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Effects of Recombinant Human Erythropoietin on Fetal and Adult Hemoglobin in Preterm Infants

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ABSTRACT

In the present study we assess the effect of recombinant human erythropoietin (r-HuEpo) upon levels of fetal Hb (HbF) and adult Hb (HbA) in preterm infants. Twenty-eight "healthy," appropriate for gestational age infants with birth weights 900-1400 g entered the study at 3 wk of age. Fourteen infants were randomized to receive r-HuEpo, and 14 infants served as controls. Four controls and six r-HuEpo treated infants had been transfused before study start, whereas four control infants were transfused in the course of the study. The untransfused infants showed a high HbF/Hb ratio during the study with only a weak tendency to decline toward the expected time of delivery. The total Hb mass increased ($p < 0.05$) more in the r-HuEpo-treated infants than in the untreated, whereas the rise in HbF mass was similar in the two groups. After each transfusion, the HbF/Hb ratio reverted gradually to the ratio expected at the infant's postconceptional age. There was no difference in the production rate of HbF between r-HuEpo-treated infants and controls. The present data indicate that the HbF/HbA ratio in preterm infants is subject to the same programmed mechanisms which govern intrauterine erythropoiesis until term and that exogenous r-HuEpo does not influence this pattern significantly. (***Pediatr Res* 38: 729–732, 1995**)

Abbreviations
r-HuEpo, recombinant human erythropoietin
HbF, fetal Hb
HbA, adult Hb

Before 35 wk of gestation more than 90% of the Hb in the blood is fetal. Thereafter, the HbF/Hb ratio decreases slowly, and amounts to about 80% in full-term infants at birth (1). The mechanisms regulating the switch from synthesis of fetal to HbA as well as the timing of this switch have remained controversial (2). Some studies conclude that environmental factors, *e.g.* birth, seem to be accompanied by a gradual decrease in the synthesis of HbF (2–4). Studies in preterm infants (5, 6) and experimental animal studies (7) indicate that the switch is related to the postconceptional age, and that a "developmental clock" rather than environmental factors controls the Hb switching. The time of the switching has, however, not been clarified.

Recent studies have shown that r-HuEpo treatment increases the postnatal red cell production in very low birth weight infants (8–13). This increased red cell production has been reported to favor HbF production (8, 12, 13). It is, however, still an open question whether, or in which way, the r-HuEpo-induced rise in erythropoiesis influences the HbF/Hb ratio, and further studies addressing this problem have been called for (14).

The purpose of this study was to estimate and compare the net changes in HbF and HbA during the postnatal period in very low birth weight infants, with and without r-HuEpo treatment.

METHODS

The data were collected from the 28 preterm infants who completed a previously described randomized investigation of the influence of r-HuEpo treatment on the erythropoiesis during the anemia of prematurity (11). The infants in this study were all appropriate for gestational age with birth weight between 900 and 1400 g, healthy and without ongoing infection. None needed artificial ventilation and fraction of inspired air (Fio_2) was less than 40% oxygen. The study period lasted from age 3 wk to age 8 wk with an additional assessment at 16 wk. They were all fed human milk (mother's milk or bank milk) fortified with human milk protein to yield a daily protein intake of about 3 g/kg. Eighteen milligrams of iron were given daily from the start of the study and doubled if serum iron fell below 16.0 μmol/L. Fourteen infants were randomized to

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receive 100 U/kg r-HuEpo (Eprex, Cilag, 2000 U/mL) s.c. thrice weekly from wk 3 to wk 7, whereas 14 infants served as controls. Blood was sampled weekly in the amount of about 1.2 mL/week.

Six of 14 infants in the r-HuEpo group and 8 of 14 in the control group received HbA blood transfusions, either before or during the period of observation ("transfusion group"). These infants were slightly younger, with lower body weight and Hb concentration than the nontransfused infants. There were no differences with respect to gestational age, birth weight, weight gain, or initial Hb concentration between the r-HuEpo treated infants and the controls (Table 1).

The transfused blood consisted of HbA red blood cells from freshly tapped O blood, suspended in plasma from AB blood to give a hematocrit of 60%.

Blood for determination of HbF was collected at postnatal age 3, 5, 7, 8, and 16 wk (some infants also at wk 4 and 6). The percentage HbF/Hb (HbF%) was determined with a modification of the spectrophotometric method of Fogh-Andersen *et al.* (15) using a Radiometer OSM3 Hemoximeter. To minimize the the blood sampling the analysis was performed in 100-μL samples of packed heparinized red blood cells, *i.e.* the rest after removing plasma for other analyses, suspended in 100 μL of 0.9% NaCl. In a pilot study on 11 individual full-term infants at birth, mean HbF% was 79.8 (range: 71–89). In seven samples taken from one pool of newborn infants HbF% was 81.1 (79–82). Dilution of a blood sample with HbF% of 89 with HbA blood to 1/2, 1/4, and 1/8 of the original gave HbF% values of 46, 22, and 12, respectively.

Hb mass was calculated as Hb (g/dL) × actual body weight (g) × 0.08, whereas HbF and HbA mass were calculated as Hb mass × HbF% and Hb mass × (100 − HbF)%, respectively.

Statistics. Test of group differences were based on *t* tests or regression analyses. Initial values (at age 3 wk) were, when appropriate, used as covariates to compensate for differences in initial values and to increase the power of the tests. When describing the HbF% *versus* time relationship, we used a smoothing spline constructed by the JPM statistical system (smoothness parameter = 100).

RESULTS

Figure 1 shows the relationships between HbF% and postconceptional age in nontransfused control and r-HuEpo-treated infants. HbF% remained high until the age of 38–40 wk in both groups. Thereafter a marked decline in HbF% was observed. There was no statistically significant difference in HbF% between the controls and the r-HuEpo-treated infants.

Table 1. *Some characteristics of nontransfused infants*

	Controls	r-HuEpo
No. of infants	6	8
Gestational age (wk)	30.7 (0.9)	30.4 (0.6)
Birth weight (g)	1280 (42)	1297 (29)
Weight (g), wk 3	1552 (54)	1503 (30)
Weight (g), wk 8	2693 (69)	2479 (62)
Initial Hb (g/dL), wk 3	13.6 (0.8)	13.2 (0.6)
HbF%, wk 3	88.5 (1.8)	91.3 (1.6)

Data are presented as mean (SEM). There were no differences between the treatment groups (r-HuEpo and controls).

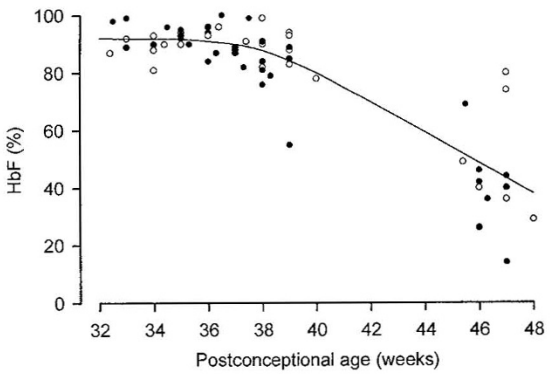

Figure 1. HbF levels (%) in individual, nontransfused infants *vs* postconceptional age. Infants treated with r-HuEpo (●), controls (○). There were no statistically significant differences between the two groups. The line represents the combined spline curve for both treatment groups (see text).

Figure 2 shows the changes in HbF and HbA mass in the nontransfused infants. During r-HuEpo treatment, from wk 3 to wk 7 postnatally, the total Hb mass showed a marked rise in the r-HuEpo-treated infants ($p < 0.01$), but only a minor increase was found in the control infants. For both groups the major part of the rise in total Hb mass during wk 3 to 8 was accounted for by an increase in HbF mass, 4 g in the controls and 5 g in the r-HuEpo-treated infants. The absolute increase in HbA mass was smaller, 3 g in the r-HuEpo-treated infants and 1 g in the controls. From wk 3 to wk 8 the production of HbA was slightly higher in the r-HuEpo-treated infants than in the controls, but this difference between the groups did not reach statistical significance. From the 8th to the 16th wk the HbF mass remained essentially unchanged in both groups, and the further marked rise in Hb mass was due to a rise in HbA mass only.

After the depression of HbF% caused by HbA transfusion, there was a rise in HbF% toward the level observed in the nontransfused infants, both in the r-HuEpo-treated and control infants. Figure 3 illustrates the changes in HbF% following transfusions for 5 of the 14 transfused infants, all with HbF measurements determined within 1 wk after the last blood transfusion.

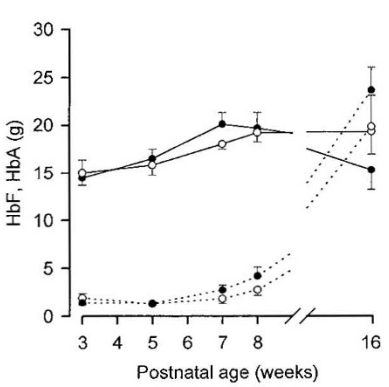

Figure 2. Mean values (SEM) of HbF (——) and HbA (·····) masses *vs* postnatal age for infants not being transfused before or during the study. r-HuEpo-treated infants (●); controls (○).

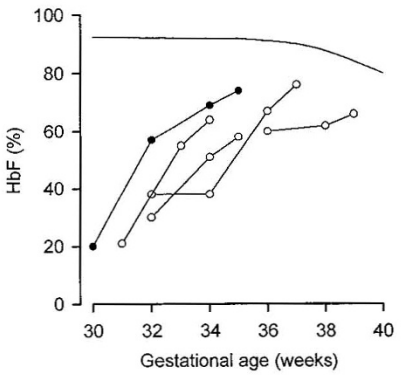

Figure 3. HbF (%) *vs* postconceptional age in individual preterm infants transfused with red blod cells within 1 wk before a HbF measurement. This and the subsequent measurements until age 8 wk are displayed. (r-HuEpo-treated (●) and control infants (○).) The *line* represents the spline curve for HbF development in untransfused preterm infants (see Fig. 1).

DISCUSSION

Previous studies in preterm infants indicate that the switch from HbF to HbA synthesis is related to postconceptional age rather than to time of birth (5, 6), although the mechanisms controlling this switch are not fully understood (2, 7). Although some studies report a gradual fall in HbF levels in premature infants with increasing gestational age (5, 6, 16, 17), the present results suggest a stable level of HbF% until close to the expected date of delivery (wk 38–40 postconceptionally). In these nontransfused infants born at a mean postconceptional age of 30.5 wk, HbF remained high at about 90% of total Hb until wk 38–40. The net rise in HbF mass during this period was greater than that of HbA mass. During these weeks the active erythropoiesis (11) produces predominantly fetal Hb. The postnatal continuation of HbF production is also clearly demonstrated in the slightly younger, more anemic infants with several HbA blood transfusions before or during the study. Reduced HbF% levels are initially observed, and then a net rise in HbF takes place (Fig. 3). The present results support the work by Brown *et al.* (18) also suggesting continued HbF production postnatally in preterm infants.

In experimental animal studies (19, 20) and in sickle cell diseases (21), Epo stimulation induces increased HbF production. Recent studies of the effect of r-HuEpo treatment on erythropoiesis in very low birth weight infants apparently indicate that r-HuEpo treatment favors production of HbF at the cost of HbA (8, 12, 13). This is in contrast to our current findings in the nontransfused infants where r-HuEpo had a very small influence on the HbF/HbA ratio.

In the study of Emmerson *et al.* (12) Hb was kept at a certain level either by r-HuEpo treatment or by transfusions. A reduction in the amount of HbA blood transfused by r-HuEpo must necessarily have led to higher HbF in the r-HuEpo group than in the controls. Likewise, in the study by Shannon *et al.* (10) previously transfused infants with initially low HbF and no further transfusions during the r-HuEpo treatment were bound to have a rise in HbF. This finding is consistent with our observation of a strong tendency to re-establish a high HbF% after blood transfusions. In the work by Bard and Widness (13) the increased HbF synthesis observed in the r-HuEpo-treated infants was due to a skew age distribution: the r-HuEpo treated infants were slightly younger with a synthesis of HbF lasting longer than seen in the placebo treated infants (Widness J, personal communication).

In the present study the transfused control infants had the same rapid increase in HbF as had the infants treated with r-HuEpo (Fig. 3). There was no evidence to indicate that r-HuEpo influenced the relationship between HbF and HbA in these transfused infants.

The prolonged maintenance of a high HbF level and the consequent delay in shift of the Hb O_2 dissociation to the right is in theory unfavorable for O_2 delivery to the tissues in anemic infants. Thus, in an anemic preterm infant a given therapeutic rise in Hb would theoretically produce a greater quantity of available O_2 when the rise in Hb is caused by HbA blood transfusion than if it is due to the infant's own production, spontaneous or r-HuEpo-induced (22). However, adult blood has clearly other disadvantages (*e.g.* blood-borne infections, human immunodeficiency virus, cytomegalovirus, hepatitis) (23) that make red cell transfusions undesirable for preterm infants.

In summary, the present data indicate that at least up to the expected date of delivery the HbF/Hb ratio in preterm infants is subject to the same programmed mechanisms which govern the intrauterine erythropoiesis until term. Exogenous r-HuEpo influences this pattern only marginally.

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