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Antioxidant Status and Lipid Peroxidation in Hemodialysis Patients Undergoing Erythropoietin and Erythropoietin–Vitamin E Combined Therapy

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In this study, plasma and red blood cell (RBC) antioxidant status and plasma lipid peroxidation were investigated in 46 hemodialysis patients. In addition, the effect of erythropoietin (EPO) and EPO–vitamin E combination therapy on plasma and RBC antioxidant status, and plasma lipid peroxidation were examined.

There were 10 healthy subjects in the control group and 10 hemodialysis patients in the untreated group. The third group included 36 hemodialysis patients that were given EPO (100 U/kg) for 3 months, 3 times per week. The fourth group included 36 hemodialysis-patients from the EPO group that were given EPO at a 50% decreased dose + vitamin E (300 mg/day) for 3 months.

MDA levels in the untreated group, the EPO group and the EPO + vitamin E groups were found to be higher than the control group ($p < 0.001$, in both). Furthermore, MDA levels in both of the treatment groups were lower when compared to the untreated group ($p < 0.001$, in both). Plasma vitamin E levels in the untreated, the EPO group and EPO + vitamin E groups were lower than the control group ($p < 0.001$). In contrast, plasma vitamin E levels in the treatment groups were higher in comparison with the control group ($p < 0.05$). SOD activities in the untreated, the EPO group and the EPO + vitamin E groups were found to be lower than the control group ($p < 0.001$). SOD activities in the treatment groups were higher than the control group ($p < 0.001$). The SOD activities in the EPO + vitamin E group increased when compared to the EPO group ($p < 0.001$). CAT activities in the untreated, the EPO group and the EPO + vitamin E groups were found to be lower than the control group ($p < 0.001$ in untreated and EPO groups, $p < 0.01$ in EPO + vitamin E group). CAT activities in EPO and EPO + vitamin E groups were increased when compared to the untreated group ($p < 0.01$).

In conclusion, our findings have shown that antioxidant status decreased and lipid peroxidation increased in hemodialysis patients. EPO has an antioxidant effect on the RBC and plasma antioxidant status, and plasma lipid peroxidation. These effects were moderately increased by the combination of vitamin E and EPO.

Keywords: Hemodialysis, erythropoietin, vitamin E, superoxide dismutase, catalase, malondialdehyde

Abbreviations: EPO, Erythropoiętin; MDA, Malondialdehyde; RBC, Red blood cell; SOD, Superoxide dismutase; CAT, Catalase; HMP, Hexose monophosphate; $^{\bullet}OH$, Hydroxyl radical; GP_x, Glutothione peroxidase; H_2O_2, Hydrogen peroxide; $O_2^{\bullet -}$, Superoxide anion

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INTRODUCTION

It has been shown that chronic renal failure (CRF) patients are generally anemic. A reduction in the erythropoietin (EPO) synthesis causes anemia in these patients. In addition to this, the erythrocyte life span in anemic patients is shortened. EPO is highly effective for the treatment of the anemia of chronic renal failure.[1–3]

For most human diseases, the increased formation of reactive oxygen species is secondary to the primary disease process. Free radicals are thought to be involved in various kidney pathologies, including acute renal failure. The deformation of damaged RBC, increased RBC hemolysis and platelet dysfunction may be due to the increased production of free radicals. It has been suggested by previous studies that a defect in the hexose monophosphate (HMP) shunt leads to the production of free radicals such as superoxide anion O_2^-, hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide. In hemodialysis patients, these oxidant agents react with polyunsaturated fatty acids in membranes and form lipid peroxidation products such as malondialdehyde (MDA). Lipid peroxidation by oxygen free radicals (OFR) has been suggested to be an important cause of RBC hemolysis.[4–6]

In the RBC, there are many antioxidant mechanisms which remove reactive oxygen species. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), reduced glutathione (GSH) and vitamin E are the most important intracellular antioxidant substances. The most important plasma antioxidants may be vitamin E, urate and albumin bound bilirubin. Moreover, transferrin and ceruloplasmin are the antioxidant substances carrying transition metals. Vitamin E, which prevents the hemolytic anemia is a lipid-soluble vitamin. Moreover, vitamin E is the most important antioxidant agent protecting polyunsaturated fatty acids against lipid peroxidation.[4,7,8]

In our study, on the chronic hemodialysis patients, we investigated antioxidant status in plasma and RBC, and examined lipid peroxidation. In addition, we investigated the protective role of both recombinant human EPO and EPO + vitamin E against plasma lipid peroxidation. Furthermore, we looked for the effects of the treatment on the erythrocyte antioxidant capacity. For this reason, erythrocyte SOD and CAT activity and plasma MDA and vitamin E levels were measured in hemodialysis patients.

MATERIALS AND METHODS

Subjects

The study included 46 patients (30 females, 16 males, 21–62 years old, mean age was 39) on maintenance hemodialysis for an average of 65 months and also 10 healthy subjects. All patients were dialyzed 3 times weekly, each session lasting 4 h. Cellulosic membranes (cuprophane and cellulose acetate) were used as the dialyzer. Patients with systemic diseases such as diabetes mellitus, liver disorders, alcoholics and heavy smokers were rejected from the study. None of them had received either blood or plasma infusions or drugs which could interfere with the measured parameters. All patients gave informed consent to enter the study. Patients were divided into 4 groups.

Group I (control group): There were 10 healthy subjects (7 females, 3 males, 29–55 years old, mean age was 36.4 ± 7.8) in this group.

Group II (untreated group): There were 10 hemodialysis patients (6 females, 4 males, 21–59 years old, mean age was 33.7 ± 6.9; Hemodialysis time 64.3 ± 4.2 months) in this group.

Group III (EPO treatment group): This group included 36 hemodialysis patients (24 females, 12 males, 21–62 years old, mean age was 40.6 ± 7.2; Hemodialysis time: 66 ± 3.6 months). EPO (100 U/kg) applied subcutaneously 3 times weekly for 3 months.

Group IV (EPO and vitamin E treatment group): Thirty-six hemodialysis patients from group III

were taken. EPO (50 U/kg) and vitamin E (300 mg/day) were administered for 3 months.

Methods

Heparinized arterial blood samples were obtained just before dialysis. Samples were immediately centrifuged at 1500 g for 5 min. Plasma and buffy-coat leukocytes were carefully separated and the erythrocyte pellet was washed 3 times with 0.9% NaCl solution. These pellets were maintained until assay. Hemolysates were prepared from these pellets. SOD activities were measured according to Winterbourn *et al.*[9] and CAT activities were measured by the Beutler method.[10] The results were expressed as U/g Hb. In the separated plasma, vitamin E levels were determined according to the Hashim method and MDA levels were measured according to Okhawa *et al.*[11,12] The results were expressed as mg/dl for vitamin E and nmol/ml MDA.

Statistical Analysis

All results were expressed as the mean ± SEM. The data was analyzed by using one-way variance analysis and Tukey-ω test together.

RESULTS

Enzyme Activities in RBC

RBC SOD and CAT activities are shown in Table I. The measured CAT activities in groups II (953 ± 61.1 U/g Hb), III (1096 ± 29.4 U/g Hb) and IV (1189 ± 31.1 U/g Hb) were decreased significantly when compared to the control group (1494 ± 191.3 U/g Hb) (in groups II and III, $p < 0.001$; and IV, $p < 0.01$). CAT activities in the groups III and IV were found to be lower than group II ($p < 0.001$). The CAT acvities in the combined treatment group were moderately increased as compared to the erythropoietin treatment group. However, this increase was not statistically significant.

TABLE I Erythrocyte SOD and CAT activities in the control subjects and the hemodialysis patients (Mean ± SEM)

	SOD (U/g Hb)	CAT (U/g Hb)
Control ($n = 10$)	3988 ± 161.3	1494 ± 191.3
Untreated group ($n = 10$)	2550 ± 66.3[a]	953 ± 61.1[d]
EPO treatment group ($n = 36$)	2931 ± 38.5[a,b]	1096 ± 29.4[d,f]
EPO + vitamin E group ($n = 36$)	3352 ± 49.0[a,b,c]	1189 ± 31.1[e,f]

[a]As compared with controls; $p < 0.001$.
[b]As compared with untreated group; $p < 0.001$.
[c]As compared with EPO group; $p < 0.001$.
[d]As compared with control; $p < 0.001$.
[e]As compared with controls; $p < 0.01$.
[f]As compared with untreated group; $p < 0.01$.

RBC SOD activities in untreated (2550 ± 66.3 U/g Hb), EPO treatment (2931 ± 38.5 U/g Hb) and EPO and vitamin E treatment (3352 ± 49.0 U/g Hb) groups were decreased significantly as compared to the control group (3988 ± 161.3 U/g Hb) ($p < 0.001$). Erythrocyte SOD activities in both treatment groups were significantly higher than in the untreated group ($p < 0.001$). In addition, SOD activities in combined treatment group were significantly increased when compared to only the EPO treatment group ($p < 0.001$).

Plasma MDA and Vitamin E Results

Plasma vitamin E and MDA levels are shown in Table II. vitamin E levels in groups II (0.901 ± 0.034 mg/dl), III (1.057 ± 0.037 mg/dl) and IV (1.097 ± 0.028 mg/dl) were significantly lower than the control group (1.35 ± 0.04 mg/dl) ($p < 0.001$). Vitamin E levels in both treatment groups were increased when compared to the untreated group ($p < 0.05$).

Plasma MDA levels in groups II (5.24 ± 0.19 nmol/ml), III (4.05 ± 0.052 nmol/ml) and IV (3.81 ± 0.085 nmol/ml) were found to be high in comparison with the control group (2.24 ± 0.06 nmol/ml) ($p < 0.001$). On the contrary, the MDA levels were found to be low when compared to the untreated group ($p < 0.001$).

TABLE II Plasma MDA and vitamin E levels in the control subjects and the hemodialysis patients (mean ± SEM)

	MDA (nmol/ml)	Vitamin E (mg/dl)
Control group ($n = 10$)	2.24 ± 0.06	1.35 ± 0.04
Untreated group ($n = 10$)	5.24 ± 0.19^{a}	0.90 ± 0.03^{c}
EPO treatment group ($n = 36$)	$4.05 \pm 0.05^{a,b}$	$1.06 \pm 004^{c,d}$
EPO + vitamin E group ($n = 36$)	$3.81 \pm 0.09^{a,b}$	$1.10 \pm 0.03^{c,d}$

[a]As compared with control; $p < 0.001$.
[b]As compared with untreated group; $p < 0.001$.
[c]As compared with control group; $p < 0.001$.
[d]As compared with untreated group; $p < 0.05$.

DISCUSSION

Cu–Zn SOD is one of the enzymes that prevents free radical-induced injury within the erythrocyte. This enzyme catalyzes the dismutation of O_2^- into H_2O_2 and molecular oxygen. H_2O_2 is also catabolized by glutathione peroxidase and catalase. Earlier results have shown that SOD enzyme activities were decreased within the erythrocyte in the hemodialysis patients. The decreased SOD activity may cause the reduction in the survival of cells. It has been suggested that decreased SOD activity may be related to deficiency of trace elements seen in hemodialysis patients. SOD enzyme depends on copper and zinc. Therefore, the deficiency of these two ions may contribute to the decrease of SOD enzyme activity. Furthermore, it has been shown that excessive levels of aluminium are found in serum of uremic patients. An inhibition of SOD activity by increased aluminium levels has also been reported. On the other hand, SOD activity might be directly decreased in accordance with the increase of O_2^- and H_2O_2 concentrations in the hemodialysis patients.[2,4,13,14] Hernandez-de-Rojas and Mateo have reported that CAT activity increased in the hemodialysis patients.[15] Rud'ko *et al.* have shown that red cell catalase activities were normal in patients with terminal stage chronic renal failure.[16] However, Turi *et al.* indicated that erythrocyte catalase was low in children with chronic renal failure.[17]

It has been suggested that reactive oxygen species may lead to RBC membrane-lipid oxidation and consumption of RBC tocopherols.[6] Yalçın *et al.* reported that MDA levels were markedly increased in the plasma of hemodialysis patients.[18] Nenov *et al.* reported that marked vitamin E deficiency was established in RBC as well as in plasma.[19] Our results are similar to these previous studies. The increased lipid peroxidation presumably enhances hemolysis seen in the hemodialysis patients by increasing peroxidative injury in the erythrocyte membranes.[5,8,18]

These findings have shown that antioxidant levels decreased and lipid peroxidation increased in the hemodialysis patients. Vanella *et al.* have suggested that a decrease of scavenger systems of activated oxygen species could contribute, in part, to the anemia of chronic renal disease.[2]

Recently, recombinant human EPO has been used in renal anemia treatment. The hematocrit increased by the stimulation of erythropoiesis during EPO treatment leading to regulation in the consumption of oxygen. For this reason, many metabolic functions are affected. Data on the improvement of protein metabolism during recombinant human EPO therapy has already been reported.[1] In our study, we found that SOD and CAT activities were significantly increased with the application of EPO when compared to untreated hemodialysis patients. This increase in the SOD and CAT activities may be explained by the increasing amount of young erythrocytes that depends on EPO circulation in uremic patients. Siems *et al.*[1] reported that during EPO therapy, catalytic activity of aspartate aminotransferase increases in the young erythrocytes, reaching a maximum one week after the start of therapy. Besides, Melissinos *et al.*[20] reported that the increased glutathione reductase activity in the uremic patients could be attributed to an increased proportion of circulating young erythrocytes.

In our study, we found that the administration of EPO increased plasma vitamin E levels significantly as compared to untreated hemodialysis

patients. In addition, we have established a decrease in lipid peroxidation. EPO is related to iron metabolism. This element has a role in the Fenton reaction where •OH is formed from H_2O_2. The response to EPO requires adequate stores of iron, since the rate of cell production correlates with the supply of iron to the erythroid marrow. Serum iron levels in the hemodialysis patients have been found to decrease after EPO treatment for 12 months.[3,7,16] Mohammed-Bany *et al.* have hypothesized that administration of EPO mobilizes iron from plasma and inhibits iron-catalysed reactions.[21] In our study, the decrease of MDA levels and the increase of vitamin E levels after EPO therapy during 3 months may be attributed to the effects of EPO. According to our findings, we suggest that EPO therapy has a possible antioxidant effect in hemodialysis patients.

It has been shown that the parenteral application of alpha-tocopherol acetate caused a marked decrease in lipid peroxidation in erythrocyte membranes.[5,8,18] In our study, SOD enzymes were increased markedly by vitamin E treatment given with a 50% decreased dose of EPO to hemodialysis patients as compared to EPO treatment. This situation has also been observed in the CAT, vitamin E and MDA levels moderately. Our findings have shown that antioxidant efficiency of EPO increased with vitamin E therapy. In our previous study, vitamin E therapy (200 mg/day) for 10 months increased SOD and CAT activity and decreased MDA levels as compared to untreated hemodialysis patients.[22] Therefore, we suggest that the effect of EPO + vitamin E combined therapy may be additive. Hemodialysis time was not changed among the 4 groups. But, hemoglobin and hematocrit levels increased in the EPO and EPO + vitamin E therapy groups according to untreated hemodialysis patients. Furthermore, the blood requirement was decreased by EPO and EPO + vitamin E as compared to untreated group.

In conclusion, our findings have shown that chronic renal failure causes oxidative stress as indicated by decreased antioxidants and enhanced lipid peroxidation. The application of vitamin E increased this effect moderately by decreasing the EPO dose in the following treatment. This finding is important and further studies are needed related to the EPO and vitamin E dose.

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