Original Article

The Effect of Early Subcutaneous Administration of Erythropoietin on Hematopoiesis and Weight Gain Velocity in Preterm Infants

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Abstract

Introduction

Anemia in preterm infants is identified as hemoglobin lower than 7-10g/dl around 1-3 months after birth. The aim of this study was to evaluate the effect of early subcutaneous administration of erythropoietin on hematopoiesis and weight gain velocity in preterm infants.

Materials and Methods

The present study was clinical trial carried out on 42 preterm infants. Those whose weight was lower than 1800 g at birth and gestational age of less than 34 weeks were included in the study. The subjects were randomly assigned into two groups. Intervention (IG) and control (CG).

The IG received 500 IU/kg of subcutaneous erythropoietin twice a week while there was no intervention with the control group. Both groups received iron supplement, vitamin A, D, E and folate daily. Measuring ferritin as well as reticulocyte, hematocrit, and hemoglobin were accomplished in each group before and after the treatment. SPSS11 software was used to analyze the data.

Results

Totally there were 19 boys (51.4%) and 18 girls (48.6%) in this study. there was an increase in the number of reticulocytes in IG after the intervention (P=0.004). Weight gain was also higher in this group (P=0.005). The mean systolic blood pressure, however, was not significant in both groups after the intervention (P=0.36). Two infants needed transfusion of packed RBCs in the CG while for IG this was not happened.

Conclusion

Early use of human recombinant erythropoietin results in erythropoiesis as well as weight gain in preterm infants.

Key words

Infant, Erythropoietin, Anemia, Blood Transfusion, Weight Gain

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Introduction

Erythropoietin is the major hormone which regulates hematopoiesis in the body. This hormone is a contributory factor for hematopoiesis in the fetus, infants and adults and by inhibiting apoptosis of precursors of erythyroid.It causes their proliferation and differentiation to normoblasts thus leading to RBCs longevity (1-2). This hormone is produced in the liver of the fetus but in adults is mainly built up from pretubular cells of the kidney and slightly in liver (1, 3). At first, it was believed that erythropoietin was the only effective cytokine for hematopoiesis. However, it is now understood that this hormone affects far more receptors thus effective in developing vascular endothelial cells of the gastrointestinal tract and brain (4).

Anemia of prematurity always being found in very low birth weight infants is a hypogenerative anemia which usually appears from the second week of life and reaches to its most severity at the age of two months (1,2,5). This is a normochrome-normocytic anemia which associated with reticulocytopenia (6,7). It seems that low serum concentration of erythropoietin is the major cause of reticulocytopenia and normochrome normocytic anemia in preterm infants (8).

In 1985, the trade form of human recombinant erythropoietin was manufactured for prevention and treatment of preterm anemia (4). Several clinical trials were designed to investigate the benefits, safety, effectiveness, and cost-effectiveness of erythropoietin administration but results reveal controversial (6-9).

Although few studies have been conducted on the effect of early administration of erythropoietin on hematopoiesis and reducing the need for blood transfusion they show that it can be effective in preventing anemia of prematurity thus decreasing the need for blood transfusion (9-10). Regarding the limited number of works carried out in this field, the present study was conducted to evaluate the effect of early administration of erythropoietin on hematopoiesis and needed amount of packed RBCs to be transfused into preterm infants.

Materials and Methods

Design: This was a clinical trial conducted on 42 preterm infants in Afzalipour Medical Center, Kerman, Iran. The sample size, determined on the basis of the previous studies (9) with power=80%, α=0.05 and d=5, was counted 15 for each group that by considering "loss to follow up" the number of the subjects for each group increased to 21. Those whose weight was less than 1800 g and gestational age of less than 34 weeks were included in the study. The subjects had to have stable respiratory and circulatory status with daily intake of at least 50 ml/kg of milk at the beginning of the intervention. Those with major congenital defects, coagulation disorders, severe asphyxia, intraventricular hemorrhage degrees 3 and 4, positive direct coombes test with the clinical signs of hemolytic anemia, hemoglobin-at-birth higher or lower than 2 standard deviation from mean hemoglobin for pregnancy age, surgical problem, exchange transfusion, any acute cardio respiratory disease requiring oxygen higher than 40% supplied by head box or by mechanical ventilation, systolic blood pressure of higher than 100 mmHg and the ANC(absolute neutrophil count) lower than or equal to 500 were excluded. The infants were Simple random assigned into intervention and control groups (IG & CG).

Drug dose and intervention period: The infants in the IG received500 IU/kg of subcutaneous erythropoietin twice a week in the anterolateral part of the thigh. This drug had been manufactured by Pooyesh Drug Company in Iran. Both groups received supportive treatment but the CG did not receive erythropoietin. The intervention pretended for four weeks. In case of seizure or constant hypertension the intervention was stopped. Systolic blood pressure higher than 100 mmHg during the first two weeks after birth and higher than 120 mmHg after two weeks.

Each infant received 3 mg/kg/day iron (vita co.). Moreover both groups received 50 microgram/kg/day oral folic acid (Drug Co.; Tehran; Iran); 1 ml/day vitamin A and D (Amin Drug Co.; Tehran; Iran) and 1 ml/day vitamin E (Behsa Drug Co.; Tehran; Iran). Each milliliter of vitamin A and D contained 1500 IU of vitamin A and 400 IU of vitamin D, while each milliliter of vitamin E contained 10 IU of this vitamin(9).

Blood transfusion was recorded during intervention period in terms of ml/kg in both groups. Blood transfusion was performed under the US Blood Bank guidance.

Experiments: In the beginning and at the end of investigation, the serum ferritin, reticulocytes, hemoglobin (Hgb) and hematocrit (Hct) were measured in both groups. The serum ferritin was measured by radioimmunoassay method in which reticulocytes were counted in a microscopic field and then it was measured on the basis of the number of RBCs in each cubic millimeter. Hgb and Hct were both measured by coulter counter machine. B p was measured by oscilometr.

The background variables such as: gestational age on the basis of the last menstrual period (LMP), birth weight (g), and sex of infants were recorded in the questioner. The information was gathered in the beginning and at the end of the study and was then analyzed.

Ethical consideration: The study protocol was approved by Ethical Committee in Kerman University of Medical Sciences. There was the possibility of ruling out the infants at any time of the study if the parents wished.

Statistical analysis: Data were analyzed through SPSS package Version 11(SPSS Inc., Chicago, IL, USA) by chi-square, student t-test and paired t-test. All values showed in mean ± SD.The P-value<0.05 was considered to be significant. All P-values were two-tailed.

Result

Two infants were excluded during the study (one from CG due to meningitis and the other from IG due to sepsis). Moreover, three others were ruled out due to using drugs inappropriately or not referring afterwards (one from CG and 2 from IG). Finally the study was accomplished with 18 infants in IG and 19 in CG.

Totally, there were 19 boys (51.4%) and 18 girls (48.6%). The frequency distribution was not statistically significant in both groups (p=0.37).

Table 1 indicates the clinical and laboratory characteristics of both groups before the intervention. None of the measured variables shows a significant difference in both groups.

Table 2 indicates the means of the variables before and after the intervention in both groups. As it indicates the mean of Hgb and Hct decreased in both groups after the intervention. The mean differences of these two variables in both groups were not significant at the beginning of the intervention, while statistically higher in IG at the end of intervention. Table 2 also shows that although the mean of serum ferritin was not significantly different between two groups at the beginning, however after the intervention it increased significantly only in the IG (P=0.01).

Although the mean reticulocytes count was not statistically different before the intervention; the mean increased significantly only in IG after the intervention i.e., it stood at 53353 ± 24231 and 92382 ± 54828 No/ml in CG and IG respectively (P=0.008) (Table 2).

As table 2 shows, the mean of weight gain in IG group was significantly higher than that of CG group.

Although mean systolic blood pressure between the two groups before and after the intervention was not significant, it was significant in both groups after the study (compared to the beginning) (Table 2).

Two infants in CG needed 10 ml/kg transfusion of packed RBCs (one at 36th day and the other at 32nd day of their birth) while none of the infants in the other group had such a need. In none of the infants the treatment was stopped due to neutropenia, hypertension or clinical

seizure. The drugs administered were tolerated favorably in both groups and there was no adverse reaction.

Table 1: Comparison of the means of the variables during the study in control group (CG) and intervention group (IG) before the study

Variable	CG (No=19)	IG (No=18)	P-value *
Pregnancy age	32.31± 1.63	31.94±1.34	**0.37**
Weight at Birth (g)	1533.68±229.06	1514.44±245.65	**0.80**
Systolic BP(mmHg)	63.89±3.16	62.58±3.01	**0.17**
Hematocrit (%)	50.42±5.98	48.63±5.61	**0.35**
Hemoglobin(g/dl)	18.19±2.06	17.61±1.85	**0.3**
Reticulocytes (No/ml)	48446 ±23323	49666±24455	**0.80**
Serum Ferritin (ng/ml)	**141.1±97.21**	**135.28±90.66**	**.85**

* Student t-test

Table 2: Comparison of the variables during the study in control group (CG) and intervention group (IG) before and after the study

Variable	CG (No=19)	IG (No=18)	P-value*
Hematocrit (%)			
Before	50.42±5.98	48.63. ±5.61	**0.35**
After	32.91±4.00	37.64±5.09	**0.005**
P-value**	< 0 .001	< 0. 001	
Hemoglobin (g/dl)			
Before	18.19±2.06	17.61±1.85	**0.37**
After	11.21±1.60	12.93±1.93	**0.008**
P-value	< 0.001	< 0 001	
Serum Ferritin (ng/ml)			
Before	141.1±97.21	135.28±90.66	**0.85**
After	141.68±91.46	142.06±95.97	**0.75**
P-value	0 .91	0 .01	
Reticulocytes (No/ml)			
Before	48446 ±23323	49666±24455	**0.80**
After	53353±24231	92282±54828	**0.008**
P-value	0.20	0.004	
Systolic BP(mmHg)			
Before	63.89±3.16	62.58±3.01	**0.17**
After	70.26±4.24	69.00±4.20	**0.36**
P-value	< 0 .001	< 0 .001	
Weight gain velocity (g/daily)	**18.89±3.89**	**23.96±6.07**	**0.005**

- Student t-test; ** Paired t-test

Discussion

Anemia of prematurity started 1-3 months after birth is accompanied by Hgb below 7-10 g/dl and manifests itself as pallor, poor weight gain, poor activity, tachypenia, tachycardia and feeding problems (2). The factors resulting in anemia of prematurity are: frequent venipuncture for blood tests, reduction of RBCs longevity, rapid growth and transient physiologic effects from fetal life (low arterial oxygen pressure and Hgb saturation) to infant life (high arterial oxygen pressure and hemoglobin saturation) (2, 11). The oxygen accessible to the tissues of a infant is lower than that of an adult; however an infant's erythropoietin

response is lower compared with the severity of anemia and leads to the reduction of Hgb as well as reticulocytes (1). Treatment of anemia in infants is possible through blood transfusion which is dependent on the signs, severity, Hgb concentration, and coexisting diseases (2, 11). Very low birth weight infants are the major group requiring blood transfusion during the first two weeks of their life (12-13). The need for blood transfusion has to be assessed against the risks resulting from transfusion. These are hemolytic reactions due to transfusion, contact with the preserver substances in blood products and other potential poisons, volume overload, increase in the risk of retinopathy of prematurity and necrotizing entrocollitis, graft-versus–host disease (GVHD) and infections transmitted through transfusion i.e., CMV, HIV, parovirus, hepatitis B and C (2,11). An attempt should be made to have the least possible transfusion for the subjects.

In 1985, when human recombinant erythropoietin was marketed, different studies were conducted to evaluate the effect of this drug on therapy and prevention of anemia of prematurity (6,7,8,14). The results are scientifically controversial. In addition to having hematopoietic effect, this drug has an important role in the development and protection of the nervous system (15).

In this study, the effect of early administration of erythropoietin on infants' hematopoiesis was assessed. To control anemia of prematurity we administered erythropoietin before the 8^{th} day of birth (9). There are records of oral and intravascular injection of erythropoietin in preterm infants (12, 16), however we started using it subcutaneously (8,9).

In this study the number of reticulocytes and the percentage of final Hct and mean Hgb were significantly higher in IG compared with CG representing bone marrow stimulation and the increase in hematopoiesis. In previous studies there were such reports as well (9, 12, 17).

As it was previously described, each infant took 3 mg/kg/day iron supplement and 50 microgram/kg/day folic acid. Iron and folic acid doses were adjusted each week on the basis of infants' body weight. Evidently iron supplement and folic acid are needed for hematopoiesis (18, 19, and 20). Also in other studies, there existed 3 mg/kg/day administration of iron supplement for those infants who could tolerate oral intake (9,13).The significant increase in concentration of serum ferritin in IG (compared with its basic mean) represents a relative increase in body's iron store and thus ameliorating anemia.

Weight gain in our study was significantly higher in IG. This can likely be attributed to the increase in Hct and Hgb and a better feeding tolerance subsequently. However further studies are needed to illustrate the cause. The results of some studies are in contrast with those of ours (9). In Khatami's study, for example, weight gain was reported higher in the control group rather than the group receiving erythropoietin but no reason has been mentioned for this (9).

In this study two infants in CG received 10 ml/kg packed RBCs only once whereas none of the infants in IG received blood. Although difference, was not significant, blood transfusion in preterm infants is accompanied by many complications. The cost-effectiveness of this method should be assessed through further studies and with more cases (in this study the cost per patient was 43.3 in IG vs. $5.9 in CG). Other studies also indicated that erythropoietin stimulates hematopoiesis in the body but it does not inhibit or decrease the need for blood transfusion (18, 20and 21). In another study (9) it was demonstrated that erythropoietin significantly decreased the number of transfusions and contact with the donor in IG.

In this study no difference found between the two groups regarding the morbidity and side-effects. Still for other studies no such morbidity difference were found (9, 20).

Lack of measuring erythropoietin, iron, transferrin saturation percent and some more precise indexes to assess blood status can be taken as the limitations of this study. If these tests could be accomplished, assessment would become more precise. On the other hand, the intervention took four weeks resulting in the impossibility of measuring other parameters related to hematopoiesis and growth which are suggested to be carried out in the further studies.

Conclusion

Early use of human recombinant erythropoietin leads to stimulation of hematopoiesis and weight gain in preterm infants.

Acknowledgement

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Conflict of Interest

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