MCV and MCH than the other groups (fig 2). Plasma ferritin was the same in the control and the EPO+iron groups; both groups exhibited significantly higher values than the EPO group. Platelet, white cell, and neutrophil counts were the same. No differences were found for several biochemical variables including plasma urea, creatinine, uric acid, cholesterol, triglycerides, total proteins, albumin, bilirubin and total iron (data not shown).

Discussion

The ultimate end point for any study using r-HuEPO in preterm infants must be, in our view, a reduction in the number of blood transfusions and of donor exposures. We confirmed in a larger number of subjects our previous finding¹³ that high doses of r-HuEPO stimulate erythropoiesis and reduce the number of blood transfusions in preterm infants. Few studies, as far as we are aware, have clearly addressed this issue. In a double blind study using 200 IU/kg/week intravenously from week 3 of age, there was no significant difference in transfusion between the r-HuEPO and the placebo groups.²² In a second double blind study using

similar doses, but with treatment beginning after the first week, there was a 41% reduction in the number of transfusions required, although this did not reach significance.¹⁴ Soubasi *et al.*,²³ using 300 IU/kg/week, showed that the need for transfusion was significantly reduced only in “uncomplicated infants”—those without severe respiratory distress syndrome or sepsis (0.37 (0.74) *vs* 1.1 2 (0.83), in treated *vs* non-treated infants). No difference was found, however, in the “complicated infants” who received 8.25 (5) (treated) *vs* 7.75 (3.7) (not treated) transfusions. The European Multicentre Erythropoietin Study Group¹⁶ has recently reported on the efficacy of r-HuEPO (750 IU kg/week) in reducing the need for blood transfusion in a large number of infants. The difference in blood transfusions between groups, although significant, was relatively modest, from a clinical point of view.²⁴ r-HuEPO treatment reduced the transfusion need by only 0.38 transfusion per patient. Similarly clinically modest results were found in a recent study in the USA.¹⁸ These authors used 500 IU/kg/week and found a modest although significant reduction of 0.5 transfusions per patient (1.1 (1.5) *vs* 1.6 (1.7) r-HuEPO *vs* controls, respectively).

Although differences in study design make comparisons rather difficult, blood losses from phlebotomies were not markedly different between our and the two above mentioned studies.^{16, 18} Furthermore, although differences exist between the patient populations, we are convinced that the better outcome of our study can best be explained by the higher doses of r-HuEPO and by the timing of its administration. In effect, what we need in these very young patients, is a prompt and sustained stimulation of erythropoiesis, to expand rapidly the red blood cell mass. This result can be accomplished only with very high doses of the recombinant hormone. The clinical experience derived from end stage renal failure²⁵ used to establish the initial doses, has a very different theoretical background (chronic anaemia) and clinical need (chronic population).

The second major finding of our study is that iron supplementation is associated with enhanced erythropoiesis (higher reticulocytes and haematocrit values) compared with infants treated with r-HuEPO alone. These infants developed signs of reduced iron stores as early as 5–6 weeks of age. Supplemental iron in nearly all published r-HuEPO data was given orally and in most cases it ranged from 2 to 8 mg/kg/day. In only one study were higher doses (18 mg/day) given.²⁶ We chose the intravenous route and the dose of 20 mg/kg once a week that corresponds to 3 mg/kg/day. The rationale for such a dose was to minimise the risk that a marginal iron deficiency would diminish the clinical response to the high doses of r-HuEPO. In studies where iron supplementation and doses of r-HuEPO were far lower than ours, the patients developed low serum concentrations of iron or ferritin.^{11, 12, 23, 27, 28} Ohls *et al.*⁹ administered iron intravenously at 1 mg/kg/day in total parenteral nutrition solution. Serum ferritin in their patients was meas-

ured after 10 days of intravenous administration of iron dextran and was 225 (42) ng/ml. Interestingly, in adults iron deficient erythropoiesis develops even in iron replete subjects.²⁹ Our data are consistent with previous observations that iron stores are critical to the rate of red blood cells produced.³⁰ The development of iron deficiency occurs in stages: first, body iron stores are depleted, reflected by a fall in serum ferritin; then transferrin saturation becomes abnormal; and finally anaemia develops. Red blood cells with a low MCHC are usually not seen until this final stage.^{31, 32} In our study preterm infants receiving r-HuEPO alone exhibited after the fourth week of life lower plasma ferritin, lower reticulocytes, and lower haematocrit than those receiving r-HuEPO+iron (fig 2). Thus our data clearly show the need for iron supplementation in patients treated with r-HuEPO. In all infants the umbilical cords were clamped immediately at birth and no placental transfusion was allowed. Under these conditions the infants in the study might have become more rapidly depleted of their iron stores (especially those assigned to the EPO treated group) than if they had received additional blood at birth.^{33, 34} The MCV was also lower in the EPO group, although we did not find significant differences in the MCHC. Future studies examining the serum concentrations of soluble transferrin receptors^{35, 36} and the proportion of red blood cells containing a low concentration of haemoglobin^{28, 29, 37} will identify earlier signs of iron deficiency. Our data clearly show that preterm infants receiving high doses of r-HuEPO need generous iron supplements. Whether large oral doses would be as effective as our intravenous regimen, or whether similar effects could be achieved by a lower intravenous dose, remain to be evaluated. A recent study²⁰ has shown that high doses of both oral (12 mg/kg/day) and intravenous (6 mg/kg/week) iron seem to supply sufficient iron for erythropoiesis in stable infants, but storage iron becomes depleted in the oral group. An intravenous supplementation of iron in rHuEPO treated preterm infants is therefore recommended, especially if serum ferritin concentrations are low. Starting iron supplements later in life could have the advantage of minimising the peroxidative damage which may be enhanced by iron administration.³⁸ The number of infants we studied is clearly too small to demonstrate any effect of intravenous iron in terms of potentiation of infection, nor this was one of our study objectives. However, we did not find any differences in the incidence of infections nor in the diseases of prematurity in which oxygen radicals are thought to have a role (table 2).³⁸ We choose not to study a group of infants receiving iron alone. In theory, iron alone could stimulate erythropoiesis by increasing serum iron concentration, but we felt that because of the paucity of information on intravenous iron in preterm infants, this treatment was not justified without the associated benefits of the r-HuEPO treatment. Furthermore, we believe that iron supplementation is not justified in an allegedly iron replete group such as a group of

infants receiving multiple blood transfusions during the first 2 months of life.

In conclusion, we found that early administration of high doses r-HuEPO to preterm infants with iron supplements significantly reduces the need for blood transfusions, iron supplements in conjunction with r-HuEPO yield higher reticulocytes and haematocrit after the fourth week of life, and infants treated with r-HuEPO alone show signs of iron deficiency. We emphasise that further studies are needed on the safety, timing, and efficacy of iron supplements during r-HuEPO treatment.

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