The Effect of Human Recombinant Erythropoietin on Prevention of Anemia of Prematurity

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Abstract

Objective: Premature infants often develop significant anemia that requires blood transfusion, this carries significant risks. This study was carried out to determine the effect of recombinant human erythropoietin (r-HuEPO) on prevention of anemia of prematurity.

Material & Methods: From April 2001 to March 2002, 24 neonates in newborn services at Amirkola children's hospital randomly were assigned to erythropoietin group and control (no treatment) group. Inclusion criteria were birth weight of ≤1750 grams and gestational age ≤34 weeks. Exclusion criteria were problems of hemolytic anemia, congenital infections, congenital malformations, severe asphyxia, intraventricular hemorrhage (grade III and IV), need for exchange transfusion and death during the first week of life. Erythropoietin group received r-HuEPO400 unit/kg/dose subcutaneously three times a week plus 4 mg/kg/day iron orally. White blood cell, hemoglobin (Hgb), hematocrit (Hct), platelet and reticulocyte count were obtained every 2 weeks until the 42nd day of life. Anemia was defined as Hgb≤8gr/dl and Hct≤24%. Student t test and Fisher exact were used to evaluate differences between the two groups.

Findings: Hemoglobin and hematocrit values were significantly higher in erythropoietin group than the control group after the 14th day of the study (P<0.04) and this difference was getting higher until the end of the trial (P<0.001). Five neonates developed anemia; all of them were from control group. One of these neonates required transfusion. None of the erythropoietin group newborns developed anemia.

Conclusion: The results of this study confirm the efficacy of recombinant human erythropoietin in the prevention of anemia of prematurity.

Key Words: Neonate, Anemia, Prematurity, Erythropoietin, Anemia prevention
**Introduction**

Premature infants experience a significant drop of hemoglobin (Hgb) blood levels during the first 2 months of life, with onset of clinical symptoms of anemia that may necessitate repeated blood transfusions. The anemia of prematurity is related to reduced erythropoietin response by the liver, which is considered the primary site of erythropoietin production in preterm infants and is less sensitive to anemia and tissue hypoxia than the kidney \[1,2\]. In addition, frequent blood sampling for diagnostic tests in sick preterm infants, not compensated by increase of serum erythropoietin concentrations\[3\], is another significant factor in the anemia of prematurity. Since anemia may decrease the amount of available oxygen to a critical level\[4\], preterm infants receive frequent transfusions according to the amount of blood drawn\[5\], but this practice carries significant risks to the low birth weight infants.

In vitro studies have indicated that recombinant human erythropoietin stimulates erythroid progenitors from preterm infants in a normal dose-response relation\[6,7,8\]. Pilot studies in small numbers of preterm infants suggested that low doses of this agent have some effect\[9,10,11\], but this finding was not confirmed in subsequent controlled trials\[12,13\].

We undertook the present study to determine whether recombinant human erythropoietin (r-HuEPO), given as recombinant human erythropoietin 400 unit/kg/dose three times a week, would prevent anemia of prematurity in premature infants.

**Material & Methods**

A randomized clinical trial was performed in newborn services at Amirkola children's hospital, from April 2001 to March 2002, with the consent of appropriate Ethics Committee on Research Affairs and that of the infants' parents. Eligible infants were randomly assigned to a control group or an erythropoietin treated group on the fifth day of life, by means of numbered, sealed envelopes. Block randomization was performed at each group.

The infants were eligible for the study if they met the following criteria: birth weight ≤1750 grams and gestational age ≤34 weeks. Exclusion criteria were problems of hemolytic anemia, congenital infections, congenital malformations, severe asphyxia, intraventricular hemorrhage (grade III and IV), need for exchange transfusion and death during the first week of life.

Erythropoietin group received recombinant human erythropoietin (r-HuEPO Cimba Co. Cuba), 400 unit/kg/dose subcutaneous three times a week plus 4 mg/kg/day iron orally. The iron was increased to 6 mg/kg/day from second week of life. All patients in both groups were given oral iron as ferrous sulfate and folic acid 25mg/day and Vitamin E 12.5 mg/day plus standard multivitamin drop starting as soon as enteral feeding was initiated and continuing during the entire treatment period. This regimen continued up to the 6th week of life. White blood cell, hemoglobin, hematocrit (Hct), platelet and reticulocyte count were done by Sysmex k1000 machine every 2 weeks until the 42nd day of life.

Sample size consisted of 24 neonates; 12 neonates for each (with a power of 80% and sigma 3.5). Anemia is defined as Hgb ≤8 gr/dl and Hct ≤24%. Data are analyzed by SPSS software, using Student t test and Fisher exact to evaluate differences between the two groups.

**Findings**

Twelve neonates in erythropoietin group are compared with 12 neonates in control (no treatment) group. The baseline characteristics in the two groups have no significant differences at the entry to the trial (Table 1).

After 14 days of study the Hgb, Hct and reticulocyte count started to increase and showed significant difference between the two groups. These differences were more prominent in Hgb and Hct than reticulocyte count values at the end of the study (P<0.001) (Table 2).

According to our definition, 5 neonates developed anemia. These neonates belonged entirely to control group. No cases of erythropoietin group developed anemia. No cases
### Table 1- Clinical and laboratory characteristics in control and erythropoietin group before the trial

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (n=12) Mean±SD</th>
<th>Erythropoietin group (n=12) Mean±SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (WK)</td>
<td>31.5±1.7</td>
<td>30.2±1.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Birth Weight (gr)</td>
<td>1245.8 ± 208.3</td>
<td>1779.1±208.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Volume of blood sampling for tests (ml)</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Hgb (gr/dl)</td>
<td>15.3±1.8</td>
<td>15.5±1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Hematocrit (gr%)</td>
<td>47.3±4.2</td>
<td>47.7±4.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>0.86±0.47</td>
<td>0.82±0.37</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Table 2- Laboratory characteristics in control and erythropoietin group after 14, 28 and 42 days of the trial

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (n=12) Mean±SD</th>
<th>Erythropoietin group (n=12) Mean±SD</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gr/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day14</td>
<td>12.6±1.6</td>
<td>14.3±2.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Day28</td>
<td>9.3±2.5</td>
<td>12.6±2.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Day42</td>
<td>9.1±1.6</td>
<td>11.8±1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Hematocrit (gr%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day14</td>
<td>39.9±5.4</td>
<td>43.8±7.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Day28</td>
<td>30.2±7.7</td>
<td>39.9±6.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Day42</td>
<td>29.1±5.4</td>
<td>38.9±3.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Reticulocyte count(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day14</td>
<td>0.6±0.2</td>
<td>1.8±0.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Day28</td>
<td>1±0.5</td>
<td>2.1±1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Day42</td>
<td>1±0.5</td>
<td>1.7±0.9</td>
<td>0.07</td>
</tr>
</tbody>
</table>

in erythropoietin group showed leukopenia, thrombocytopenia or thrombocytosis as side effects of erythropoietin.

### Discussion

Our study confirmed the efficacy of erythropoietin on prevention of anemia of prematurity in
premature infants with a stable cardiovascular status. The difference in hemoglobin and hematocrit after second week of trial was getting higher. So, this finding reveals the effect of erythropoietin on erythropoiesis and prevention of anemia of prematurity. All of the five babies, who developed anemia according to our definition, belonged to the control group and the one neonate that required packed cell transfusion also was from control group. Results of this study like other investigations\[14-19\] suggest that recombinant human erythropoietin could prevent anemia of prematurity. Despite stimulated erythropoiesis, the frequency of transfusions could not be reduced with r-HuEPO therapy and reduction in the number of transfusions is not conclusive\[19,20\].

Carnielli and coworkers were first to evaluate the effect of erythropoietin on preventing the anemia of prematurity. To determine whether prophylactic treatment with recombinant human erythropoietin and iron would reduce the need for blood transfusions, they randomly assigned 22 premature infants with gestational ages less than or equal to 32 weeks and birth weights less than or equal to 1.75 kg, to receive r-HuEPO, 400 IU/kg three times a week, plus iron, 20 mg/wk intravenously from the second day of life (11 infants), or no r-HuEPO and no iron (11 infants). The two groups had similar birth weights and clinical variables. The treated infants required fewer blood transfusions and less volume of packed erythrocytes. Reticulocyte and hematocrit values were higher in the treated group. These data indicate that r-HuEPO, in combination with iron supplementation, is effective in reducing the need for blood transfusions in the premature infant\[21\].

The difference in reticulocyte count between the two groups after 2 weeks in our study was significant but there was no significant difference in reticulocyte counts between the two groups at the end. It may relate to the 5 neonates in control group, which developed anemia.

Maier et al investigated whether early treatment of preterm infants with very low birth weight with recombinant human erythropoietin would reduce their need for transfusions in 241 infants with stable condition (off mechanical ventilation) at 12 centers in six European countries\[14\]. When these infants were three days old, they were randomly assigned either to the erythropoietin group or to the control group. Those in the erythropoietin group received 250 IU per kilogram of body weight subcutaneously three times a week from day 3 to day 42 (for a total of 17 doses); those in the control group did not receive this drug. Infants in both groups received oral iron (2 mg per day) from day 14 onward. The control infants needed a mean of 1.25 transfusions each, as compared with 0.87 transfusions for erythropoietin treated infants. The rate of success, defined as an absence of need for transfusions and a hematocrit that never fell below 32%, was 4.1% in the control group and 27.5% in the erythropoietin group. They concluded that infants with very low birth weight have less need of transfusions if given erythropoietin during the first six weeks of life (250 IU per kilogram three times a week). So they recommend early erythropoietin treatment for all such infants\[14\].

Most of the babies in our study had a stable clinical and cardiorespiratory condition. In order to assess in which neonates Epo treatment was effective, Soubasi and coworkers evaluated the effect of erythropoietin on transfusion requirement in uncomplicated (off mechanical ventilation) and complicated (need for mechanical ventilation)\[22\]. They concluded that early Epo administration reduces the need for transfusion in uncomplicated premature neonates, but this reduction did not occur in neonates requiring artificial ventilation\[22\]. Darveau et al also reviewed the literature concerning the role of r-HuEPO in reducing the need for transfusion in critically ill newborn. They concluded that r-HuEPO could not be recommended to reduce the need for red blood cell transfusions in anemic, critically ill patients\[23\]. Unlike these findings, Givens and Lapointe showed that the use of erythropoietin in critically ill patients can decrease the number of blood transfusions required during hospitalization, and potentially result in transfusion avoidance\[24\]. They concluded that because of the scarce amount of evidence and the diversity of dosing regimens used, no strict recommendations can be drawn from this review\[24\].
Many controversial questions regarding the use of r-HuEPO in attempts to either diminish the severity of or to treat the anemia of prematurity remains still unanswered. Strauss stated that r-HuEPO has efficacy in stimulating erythropoiesis in preterm infants, but success in the elimination or marked reduction in the need for RBC transfusions has not been definitively demonstrated[25]. But Türker and his colleagues randomly assigned 93 premature infants<1500 g in to study and control group. Study group received recombinant human erythropoietin (r-HuEPO) 750 U/kg per week subcutaneously from day 5 to 40 and enteral iron supplementation of 2 to 6 mg/kg/d beginning on day 14 that a 12-week monitoring period. They concluded that r-HuEPO combined with early enteral iron reduced transfusion needs only in the subgroup<1000g. So r-HuEPO combined with early enteral iron is both effective and safe in infants<1000g[26].

The early administration of parenteral r-HuEPO for preventing red blood cell transfusion in preterm and/or low birth weight infants was recently reviewed by Ohlsson and Aher as part of The Cochrane Collaboration Review. Review of 23 studies with enrollment of 2074 preterm infants, revealed that parenteral early administration of EPO reduces the use one or more red blood cell transfusions, the volume of red blood cells transfused, and the number of donors and transfusions[27].

**Conclusion**

We conclude that the early administration combination of r-HuEPO and iron stimulates erythropoiesis, maintain hematocrit and hemoglobin at higher level and is safe in very low birth weight infants.

**Acknowledgments**

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**References**

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