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Superoxide Dismutase and Glutathione Peroxidase in Erythrocytes of Patients with Iron Deficiency Anemia: Effects of Different Treatment Modalities

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Aim. To test whether the activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) can be affected by oral iron (OI) treatment, parenteral iron (PI) treatment, and parenteral iron treatment with vitamin E supplementation (PIE) in iron deficiency anemia.

Methods. Twenty-eight patients with iron deficiency anemia and 18 healthy controls were included in the study. Anemic patients were systematically allocated into 3 treatment groups. The first group (n = 8) received OI, the second group (n = 10) PI, and the third group (n = 10) PIE. SOD and GSH-Px activities were determined using commercial kits.

Results. Before the treatment, SOD activity was significantly lower in anemic patients than in the control group (Kruskal-Wallis test, $p < 0.05$). After the treatment, SOD activity significantly increased in all three patient groups and reached the values found in the control group (Wicoxon signed-rank test, $p = 0.017$ for OI, $p = 0.047$ for PI, and $p = 0.037$ for PIE group). Before the treatment, GSH-Px activities in anemic patients were similar to that of control group (Kruskal-Wallis test, $p > 0.05$). Although there was no significant decrease in GSH-Px activity after OI treatment, both PI and PIE treatments significantly decreased GSH-Px activity (Wilcoxon signed-ranks test, $p = 0.007$ for PI and $p = 0.005$ for PIE). PIE was more effective than PI treatment in maintaining GSH-PX activity.

Conclusion. Oral iron treatment improved the iron deficiency anemia and recovered antioxidant defense system by increasing SOD activity and maintaining GSH-Px activity at normal level. When parenteral iron treatment is inevitable, vitamin E supplementation together with PI treatment may be effective in partially restoring the antioxidant status in this type of anemia.

Key words: *anemia, iron deficiency; glutathione peroxidase; iron; superoxide dismutase; vitamin E*

Reduction in the serum iron concentration causes insufficient hemoglobin synthesis, with a subsequent reduction in erythrocyte proliferation. Furthermore, reduced red cell survival may be present in patients with iron deficiency anemia (1). Iron deficiency does not only affect the production of hemoglobin, but also the production of other proteins containing Fe^{2+}, such as cytochromes, myoglobin, catalase, and peroxidase. Impairment of the antioxidant defense system and reduced cellular immunity and myeloperoxidase activity were previously reported in patients with iron deficiency anemia (2,3). All of these may contribute to inadequate erythrocyte survival. The literature offers contradictory and limited data on oxidative stress and antioxidant defense in patients with iron deficiency anemia (2,4-7). Also, alterations in antioxidant status by iron treatment in iron deficiency anemia have not been clarified.

The aim of this study was to determine erythrocyte antioxidant enzyme activities in patients with iron deficiency anemia and alterations of enzyme activities after the treatment with oral iron, parenteral iron, or parental iron plus vitamin E supplementation. To our knowledge, the effects of different therapeutic modalities on antioxidant enzymes in iron deficiency anemia have not yet been investigated.

Subjects and Methods

Setting

The study was conducted at the Departments of Internal Medicine and Biochemistry and Clinical Biochemistry, Suleyman Demirel University, from November 1997 to July 1998. The research was performed according to the Helsinki Declaration principles.

Study Criteria

Description criteria for iron deficiency anemia are microcytic hypochromic erythrocytes; with a mean corpuscular volume < 80 fL, hemoglobin concentration < 12 g/dL, serum iron concentration < 45 g/dL, and serum ferritin concentrations for women and men < 15 ng/mL and < 25 ng/mL, respectively. Causes of anemia in the patients were chronic gastrointestinal blood loss or hypermenorrhea. Exclusion criteria were acute

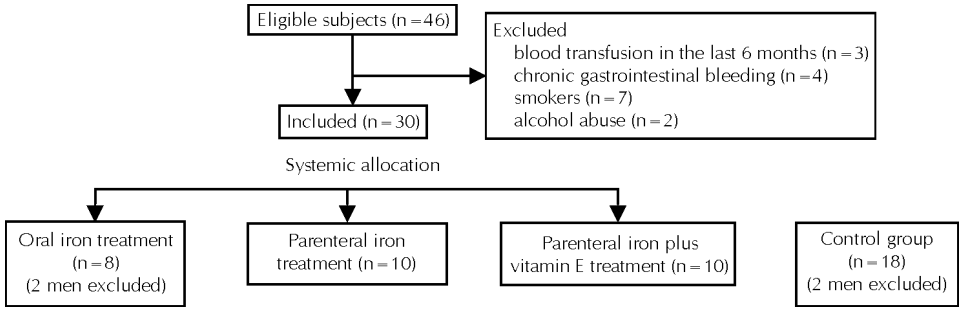

Figure 1. Profile of the study.

bleeding and history of blood transfusion within 6 months before the study (Fig. 1). Anemic patients who needed blood transfusion were also excluded. None of the subjects smoked nor drank alcohol or vitamin pills, and all had normal routine biochemical tests.

Subjects

Thirty patients were systematically allocated to the 3 treatment groups (Fig. 1). The first group of patients received oral iron (567.7 mg bid ferroglycine sulphate; Ferro-Sanol® duodenal capsule, Adeka, Istanbul, Turkey). The second patient group received parenteral iron (ferro III hydroxide polymaltose; 200 mg IM, every other day; Ferrum Hausmann® ampule, Abdi Ibrahim, Istanbul, Turkey). The third group received parenteral iron plus oral vitamin E (Ephynal® capsule, Roche, Istanbul, Turkey, 300 mg/day orally). Patients were chosen and systematically allocated to the treatment groups as follows: the first patient who visited the clinic during the study period and fulfilled the study inclusion criteria was allocated to the oral iron treatment group; the next eligible patient was allocated to the parenteral iron treatment group, and the next one to the parenteral iron plus vitamin E treatment group. Patients were allocated in this order (oral iron, parenteral iron, and parenteral iron plus vitamin E treatments) until all treatment groups had 10 subjects.

The control group consisted of 20 healthy subjects, members of the hospital staff, who had to fulfill the following criteria: have hemoglobin >12.5 g/dL and normochromic-normocytic erythrocytes, not to smoke, use alcohol, or drugs. In addition, routine biochemical parameters of selected control subjects had to be in the normal range.

Two men from the oral iron treatment group and 2 from the control group were excluded from the analysis since two men scheduled for the treatment were by chance allocated to the same group.

Oral iron treatment period lasted up to 6 months, whereas parenteral iron and parenteral iron plus vitamin E treatment lasted up to a month. The oral iron treatment group received the treatment for at least 4 months; at the end of that period, the subjects having hemoglobin concentration (Hb) below 12 g/dL continued with the same treatment for another 2 months (1).

Parenteral iron requirement was calculated by the following formula:

Iron requirement = $(Hb_{normal} - Hb_{patients})$ body weight 3.

Before the therapy, blood samples were taken from all the subjects for routine hematological and biochemical analyses to determine serum iron, iron binding capacity (SIBC), ferritin, and activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). All measurements were repeated at the end of each treatment period.

Assays

Subjects' venous blood samples were obtained from median cubital vein between 8 and 9 a.m. after overnight fasting, and collected into polystyrene tubes and standard tubes containing ethylene diamine tetraacetic acid (EDTA). Blood serum was immediately separated by centrifugation (4,000 G, for 10 min) to determine iron level, serum iron binding capacity (SIBC), and ferritin level. Blood samples in standard EDTA-containing tubes were used for hematological assays and determination of the activities of SOD and GSH-Px. Hemolyzate was prepared immediately and stored at -20 C until analysis. SOD and GSH-Px activities were measured with Ransod and Ransel kits (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) and expressed as U/g Hb. Serum iron and SIBC were determined with commercially available kits on Vitros 750 analyzer (Johnson&Johnson, Clinical Diagnostic, UK). Serum ferritin concentration was determined with chemiluminescence method on an ACS-180 analyzer (Chiron Diagnostic, East Walpole, MA, USA).

Statistical Analysis

Data were expressed as median and range (minimum and maximum). Kruskal-Wallis test was used to determine the significant effects of different treatments on the experimental variables. For double comparison, Bonferroni-adjusted Mann-Whitney U-test was performed. Before- and after-treatment changes were compared by Wilcoxon signed-ranks test. Correlation analysis was performed by Pearson correlation test. Significant level was set at $p<0.05$. All statistical analyses were done with the SPSS for Windows 7.0 statistical package.

Results

No subject was lost during the course of the study. The median age of subjects was 32 years (range, 20-55) in the of oral iron treatment group, 37 years (range, 18-48) in the parenteral iron treatment group, 40 years (range, 20-49) in the parenteral iron plus vitamin E treatment group, and 35 years (range, 20-54) in the control group. There was no significant difference in age ($p=0.564$) among the groups. After the treatments, Hb concentrations increased from 9.85 (7.2-11.0) g/dL to 12.2 (11.2-14.3) g/dL in patients treated with oral iron, from 9.3 (8.0-11.8) g/dL to 14.1 (10.9-16.4) g/dL in patients treated with parenteral iron, and from 9.35 (7.7-10.1) g/dL to 12.2 (11.0-13.2) g/dL in patients treated with parenteral iron plus vitamin E ($p<0.001$ for all groups).

After the treatment, serum iron and ferritin concentrations increased, whereas SIBC decreased in all treatment groups (data not shown).

Before the therapy, SOD activities were not significantly different among the oral iron, parenteral iron, and parenteral iron plus vitamin E treatment groups, but they differed significantly from the SOD activity values found in the control group (Table 1). SOD activity in the oral iron, parenteral iron, and parenteral iron plus vitamin E treatment groups was significantly lower than that in control subjects (Table 1). After the treatment, SOD activities in all treatment groups significantly increased (Table 1). SOD activities in all treatment groups reached the level of SOD activity in the control group at the end of the treatment periods and no significant differences were observed among the groups ($p>0.05$).

Table 1. The effect of different treatment modalities on the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in patients with iron deficiency anemia

Enzyme activities (U/gHb)		Iron treatment modality (median, range)				
		oral (n = 8)	parenteral (n = 10)	parenteral + oral vitamin E (n = 10)	control (n = 18)	p[a]
SOD	before treatment	1,193 (666-2,206)	676 (312-3,571)	1,250 (549-4,630)	3,170 (2,730-3,620)[c,d,e]	< 0.001
	p[b]	0.017	0.047	0.037		
	after treatment	3,062 (630-5,172)	2,598 (634-7,798)	2,796 (1,000-5,833)	3,170 (2,730-3,620)	0.698
GSH-Px	before treatment	35.4 (26.1-50.4)	36.5 (28.3-56.9)	38.6 (33.0-48.4)	36.9 (27.3-47.8)	0.552
	p[b]	0.263	0.007	0.005		
	after treatment	31.8 (28.8-36.9)	19.4 (11.9-33.3)[f]	28.9 (23.0-35.0)	36.9 (27.3-47.8)[d,e]	< 0.001

[a]Significance of differences among enzyme activities of four groups in the same line; Kruskal Wallis test.
[b]Significance of differences between enzyme activities of the same groups before and after the treatment; Wilcoxon signed ranks test.
[c-f]Significant differences between the groups ($p < 0.05$, Bonferroni-adjusted Mann-Whitney U-test): [c]control vs oral iron treatment group; [d]control vs parenteral iron treatment group; [e]control vs parenteral iron plus vitamin E treatment group; and [f]oral vs parenteral iron treatment group.

There were no differences in GSH-Px activity among the groups before the treatment period, but afterwards the differences were significant (Table 1). In comparison with control group, GSH-Px activities in patients treated with parenteral iron and parenteral iron plus vitamin E decreased after the treatment (Table 1). GSH-Px activity in the patients treated with parenteral iron was lower than in the patients treated with oral iron and in the control group (Table 1). Iron treatment caused a decrease in GSH-Px activities in groups treated with parenteral iron and parenteral iron plus vitamin E (Table 1).

No correlations were found between serum iron concentration and SOD activity or between serum iron concentration and GSH-Px activity.

Discussion

Erythrocytes are equipped with a highly effective antioxidant defense system. In comparison with other cell types, they possess highly active antioxidant enzymes, such as SOD and GSH-Px. However, it is reported that antioxidant capacity of erythrocytes is reduced in iron deficiency anemia (4,6-10). In our study, low SOD activity in anemic patients significantly increased after every treatment modality. GSH-Px activities in anemic patients were similar to that of the controls, but significantly decreased after parenteral iron and parenteral iron plus vitamin E treatments.

The causes of increased oxidative stress and decreased antioxidant defense in iron deficiency anemia have not been completely explained, although a significant increase in lipid peroxidation has been found (6,8,10). In addition to decreased antioxidant defense system activity and increased lipid peroxidation in erythrocytes of patients with iron deficiency anemia, Kumerova et al (6) found that pentose phosphate pathway activity was also reduced in iron deficiency anemia. Bartal et al (10) reported that iron deficiency anemia erythrocytes were more susceptible to oxidation but had good capacity for recovery. Some researchers observed increased SOD activity in patients with iron deficiency anemia (2,4,5). Jansson et al (4) suggested that increased SOD formation was a compensatory factor for increased oxidant stress. Other research (6,7) showed decreased activities of antioxidant enzymes, such as SOD, GSH-Px, and catalase, in patients with iron deficiency anemia. In the present study, SOD activity in anemic patients was lower than that of control group, which might be caused by insufficient nutrition and oxidative stress under hypoxic condition. It is well known that reactive oxygen species, especially hydrogen peroxide, inhibit SOD activity (11). Furthermore, decreased SOD activity may contribute to free radical production.

Yetgin et al (12) found that selenium concentration was significantly lower in iron deficiency anemia. GSH-Px is a selenium-dependent enzyme and may be affected by selenium deficiency. Significant correlation was reported between serum iron, selenium concentration, and GSH-Px activity (7,13). Sevgi et al (14) observed decreased neutrophil GSH-Px activity in iron deficient anemic children. Cellerino et al (7) observed decreased GSH-Px activity in patients with iron deficiency anemia, and suggested that iron could be of crucial importance for erythrocyte GSH-Px activity in thalassemia and sideroblastic anemia. However, Acharya et al (2) showed that GSH-Px activity in anemia was similar to that of normal cells. We also found similar GSH-Px activities in the anemic and control groups. GSH-Px activity in our study was in accordance with the results of Acharya et al (2), and in contrast to the results of many others mentioned above. No correlations were observed between iron concentration and GSH-Px activity in our study. Therefore, we can speculate that iron is not the only cause of changes in GSH-Px and SOD activity in patients with iron deficiency anemia. Other minerals, such as Cu, Zn, Mn, and Se, which mediate the activities of these enzymes, may also play important roles in the alteration of enzyme activity. It has been reported that the activities of pentose phosphate pathway enzymes, the major source of the NADPH production, were increased in patients with iron deficiency anemia (15). Since GSH-Px activity depends on NADPH levels produced by pentose phosphate pathway, this may explain why the GSH-Px activity was within the normal level in patients with iron deficiency anemia, as opposed to significantly low SOD activity in comparison with healthy subjects.

In the present study, SOD activity increased to normal levels in all anemic groups after the treatment, and there was no difference among the experimental groups. On the other hand, GSH-Px activity signifi-

cantly decreased after parenteral iron and parenteral iron plus vitamin E treatments, as compared with the GSH-Px activity values before the treatments and GSH-Px activity of control group. We can, therefore, speculate that parenteral iron treatment failed to restore the antioxidant system, at least in the early period of the treatment. Iron treatment, especially via parenteral route, may give rise to increased free iron concentration in cells, which may induce free radical production. After parenteral iron administration, absorption may last for at least four weeks; hence free-radical formation may be prolonged (1). It is clear that iron can cause hydroxyl radical production via the Fenton and Haber-Weiss reactions and promote lipid peroxidation (16), which is why some investigators recommend the avoidance of iron overuse (17).

In the present study, we also tested whether vitamin E supplementation to parenteral iron affects erythrocyte antioxidant enzyme status. It is known that vitamin E is a strong antioxidant (18) and therefore can be expected to contribute to the normalization of impaired antioxidant enzyme status in erythrocytes. We found that vitamin E supplementation to parenteral iron therapy did not additionally increase SOD activity, but did increase GSH-Px activity, as compared with parenteral iron only treatment. However, this increase was not insufficient to normalize GSH-Px activity. This clearly indicated that administration of vitamin E together with parenteral iron might provide some benefit in restoring the antioxidant enzyme status.

In conclusion, iron replacement by either oral or parenteral route stimulates SOD activity in patients with iron deficiency anemia. However, parenteral iron treatment, unlike oral iron treatment, reduces GSH-Px activity below the normal level found before the treatment. Thus, oral iron treatment seems to be better vehicle than parenteral iron treatment because it restores the antioxidant defense system in patients with iron deficiency anemia. When parenteral iron treatment is inevitable, vitamin E supplementation can be relatively effective in restoring the antioxidant status.

The limitations of our study were the limited number of subjects, their non-random allocation, and the fact that only women were included in the analysis. Although our study showed that oral iron treatment should be preferred in the treatment of iron deficiency anemia because of its beneficial effect on antioxidant system, larger randomized studies are needed to confirm our findings.

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References

1 Lee GR. Iron deficiency and iron deficiency anemia. In: Pine JW, editor. Wintrobes clinical hematology. Middle East edition. Giza (Egypt): Williams and Wilkins; 1999. p. 979-1004.

2 Acharya J, Punchard NA, Taylor JA, Thompson RP, Pearson TC. Red cell lipid peroxidation and antioxidant enzymes in iron deficiency. Eur J Haematol 1991;47: 287-91.

3 Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron deficiency anemia. N Engl J Med 1993;329:1691-5.

4 Jansson LT, Perkkio MV, Willis WT, Refino CJ, Dallman PR. Red cell superoxide dismutase is increased in iron deficiency anemia. Acta Haematol 1985;74:218-21.

5 Panchenko LF, Lamchingiin T, Gerasimov AM, Sukhanov IS, Konoplina LA. Superoxide dismutase activity in the blood of children with iron deficiency anemia [in Russian]. Vopr Med Khim 1979;25:181-5.

6 Kumerova A, Lece A, Skesters A, Silova A, Petuhovs V. Anaemia and antioxidant defence of the red blood cells. Mater Med Pol 1998;30:12-5.

7 Cellerino R, Guidi G, Perona G. Plasma iron and erythrocytic glutathione peroxidase activity. A possible mechanism for oxidative haemolysis in iron deficiency anemia. Scand J Haematol 1976;17:111-6.

8 Vives Corrons JL, Miguel-Garcia A, Pujades MA, Miguel-Sosa A, Cambiazzo S, Linares M, et al. Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. Eur J Haematol 1995;55:327-31.

9 Macdougall LG. Red cell metabolism in iron deficiency anemia. 3. The relationship between glutathione peroxidase, catalase, serum vitamin E and susceptibility of iron-deficient cell to oxidative hemolysis. J Pediatr 1972;80:775-82.

10 Bartal M, Mazor D, Dvilansky A, Meyerstein N. Iron deficiency anemia: recovery from in vitro oxidative stress. Acta Haematol 1993;90:94-8.

11 Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. Biochemistry 1975; 14:5294-9.

12 Yetgin S, Hincal F, Basaran N, Ciliv G. Serum selenium status in children with iron deficiency anemia. Acta Haematol 1992;88:185-8.

13 Perona G, Cellerino R, Guidi GC, Moschini G, Stievano BM, Tregnaghi C. Erythrocytic glutathione peroxidase: its relationship to plasma selenium in man. Scand J Haematol 1977;19:116-20.

14 Sevgi Y, Gonenc C, Cigdem A. Neutrophil glutathione peroxidase activity in iron deficiency anaemia. Scand J Haematol 1986;36:58-60.

15 Macdougall LG. Red cell metabolism in iron deficiency anemia. J Pediatr 1968;72:303-18.

16 Gutteridge JM. Biological origin of free radicals and mechanism of antioxidant protection. Chemico-Biological Interaction 1994;91:133-40.

17 Reizenstein P. Iron, free radicals and cancer. Med Oncol Tumor Pharmacother 1991;8:229-33.

18 Fritsma GA. Vitamin E and autoxidation. Am J Med Technol 1983;49:453-6.

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