

Effects of Recombinant Human Erythropoietin on Hematological Parameters in Newborn, Young and Adult Rats

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Abstract

Recombinant human erythropoietin (rHuEpo) is a glycoprotein hormone that stimulates erythropoiesis. This study was designed to investigate the effects of rHuEpo on red blood cells (RBCs), hemoglobin (Hb), hematocrite (Hc), reticulocytes and creatinine in newborn, young, and adult rats. Three doses of rHuEpo (50, 100 and 200 IU/kg) were administered to animals via subcutaneous injections, twice a week for a period of six weeks. RBCs were counted using H1 autoanalyser. Hb, Hc, reticulocytes and creatinine contents of the blood samples were determined. The data was analysed using one-way ANOVA, Tukey test and paired samples. There were dose dependent increases in RBCs (2-12%), Hb (7-53%), Hc (5-49.6%) and creatinine (11-14%). However, reticulocytes showed a dose dependent decrease down to 2.1-6.3%. The maximum and minimum changes were observed in newborn and young rats. The results suggest that rHuEpo may affect hematopoietic stem cells and modify other progenitor cells to erythroid progenitors. Subsequently, reticulocytes were differentiated to RBCs. The kidney performance to excrete creatinine, was also reduced under the influence of rHuEpo.

Keywords: *Recombinant Human Erythropoietin, RBC, Hemoglobin, Hematocrite, Reticulocyte and Creatinine.*

Introduction

RHuEpo is a glycoprotein hormone that stimulates erythropoiesis (Steinberg *et al.*, 1986). In 1977, for the first time, erythropoietin (Epo) was isolated and purified from the urine of patients who were suffering from anemia (Erslev, 1991; Faruki and Kiss, 1995). Then

later, rHuEpo was produced using biological techniques and genetic methods (Jacobs *et al.*, 1985; McDonald *et al.*, 1986). In human, liver during fetal stage and kidneys after birth are the main sources of Epo production (Lacombe, 1997). Epo receptors are found on progenitors burst forming unit erythroid (BFu-E) and colony forming unit erythroid (CFu-E) (Koury & Boubdurant, 1990; Lacombe, 1997). Reports of the previous observations have indicated that rHuEpo increases RBCs, Hb and Hc (Vaziri & Tang, 2000). The use of Epo has been recommended for the treatment of some types of anemia. The purpose of this study was to investigate the effects of different doses of rHuEpo on RBCs, Hb, Hc, reticulocytes and creatinine in newborn, young and adult rats.

Materials and Methods

Wistar newborn (50g), young (100-150g) and adult (250-300g) rats were purchased and kept under standard conditions of heat and humidity on a twelve-hour light cycle. All animals were fed standard rat chow diet *ad libitum* throughout the experimental period. The young rats were randomly distributed into four groups of five each, including a control group and three treatment groups. The adult rats were also randomly distributed into four groups of five each, including a control group and three treatment groups. The newborn rats were randomly distributed into two groups of five each, including a control group and a treatment group. Either of the three treatment groups of adult rats received 50, 100 or 200 IU/kg rHuEpo (Eprex). Either of the three treatment groups of young rats also received 50, 100 or 200 IU/kg rHuEpo. But, the only one treatment group of newborn rats received 200 IU/kg rHuEpo. Dosing rHuEpo to all treated animals was performed via subcutaneous injections twice a week for a period of six weeks. Doses were modified weekly, according to the body weight alterations. All control rats received placebo physiological serum using same timing and methods just like treated animals. At the end of the dosing period, blood sample were obtained from orbital sinus of all animals using Stone method. RBCs, Hb and Hc contents of the blood sample were determined using an autoanalyser (H1, Technicon, U.S.A.). In order to count reticulocytes, blood samples were stained with brilliant cresyle blue. The blood

serum samples of all animals were used to determine blood creatinine contents using an autoanalyser (RA-1000) and Jaffer method.

Statistical methods

The data was analysed using one-way ANOVA, Tukey test and paired samples. Statistical significance was considered at $P < 0.05$ level.

Results

Increases in RBCs (2-12%), Hb (7-53%) and Hc (5-49%) were observed in all treated groups of animals. The maximum levels of RBCs were observed in animals that received 200 IU/kg rHuEpo ($P < 0.05$, Figure 1). However, the reticulocyte counts indicated dose dependent decreases down to 2.1-6.3%. The lowest level of decrease was observed in animals that received 100 IU/kg rHuEpo ($P < 0.05$, Figure 2). Figure 3 indicates the dose dependent influence of rHuEpo on factors causing enhancement of blood creatinine contents up to 11-14% in all treated groups of young and adult rats, but the differences were not significant. This parameter was not measured in newborn rats due to lack of enough blood to perform creatinine test.

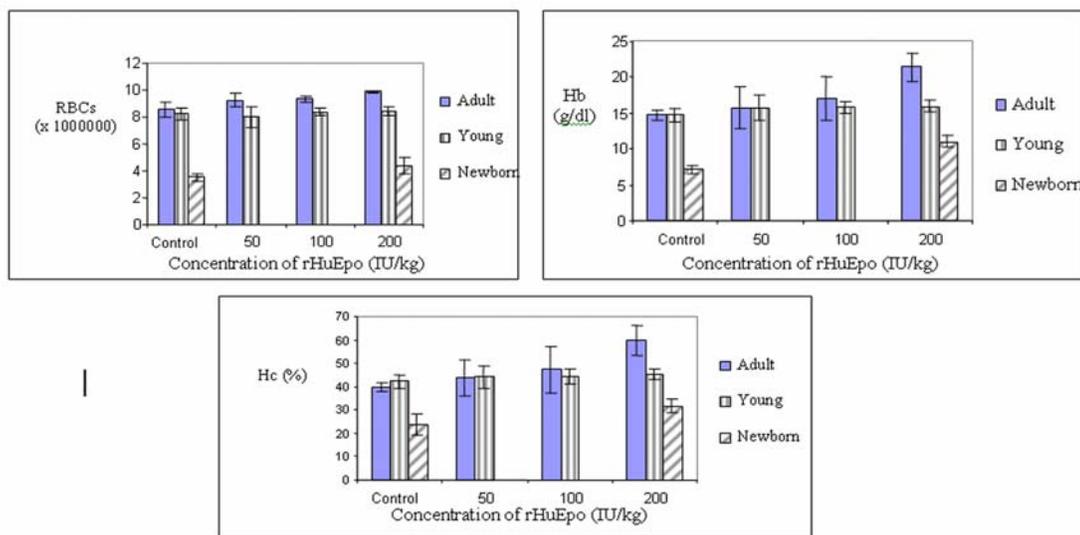


Figure 1. Effects of different doses of rHuEpo on RBC, Hb and Hc in newborn, young and adult rats.

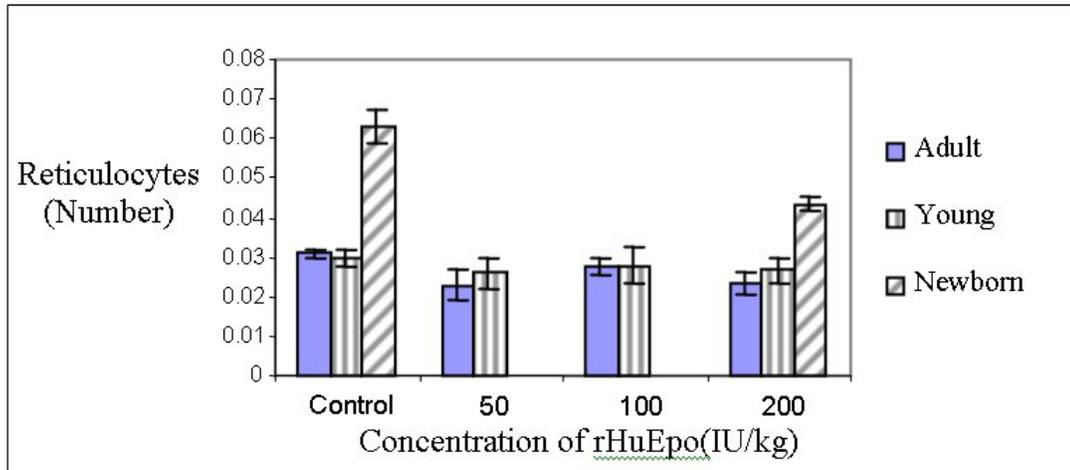


Figure 2. Effects of different doses of rHuEpo on reticulocyte in newborn, young and adult rats.

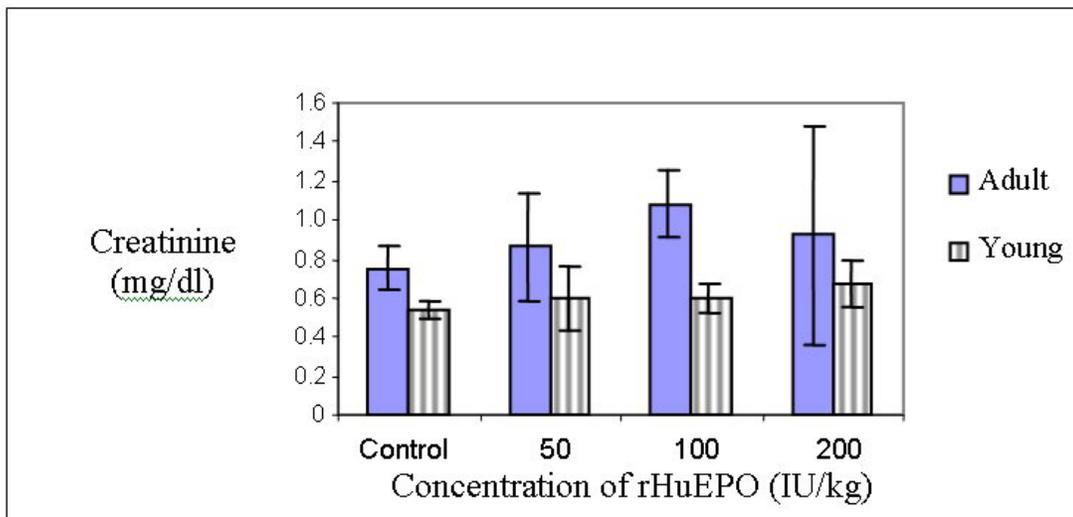


Figure 3. Effects of different doses of rHuEpo on creatinine in young and adult rats.

Discussion

The results of this study indicate a potential dose dependent increase in RBCs, Hb and Hc in all animal groups treated with rHuEpo. Increases in the number of Epo receptors up to about 1000/cell at CFu-E have been reported (Koury & Boubdurant, 1990; Lacombe, 1997). Epo was mainly considered as a survival factor, allowing both the maintenance of cell proliferation and the induction of expression of erythroid specific proteins such as membrane proteins and hemoglobin (Koury & Boubdurant, 1990). Consequently, the RBCs

counts could get increased. RhuEpo has been reported to have an effect on increasing mRNA levels and the expression of e-ALAS in progenitors, due to the existence of hemoglobin precursors compounds such as glycine and succinyl-coA (Zoller *et al.*, 2002). Hc has been also reported to be increased proportional to RBCs counts (Berglund & Ekblom, 1991; Sculco, 1995).

In this study, the maximum levels of alterations in the studied factors were observed in newborn rats due to the fact that sensitivity of erythroid progenitors to rHuEpo in early stage of the life is more compared with the later stages. A decrease in the number of reticulocytes was observed in the animals treated with rHuEpo, but this result is not in accordance with major findings in this regard (Major *et al.*, 1994). Berglund and Ekblom (1991) did not observe any significant increases in the number of reticulocytes after six weeks of treatment with rHuEpo, indicating the process of erythropoiesis performed with a sub-maximal speed and mobilization of reticulocytes. This reflects the effects of low doses of subcutaneous rHuEpo (Major *et al.*, 1994). In this study, a dose dependent increase in blood creatinine content was observed which is in accordance to Berglund's report (1991). Related to this observation, Garcia (1986) has suggested that rHuEpo causes the excretion of creatinine from kidney to be decreased ((Berglund & Ekblom, 1991; Steinberg *et al.*, 1986).

The important point in this study is the side effect of rHuEpo on blood creatinine content and the performance of kidneys. Therefore, it may be necessary to use reasonable doses of rHuEpo during different stages of life. According to the design of this study, high dose of rHuEpo (200 IU/kg) in adult animals and low dose of rHuEpo (50 IU/kg) in young animals are both more effective to be considered as reasonable treatment doses

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