Effect of Human Recombinant Erythropoietin Treatment on Erythrocyte δ -Aminolevulinic Acid Dehydratase in Patients on Maintenance Hemodialysis

V.B.		Djordjević
D.		Pavlović
M.		Pejović
N.		Lečić
D.		Koraćević
V.		Djordjević
V.		Stefanović

Institute of Nephrology and Institute of Biochemistry, Faculty of Medicine, Niš, Serbia

Prof. Dr. Vidosava Djordjević, Biohemijski institut, Medicinski fakultet, B. Taskovića 81, 18000 Niš (Serbia)

Dear Sir,

Anemia is the most common complication in patients maintained on chronic hemodialysis; it occurs in 97% of hemodialyzed patients [1]. The mechanisms underlying this anemia are complex but erythropoietin deficiency seems to be the main cause. Following the cloning of the gene for human erythropoietin, a recombinant human erythropoietin (rHuEPO) has become available for treatment of anemia in end-stage renal disease (ESRD). After the first studies, evidence is presented that renal anemia can be corrected in virtually every patient treated [2,3]. Administration of erythropoietin increases blood hemoglobin and hematocrit, and oxygenation of the tissues is better. The present study was aimed to determine the activity of erythrocyte δ -aminolevulinic acid dehydratase (ALA-D) and the concentration of reduced glutathione (GSH), the major reducing agent in erythrocytes, in patients on maintenance hemodialysis treated with rHuEPO. Both parameters may reflect the redox state of erythrocytes.

In 4 anemic patients on maintenance hemodialysis, rHuEPO was administered intravenously in a dose of 50 U/kg, after dialysis, 3 times weekly during 2 months. Iron and vitamin supplements were also given. ALA-D and GSH were determined before and 1, 2, 4 and 8 weeks after the start of erythropoietin treatment.

ALA-D was determined according to the method of Coleman modified by Mitchell et al. [4], GSH by the method of Ellman modified by Beutler et al. [5]. Hemoglobin was measured by the cyanmethemoglobin method. Results are presented in figure 1.

2,000 -i

1,000 -i

800

600

400

200

2 4

Weeks on EPO

Fig. 1. Erythrocyte hemoglobin, GSH and ALA-D activity during rHuEPO therapy.

©1993 S.Karger AG, Basel

0028-2766/93/

0653-0497\$2.75/0

Blood hemoglobin levels increased from a mean 4.8 ± 0.5 mmol/l (range 3.4-5.8) before erythropoietin treatment to 6.1 ± 0.6 mmol/l (range 5.0-7.9) after 8 weeks of treatment. ALA-D activity was low before rHuEPO treatment, 616 μmol porphobilinogen (PBG)/l blood/h. During the first 2 weeks of treatment, a slight increase in ALA-D activity was observed in 2 patients. Prolonged treatment produced an increase in ALA-D activity to $1,032 \pm 205$ and $1,533 \pm 105$ μmol PBG/l blood/h, after 1 and 2 months of rHuEPO administration, respectively. In 3 of 4 patients, ALA-D activity after rHuEPO treatment was within the normal limits. During the first 2 weeks of treatment, reduced GSH showed a decrease from a mean 759 ± 15 to 552 ± 71 μmol /l blood, and then increased to the mean value of 853 ± 63 μmol /l blood.

Erythrocyte ALA-D is an essential cyto-plasmic enzyme which participates in porphyrin heme biosynthesis by catalyzing the condensation of 2 molecules of ALA to PBG. Very low ALA-D activity was found in ESRD patients on maintenance hemodialysis [6]. This low level was confirmed in the present study. The increase in ALA-D activity could be explained by the increased needs for heme synthesis after rHuEPO challenge. Transitory changes of reduced GSH were observed. Decrease in reduced GSH is probably the result of an increased level of oxygen in the blood. Oxygen consumption produces more free radicals which may engage increased quantities of reduced GSH. After adaptation to the new metabolic state, GSH rose as well as ALA-D activity. The biochemical mechanisms involved in enzyme activity and in the redox state of erythrocytes during erythropoietin treatment have yet to be completely clarified.

References

- Charles G, Lundin AP III, Delano BG, Brown C, Friedman EA: Absence of anemia in maintenance hemodialysis. *Int J Artif Organs* 1981;4: 277-279.
- Winearls CG, Oliver DO, Reid C, Downing MR, Pippard MR, Cotes PM: Effects of human erythropoietin derived from recombinant DNA in the anaemia of patients maintained by chronic haemodialysis. *Lancet* 1986;ii:1175-1177.
- Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW: Correction of anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. *N Engl J Med* 1987;316: 73-78.
- Mitchell RA, Drake JE, Wittlin LA, Rejent TA: Erythrocyte porphobilinogen synthase (δ -aminolevulinate dehydratase) activity, a reliable and quantitative indicator of lead exposure in humans. *Clin Chem* 1977;23:105-111.
- Beutler E, Duron O, Kelly MB: Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-888.
- Djordjevic VB, Strahinjac S, Koracevic D, Miljkovic P, Djordjevic V, Pavlovic D, Stefanovic V: Erythrocyte δ -aminolevulinate dehydratase measurements in Balkan endemic nephropathy. *Kidney Int* 1991;40(suppl 34):S93-S96.