Improved erythrocyte survival with combined vitamin E and selenium therapy in children with glucose-6-phosphate dehydrogenase deficiency and mild chronic hemolysis

To study the antioxidant effect of high-dose vitamin E alone and in combination with selenium in patients with glucose-6-phosphate dehydrogenase deficiency with mild chronic hemolysis, 36 male children with such manifestations were enrolled consecutively into two equal groups. Group 1 received 800 IU vitamin E daily, and group 2 received 800 IU vitamin E in combination with 25 μg selenium. Hematologic status before and 2 months after treatment was evaluated. After treatment there was a significant change toward normal in both groups. The mean red cell half-life increased in group 1 from 16.9 to 22.8 days ($P < 0.01$), and in group 2 from 15.6 to 24.3 days ($P < 0.01$). A comparison of the mean difference of paired values in the two groups revealed a more significant increase in hemoglobin (0.9 ± 0.1 gm/dl vs 1.2 ± 0.2 gm/dl, $P < 0.05$), hematocrit (2.4% ± 0.4% vs 3.8% ± 0.3%, $P < 0.05$), and red cell half-life (5.9 ± 3.0 days vs 9.1 ± 4.4 days, $P < 0.01$), and more significant reduction in reticulocytes (−0.7% ± 0.2% vs −1.5% ± 0.4%, $P < 0.01$) in group 2. Clinical assessment and follow-up indicated no side effects related to the drugs. (J PEDIATR 1986;108:558-561)

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Vitamin E (α-tocopherol) can act as an antioxidant against the attack of molecular oxygen on polyunsaturated fatty acids by providing protons to free radicals, and thus protect cells from lysis induced by oxidant stress.[1-3] Leonard,[4] Dallman[5] and Farrell et al.[6] observed that vitamin E deficiency can contribute to the extent of red cell susceptibility to oxidative stress and lead to shortened red cell survival.

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Although vitamin E is the major biologic antioxidant,[7] endogenous antioxidant protection is provided by a number of other dietary components, including selenium, which is at the active site of glutathione peroxidase enzyme; the latter is important in the metabolism of erythrocytes through utilization of peroxides to reoxidize reduced glutathione.[8]

G-6-PD	Glucose-6-phosphate dehydrogenase

The anemia of glucose-6-phosphate dehydrogenase deficiency is a common genetic disorder. Mediterranean-type G-6-PD deficiency, commonly observed in Egypt, is char-

Table I. Effect of therapy on hematologic status

	Control (n = 14)	Group 1 (n = 18) Before treatment	Group 1 (n = 18) After treatment	P	Group 2 (n = 18) Before treatment	Group 2 (n = 18) After treatment	P
Hemoglobin (gm/dl)							
Mean	12.1 ± 0.88	9.0 ± 0.35	9.9 ± 0.41	<0.05	8.6 ± 0.41	9.8 ± 0.52	<0.01
Range	11.7-13.8	8.2-9.9	9.0-10.6		7.9-9.6	8.4-10.8	
Packed cell volume (%)							
Mean	38.1 ± 1.43	28.1 ± 1.26	30.5 ± 1.32	<0.05	26.4 ± 1.24	30.2 ± 1.41	<0.05
Range	36-42	25-31	27-34		24-30	27-33	
Reticulocytic count (%)							
Mean	0.71 ± 0.18	3.0 ± 0.16	2.3 ± 0.18	<0.05	2.8 ± 0.13	1.3 ± 0.12	<0.01
Range	0.3-1.2	2.2-4.2	1.4-2.9		2.2-3.6	0.6-2.1	
Haptoglobin (mg/day/HBC)							
Mean	196.3 ± 8.42	79.2 ± 9.16	106.7 ± 8.24	<0.01	81.9 ± 8.45	114.7 ± 7.63	<0.01
Range	164-212	38-138	0 69-144		38-130	89-196	
Red cell half-life (day)							
Mean	28.0 ± 0.72	16.9 ± 1.80	22.8 ± 4.58	<0.01	15.6 ± 1.93	24.3 ± 4.78	<0.01
Range	27.2-31.4	13.2-19.1	17-28		12.4-18.0	16-28	
Serum vitamin E (mg/dl)							
Mean	0.95 ± 0.06	0.50 ± 0.04	1.15 ± 0.13	<0.001	0.53 ± 0.03	1.36 ± 0.07	<0.001
Range	0.62-1.38	0.25-0.77	0.68-1.94		0.38-0.74	0.92-1.88	

Values represent mean ± SEM.

acterized by decreased cellular reductive capacity and is associated with mild chronic hemolysis.[9, 10] Four previous studies have been done regarding the effect of vitamin E on chronic hemolysis in patients with Gd^{Md}. Two of these studies indicated that high doses of vitamin E reduced the rate of hemolysis,[11, 12] whereas no change was observed in the other two.[13, 14]

We studied the antioxidant effect of vitamin E alone and in combination with selenium in patients with G-6-PD deficiency with mild chronic hemolysis.

METHODS

The study included 36 male children, ranging in age from 3 to 12 years. All had had a previous acute hemolytic crisis that required blood transfusion; 31 had a history of hemolysis associated with fava bean ingestion, and seven had a history of drug-induced hemolysis. Patients included in the study had chronic anemia with no identifiable cause, G-6-PD activity <1% of normal, and no complicating medical problems, and were receiving no medications. They had no history of acute hemolytic episodes or blood transfusion for at least 2 months. Patients in whom β-thalassemia trait was diagnosed on the basis of reduced red cell mean corpuscular volume and elevated A2 level were excluded from the study.

Patients were enrolled consecutively into two groups, each comprising 18 children. They received no medication except as follows, for 60 days: group 1, vitamin E 800 IU/day orally, in divided doses, four times daily; group 2, vitamin E 800 IU/day and selenium 25 μg/day. Selenium tablets (obtained from Gray Faur, Cleveland) contain a natural special yeast (not added to or fortified with) to which the Se is organically bound.

We decided to give this high dose of vitamin E because some workers found it to be effective as an antioxidant.[11] The 25 μg Se is the physiologic dose.[2, 7]

Fourteen sex- and age-matched healthy children from the same locale served as controls.

Laboratory investigations. Hemoglobin level, hematocrit, and red blood cell and reticulocyte counts were determined with standard laboratory procedures. Haptoglobin was determined with immunodiffusion plates[15] (Norpartigen, Behringwerke AG, Marburg, West Germany). Estimation of vitamin E in serum was carried out according to the method of Hashim and Schuttringer.[16]

Determination of red cell survival was carried out as follows.[17] Ten milliliters of venous blood was placed in a tube containing acid citrate dextrose. Then 30 μCi sodium chromate (^{51}Cr, obtained from Radiochemical Centre, Amersham, England) was added to the blood to reduce the unbound ^{51}Cr to chromic salt and prevent further tagging. The red cells were washed, suspended in saline solution, and reinjected into the patient. At 24 hours a blood sample was counted in a scintillation counter and designated as 100% sample. Serial blood samples were then obtained at 5 and 10 days. The activity per milliliter of blood at these intervals was compared with that of the original 24-hour

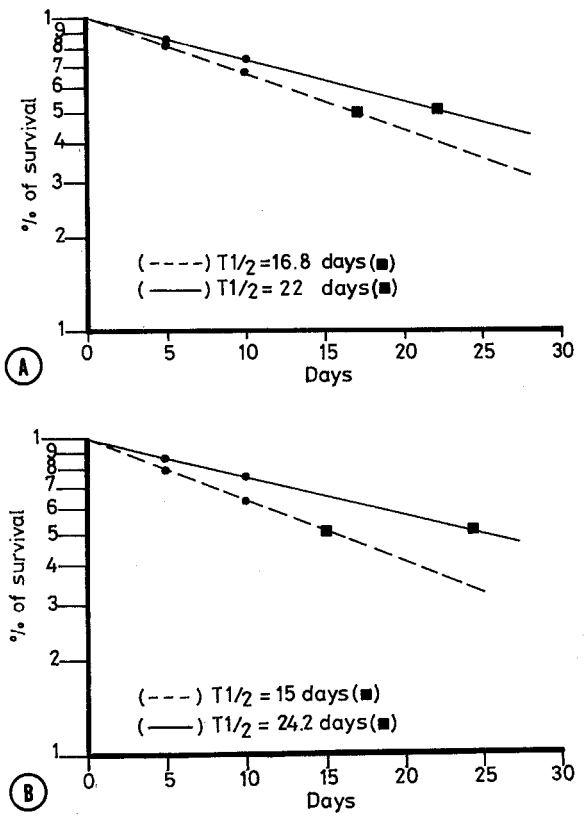

Figure. Improved red blood cell survival after 60 days of therapy with vitamin E (**A**) and with vitamin E plus selenium (**B**).

sample, and the percentage survival plotted on semilog paper. Percentage survival at any day equals counts per milliliter of whole blood on a specific day divided by counts per milliliter of the initial 24-hour sample, times 100. From the curve, the mean half-life was determined.

After 60 days of treatment we repeated the laboratory studies and red cell survival test.

Statistical analysis.

Hematologic and red cell half-life data were compared with control data, by Student t test. For the effect of therapy, each patient served as his own control. The effect of therapy in the two groups was analyzed by comparing the mean differences of paired values with the paired t test. The coefficients of variation for the determination before and after treatment were compared to exclude laboratory bias.[18]

RESULTS

Hematologic evaluation in the two groups before treatment revealed a significant difference from normal (Table I). The patients' mean baseline serum vitamin E concentration was also lower ($P < 0.005$) than that in controls.

During the 60-day treatment period, no patients had a hemolytic crisis. There were no reports of intolerance to medication. Serum vitamin E levels increased appropriately with drug administration (Table I).

Table II. Comparison between effects of therapy

	Mean difference of paired values		
	Group 1 (n = 18)	Group 2 (n = 18)	P
Hemoglobin (gm/day)	0.9 ± 0.1	1.2 ± 0.2	<0.05
Packed cell volume (%)	2.4 ± 0.4	3.8 ± 0.3	<0.05
Reticulocytic count (%)	−0.7 ± 0.2	−1.5 ± 0.4	<0.01
Haptoglobin (mg/day/ HBC)	27.5 ± 7.3	32.8 ± 6.8	NS
Red cell half-life (day)	5.9 ± 3.0	9.1 ± 4.4	<0.01
Serum vitamin E (mg/day)	6.65 ± 0.2	0.83 ± 0.3	NS

Values represent mean ± SEM.
NS, not significant.

After 60 days of vitamin E administration, the hematologic status in group 1 patients improved significantly and the mean red cell half-life (Figure, *A*) increased, from 16.9 to 22.8 ($P < 0.01$). In group 2 there was a significant response to the combined vitamin E and Se therapy. Hematologic values improved significantly, and the mean red cell half-life increased (Figure, *B*), from 15.6 to 24.3 ($P < 0.01$).

Statistical comparison between the mean difference of paired values in the two groups (Table II) revealed a more significant increase in mean hemoglobin concentration, hematocrit, and red cell half-life, and more significant reduction in mean reticulocyte count in group 2. There was no significant difference between the mean differences of haptoglobin and serum vitamin E levels.

DISCUSSION

The mean serum concentration of vitamin E was significantly below that of control values in the patients with G-6-PD deficiency. Because the controls came from the same households as the patients and were of similar age, it does not seem likely that dietary differences or age distribution could explain this finding.[19] None of the patients had a history of malabsorption to account for lower serum vitamin E levels. Because red cells in G-6-PD deficiency have a compromised capacity to produce reduced pyridine nucleotides and reduced glutathione and are thus vulnerable to oxidative damage, the role of vitamin E as an antioxidant may be more critical than in normal cells. Thus the reduction in serum vitamin E may

be the result of increased consumption while neutralizing oxidative damage by these vulnerable cells. A similar result was reported by Corash et al.[11]

Patients with Mediterranean-type G-6-PD deficiency have shortened red cell half-life and decreased hemoglobin concentration because of low-grade chronic hemolysis. However, in Egypt G-6-PD deficiency is clinically and biochemically heterogenous; some patients appear clinically normal when not being exposed to oxidant stress. We recruited patients who had clinical manifestations of G-6-PD deficiency and evidence of chronic hemolysis. After 60 days of treatment, analysis revealed a significant change toward normal in the two groups studied. The improvement seemed more significant in the group given both vitamin E and selenium, as indicated by the hematologic status and red cell life span. However, these results can be explained in metabolic terms. Vitamin E and selenium cannot be substituted for each other, but are essential alternate pathways of intermediary metabolism. Both agents participate in peroxide metabolism.[2]

Spielberg et al.[12] examined the effects of long-term administration of high doses of vitamin E on erythrocyte survival in two patients with inherited disorders of glutathione metabolism, one with a rare form of severe G-6-PD deficiency and the other with glutathione synthetase deficiency. In both patients, there was increased red cell survival and decreased reticulocyte count. Corash et al.[11] studied the effect of vitamin E on chronic hemolysis in 23 patients with Mediterranean-type G-6-PD deficiency. After 90 days of treatment there was improvement in hemolysis, as evidenced by longer red cell half-life, elevated hemoglobin level, and decreased reticulocyte count. On the other hand, studies by Johnson et al.[13] and Newman et al.[14] indicated no change in hematologic status after treatment with high doses of vitamin E.

Clinical assessment and follow-up in our patients indicated that there were no subjective or objective side effects that could be attributed to the drugs. Although the differences are of minor clinical importance, the prolongation of red cell half-life and improvement in hematologic status appear promising. However, inasmuch as these significant improvements were documented for only 60 days, we suggest that further studies of long-term vitamin E therapy, possibly combined with selenium, be conducted to determine whether it will reduce mild chronic hemolysis and reduce or ameliorate the severity of acute hemolytic crisis in patients with G-6-PD deficiency.

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