Journal of Sciences, Islamic Republic of Iran 15(2): 127-130 (2004)
University of Tehran, ISSN 1016-1104

Effects of Recombinant Human Erythropoietin (rHuEpo) on the Blood Cells and Hematopoiesis in Spleen of Rats

L. Momeni,[1,*] A. Vahdati,[1] Sh. Touri,[2] and T. Afrooz[1]

[1] *Division of Biology, Faculty of Science, Isfahan University, Isfahan, Islamic Republic of Iran*
[2] *Division of Pathology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran*

Abstract

Recombinant human erythropoietin is a glycoprotein hormone that stimulates erythropoiesis. In clonogenic assays of hematopoietic progenitors, high concentrations of erythropoietin (Epo) increase CFU-E and diminish the number of granulocytes formed per culture plate. Fetal progenitors are more sensitive to these effects of Epo than progenitors from adults. We administered doses 50, 100, 200 IU/kg of rHuEpo, subcutaneously, twice a week for six weeks. rHuEpo recipients had elevations in RBCs and reductions in WBCs, and platelet count, as well as in spleen ($p<0.05$).

Keywords: Recombinant human erythropoietin; Hematopoiesis; RBC; WBC; Platelet

Introduction

Erythropoietin (Epo) is a glycoprotein hormone that is the primary inducer of erythrocyte formation in mammals [10,11]. Epo is produced in the kidney or liver of adult and the liver of fetal or neonatal mammals. Responsive cells to Epo have been identified in the adult bone marrow, fetal liver or adult spleen [6]. Few Epo receptors (EpoR) are found on early burst-forming unit-erythroid (BFU-E), their number increases and reaches about 1000/cell at the colony-forming unit-erythroid (CFU-E) stage. Receptor expression then decreases with erythroid maturation [9]. In 1977 Miyake *et al.* isolated and purified Epo from human urine. This achievement led to the development of an accurate and easy radioimmunoassay for the measurement of plasma Epo concentrations, replacing the laborious bioassay in polycythemic mice [5]. Furthermore, investigators from two biotechnology firms and genetics institutes, determined the amino acid composition of short sequences of pure Epo. The recent cloning of the human Epo gene and its expression in CHO cells have resulted in to the production of recombinant human erythropoietin (rHuEpo) in 1985 [4,5].

rHuEpo is indicated for the treatment of symptomatic or transfusion requiring anemia associated with chronic renal failure, cancer, chemotherapy and zidovudine-treated HIV infection [9,13]. The purpose of this study was to investigate the changes that may occur in blood cells and hematopoiesis in spleen of rats who have been treated with rHuEpo. We conclude that whether or not rHuEpo treatment at different doses increases the risk of thrombosis or leukopenia in different ages. We believe that our results, as a standard medical practice, may justify the routine administration of rHuEpo to rats in this study.

[*] *E-mail: itslida@yahoo.com*

Materials and Methods

Wistar newborn (50 g), young (100-150 g) and adult rats (250-300 g) were used in this experiment. They were treated with rHuEpo (Eprex: Amgen Corp, Thousand Oaks, CA). Each of the adult and young rats were divided into four groups: one control group and three experimental groups; the newborn group was divided into two groups of experimental and control. The adult and young experimental groups received subcutaneous injections of 50, 100 and 200 IU/kg and the newborn experimental group received 200 IU/kg of rHuEpo twice a week for 6 weeks. Doses of rHuEpo were adjusted weekly according to the changes in body weight. The control groups received only physiological serum. At the end of the injection period, blood was obtained by Stone method of orbital sinus for a complete blood count. The spleens were disrupted by using a knife and fixed. They were cut to sections by Microtom (Leitz 1512, Germany) with 10 µ thickness, processed by Tissue Processing (Shandow, England) followed by Hematoxilin and Eosin staining. Blood was transferred to Autoanalyser H_1 system (Technicon H_1 89, USA) for RBCs, WBCs, and platelets count. H_1 system acts based on photocytometery.

Statistical Methods

Comparison of means were performed using one-way analysis of variance followed by Tukey test and Paired sample. Statistical significance was taken as $p<0.05$.

Results

Effect of rHuEpo Administration on RBCs Count

RBC count was performed in at least 12 groups. The panel (a) of Figure 1 shows the effect of increasing concentrations of rHuEpo on RBC count. There was a dose dependent increase in RBC count (2-12%, $p<0.05$, Table 1).

Effect of rHuEpo Administration on WBCs and Platelet Count

These studies were performed to determine whether rHuEpo results in decreased WBC and platelet generation from the progenitors of normal wistar rats. As shown in Figure 1b,c increasing concentrations of rHuEpo were associated with a decrease in WBC formation, and with diminished platelet generation from hematopoietic progenitors (10%, $p<0.05$, Table 1).

Effect of rHuEpo Administration on Spleen

Splenic sections of control rats showed white and red pulps with many sinousoids. Some sinousoids were dilated and congested (Fig. 2). In rats who had received 50 IU/kg rHuEpo, the most part of white pulp were replaced by red pupls, sinousoids were dilated and severely congested (Fig. 3). In rats who had received 100 IU/kg rHuEpo sinousoids were severely dilated in which vessels were full of red cells (Fig. 4). Splenic section of rats who had received 200 IU/kg rHuEpo showed atrophic white pulps with dilated and congested red pulp (Fig. 5).

Discussion

Recent studies have shown that Epo is crucial for the proliferation and survival of CFU-E and irreversible terminal differentiation [8,9]. Epo acts mainly as a survival factor, allowing both the maintenance of cell proliferation and the induction of expression of erythroid specific protein such as RBC membrane proteins and hemoglobin [9].

There is growing evidence that EPO-responsive precursor cell is already destined to differentiate into erythroid cells and that Epo salvages these dividing cells from programmed death, thereby allowing expansion of red cell production [1,8]. Epo also increases mRNA levels and expression of e-ALAS in RBC, consequently leads to addition hemoglobin [4].

Table 1. Number of blood cells in newborn, young and adult rats in this study

	Age	Control	50 IU/kg	100 IU/kg	200 IU/kg
RBC	Adult	8576±546	9250±488	9350±206	9862±755
(×1000/µL)	Young	8200±435	8000±775	8370±3055	8470±363
	New born	3512±281			4382±616
WBC	Adult	6636±732	6250±1948	5966±539	7626±1139
(No/µL)	Young	7344±2264	7820±401	5696±1051	7208±703
	New Born	2535±379			2752.5±67
PLT	Adult	643±73	601±104	593±112	449±94
(×1000/µL)	Young	610±42	592±107	532±47	478±100
	New born	683±50			506±105

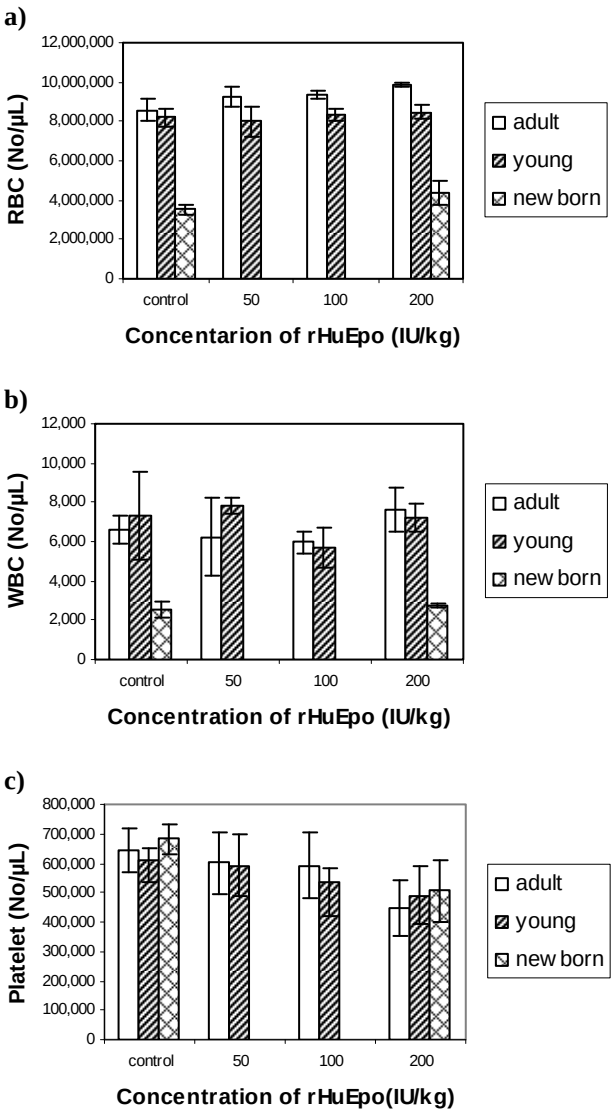

Figure 1. a) Effect of rHuEpo on RBC count in rats; b) Effect of rHuEpo on WBC count in rats; c) Effect of rHuEpo on platelet count in rats.

We proposed that rHuEpo might also have the capacity to influence WBC production. This hypothesis was based, in part, on our observation that newborn rats with high rHuEpo levels, were born with a hyporegerative leukopenia (2752.5±67 No/µL V 2535±379 No/µL). Our observations were consistent with the report of Christensen who noted a step-wise decrease in situ-identified Granulocyte-Macrophage colonies (GM) when Epo concentrations were increased in progenitor cultures from human fetal liver [2]. The mechanism by which Epo reduces WBC production is not clear from these studies.

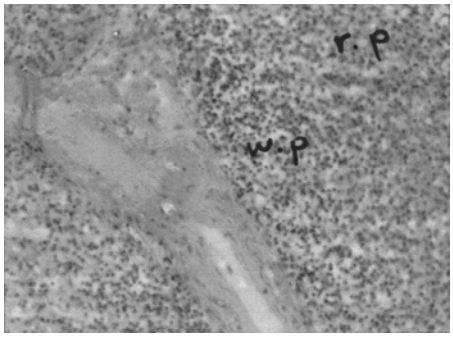

Figure 2. Section of spleen of control rats.

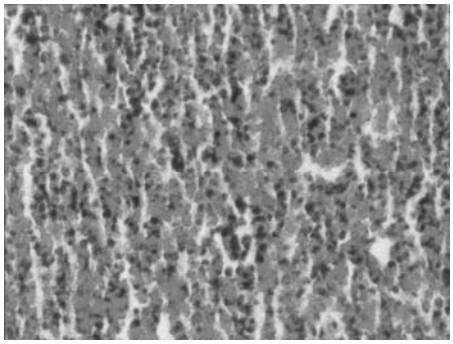

Figure 3. Section of spleen of rats received 50 IU/kg rHuEpo.

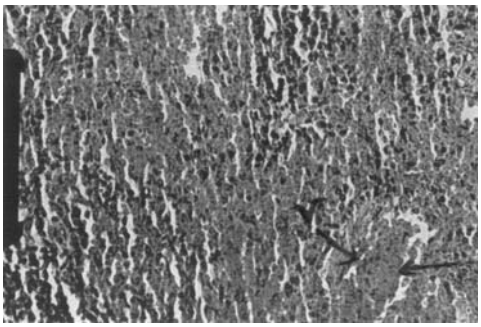

Figure 4. Section of spleen of rats received 100 IU/kg rHuEpo.

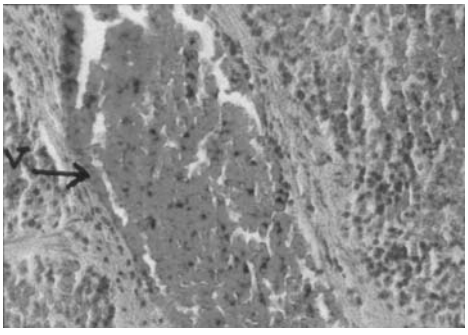

Figure 5. Section of spleen of rats received 200 IU/kg rHuEpo.

Christensen *et al.* and Koenig *et al.* [1] proposed that the involved mechanisms might be direct binding of Epo to receptors on progenitors, resulting in a subsequent down-modulation of receptors for WBC-specific growth factors, [2] induction of an inhibitor(s) of WBC production by cells within the developing clone and [3] combination of the two, [4] reduction of early GM clones into CFU-E or BFU-E colony maturation, [5] complete ablation of some of the GM clones, [6] reduction the number of granulocytes within individual GM colonies [3,7]. Studies by Van Zant and Goldwasser, Ulich *et al.*, and Christensen *et al.* suggest accelerated sufficiently, WBC production will decrease [2,14,15].

The present study has shown that rHuEpo decreases platelet count in rats. The mechanism is unclear. rHuEpo may affects megakaryoblasts and changes to CFU-E and BFU-E. Alternatively, the effect of Epo on platelet generation may not be direct, but Epo may cause thrombocytopoietic inhibitory factor production, leading to decreased platelet generation and reduced megakaryocytic clonies in cultures [4,12].

The maximum and minimum levels of changes were observed in newborn and young rats, respectively. Our reasoning was based, in part, on the observations that progenitors from neonates were more sensitive to down-modulation of WBC production by Epo *in vitro*, than those of adult and young rats [3].

Spleens of rHuEpo recipients contained fewer proliferative WBC than controls. However, Spleens of rHuEpo recipients contained more RBC than controls [3]. Sugiyama et al demonstrated that the upregulation of rHuEpo receptors is due to stimulation of target cell migration by rHuEpo [13]. We hypothesized that such difference in spleens might be due to migration of the target cells expressing Epo receptors (CFU-E) from bone marrow to spleen. rHuEpo treatment also had a similar effect on spleen. BFU-E were migrated to the spleen and differentiated there into CFU-E [13].

We conclude that treatment with different doses of rHuEpo (~200 IU/kg) does not establish the risk of thrombosis but it may render bleeding. Therefore, it is necessary to use proper doses of rHuEpo for different ages. Our results indicate that high doses of rHuEpo (200 IU/kg) in adult and low doses (50 IU/kg) in younger individuals are more effective.

References

1. Bunn H.F. Erythropoietin, current status. *Yale J. Biol. Med.*, **63**: 381-386 (1990).
2. Christensen R.D., Koenig J.M., and Viskochil D.H. Down-Modulation of neutrophil production by erythropoietin in human hematopoietic clones. *Blood*, **74**(2): 817-822 (1989).
3. Christensen R.D., Liechty K.W., and Koenig J.M. Adminisrtration of erythropoietin on newborn rats results in diminished neutrophil production. *Ibid.*, **79**(5): 1241-1246 (1991).
4. Dessypris E.N., Graber S.E., and Krantz S.B. Effects of recombinant erythropoietin on the concentration and cycling status of human hematopoietic progenitor cells in vivo. *Ibid.*, **72**(6): 2060-2062 (1988).
5. Erslev A.J. Erythropoietin. *Drug Therapy*, **324**(19): 1339-1344 (1991).
6. Jacobs K., Shoemaker Ch., Rudersdorf R., and Neill S.D. Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature*, **313**: 806-810 (1985).
7. Koenig J.M. and Christensen R.D. Effects of erythropoietin on granulocytopoiesis: in vitro and in vivo studies in weanling rats. *Ped. Res.*, **27**(6): 583-587 (1990).
8. Koury M.J. and Boundurant M.C. Control of red cell production the role of programmed cell death (apoptosis) and erythropoietin. *Transfusion*, **30**(8): 673-674 (1990).
9. Lacombe C. Erythropoietin: from molecular biology to clinical use. *Europ. Cytok. Network*, **8**(3): 308-310 (1997).
10. Lanes F. and Ceaurriz J.D. Recombinant erythropoietin in urine. *Nature*, **405**: 635-637 (2000).
11. McDonald J.D., Lin, F.K., and Asser E.G. Cloning, Sequencing and Evolutionary Analysis of the Mouse Erythropoietin. *Gene. Mol. Cell. Biol.*, **6**(3): 842-848 (1986).
12. McDonald T.P., Cottrell M.B., and Clift R.E. High doses of recombinant erythropoietin stimulate platelet production in mice. *Exp. Hematol.*, **15**: 719-721 (1987).
13. Sugiyama Y., Kato M., and Kato Y. Machanism of the upregulation of erythropoietin-induced uptake clearance by the spleen. *AJP-Endo. Metab.*, **176**(5): E887-E895 (1999).
14. Ulich T.R., Castillo J., Yin S., and Egrie J.C. The erythropoietic effects of interleukin-6 and erythropoietin in vivo. *Exp. Hematol.*, **19**: 29 (1991).
15. Van Zant G. and Goldwasser E. Competition erythropoietin and colony- stimulating factor for target cells in mouse marrow. *Blood*, **53**: 946 (1977).