Red Cell Lipid Peroxidation and Antioxidant System in Chronic Renal Failure Patients Treated with Recombinant Human Erythropoietin

S.		Bozfakioglu ^a
N.		Alptekin ^b
Ş.		Seçkin ^b
E.		Ark ^a
N.		Koçak-Toker ^b

^aDepartment of Internal Medicine, Nephrology Section, and ^bDepartment of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

N. Toker, Istanbul Faculty of Medicine, Department of Biochemistry, Çapa 34390 İstanbul (Turkey)

Dear Sir,

Erythropoietin (EPO) deficiency and shortened red cell survival are considered as main factors known to contribute to the pathogenesis of anemia almost invariably observed in chronic renal failure (CRF) [1]. Shortened red cell life span has been attributed to the susceptibility of red cell membrane lipids to autoxidation [2], and to the alteration of the antioxidant system [3]. Indeed, elevated red cell lipid peroxide levels have been observed in CRF [2].

Although the use of recombinant human EPO (rhEPO) for the correction of anemia in patients on regular hemodialysis has been reported to be effective [4], its actions on red cell lipid peroxidation and antioxidant system elements like glutathione peroxidase (GSH Px) and GSH, are not clearly established. In a recent study [5], EPO deficiency due to starvation has been reported to increase red cell lipid peroxidation and depress antioxidant system. Replenishment of EPO has been observed to reverse these effects.

In order to determine the role of correcting anemia with rhEPO on the susceptibility of red cells to lipid peroxidation and on GSH Px activity and GSH levels, 9 patients (5 women, 4 men; age 20-65 years), on maintenance

hemodialysis three times weekly for 1-8 years, were included in the study. The primary renal diseases were: 5 with chronic glomerulonephritis; 2 with chronic pyelonephritis; 1 with primary nephrosclerosis, and 1 with diabetic nephropathy. All patients were anemic, the mean hemoglobin value being 7.0 ± 1.1 g/dl. No other obvious cause for anemia besides uremia was observed. EPO (Cilag AG, International) was applied intravenously at the end of hemodialysis. The starting dose was 50 U/kg body weight three times a week. The target hemoglobin level was 10-12 g/dl. If the desired level was not reached, the rhEPO dose was increased to 75 U/kg body weight after 4 weeks. Finally 100 U/kg body weight was used.

Blood counts were monitored using Technicon H 6000 analyzer. Red cell diene conjugate levels were measured according to Goldstein and Harber [6]. The amount of thiobarbituric acid (TBA) reactive substances was determined by the method of Cynamon et al. [7]. Red cell suspensions in phosphate buffer with sodium azide were incubated with 0.75% H₂O₂ for 1 h at 37 °C. Red cell

GSH content was measured with Ellman's reagent [8]. GSH Px activity in hemodialysates was determined by the assay procedure described by Hafe-man et al. [9]. Enzyme activity was expressed as a decrease in log GSH of 0.001/min/mg hemoglobin after subtracting the decrease in log GSH per minute of the nonenzymatic reaction. Statistical analysis was performed by Student's t test.

Table 1 shows the hemoglobin, hematocrit, red cell diene conjugate, TBA-reactive substance levels, GSH content and GSH Px activity before and 1, 2, 3 and 4 months after treatment with rhEPO. Although hemoglobin and hematocrit values after 1- and 4-months treatment were increased significantly compared to the pretreatment values, elevated pretreatment red cell diene conjugate and TBA-reactive substances were not found decreased after rhEPO treatment. On the other hand GSH levels have been reported to remain unchanged, whereas GSH Px activities were observed to be decreased in CRF [3,10]. In our study, GSH Px activities have not been found to reach normal levels after treatment with rhEPO.

Although the use of rhEPO has been considered very effective in elevating the hemoglobin levels of patients with CRF, a 4-month treatment does not seem to alter red cell lipid peroxide levels and GSH Px activity.

© 1992 S. Karger AG, Basel
0028-2766/92/
0612-0228\$2.75/0

Table 1. Hemoglobin, hematocrit, red cell diene conjugate and TBA-reactive substance levels, GSH content and GSH Px activities in controls and patients before and after treatment with rhEPO (n = 9; mean ± SD)

* p < 0.01 compared to pretreatment values; ** p < 0.001 compared to pretreatment values.

References

Eschbach JW, Adamson JW: Modern aspects of the pathophysiology of renal anemia. *Contr Nephrol*. Basel, Karger, 1988, vol. 66, pp 63-70. Miguel A, Miguel A, Linares M, et al: Evidence of an increased susceptibility to lipid peroxidation in red blood cells of chronic renal failure patients. *Nephron* 1988;50:64-65. El-Rashidy FH, Al-Turk WA, Stohs SJ: Glutathione, glutathione reductase and glutathione S-transferase activities in erythrocytes and lymphocytes in chronic renal disease. *Res Commun Pathol Pharmacol* 1984;44:423-430.

Schaefer RM, Hörn WH, Massry SG: Treatment of renal anemia with recombinant human erythropoietin. *Am J Nephrol* 1989;9:353-362.

Chakraborty M, Ghosal J, Biswas T, et al: Effect of erythropoietin on membrane lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase of rat RBC. *Biochem Med Metab Biol* 1988;40:8-18.

Goldstein BD, Harber LC: Erythropoietic pro-toporphyria: Lipid peroxidation and red cell membrane damage associated with photohemolysis. *J Clin Invest* 1972;51:892-902.

Cynamon HA, Isenberg JN, Nyugen CH: Erythrocyte malondialdehyde release in vitro: A functional measure of vitamin E status. *Clin Chim Acta* 1985;151:169-176.

Beutler E, Duran O, Kelly BK: Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-888.

Hafeman DG, Sunde RA, Hoekstra WG: Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 1974;104:580-587.

10 Koçak-Toker N, Yalçın AS, Uysal M: Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with chronic renal failure. Med Bull Istanbul 1986;19:69-74.
229