REDUCTION OF OSMOTIC HAEMOLYSIS AND ANAEMIA BY HIGH DOSE VITAMIN E SUPPLEMENTATION IN REGULAR HAEMODIALYSIS PATIENTS

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Summary

In 15 haemodialysis patients receiving oral vitamin E supplementation, 600mg daily for 30 days, both plasma and RBC vitamin E concentrations were significantly increased, while in unsupplemented patients the values remained unchanged. Mean osmolarity at which in vitro haemolysis occurs at the start and end of haemodialysis decreased from 102.8±0.9 to 98.9±0.7 and 72.1±1.1 to 67.4±0.8mOsm/L, respectively in supplemented patients. In addition the Hct increased from 26.1±1.0 to 28.1±1.2 per cent (p<0.05). In conclusion, oral supplementation of vitamin E could be of clinical benefit in correcting anaemia in regular dialysis patients by reducing the fragility of red blood cells.

Introduction

Anaemia remains one of the major problems in patients with end-stage renal failure treated by regular haemodialysis. Vitamin E is known to block the peroxidation of the polyunsaturated fatty acid constituents of cell membranes including red blood cells [1] and the increased sensitivity of red cells to oxidative stress is an index of vitamin E deficiency [2]. In addition, administration of vitamin E can significantly improve the anaemia of sickle cell disease [3]. In spite of these observations, very little information is available on the importance of vitamin E in patients on regular haemodialysis, particularly in relation to anaemia. For these reasons, we have investigated the effect of oral supplementation on plasma and red blood cell vitamin E concentrations and the osmotic fragility of red blood cells.

Materials and methods

Thirty patients aged from 29 to 68 years were included in the study. They had all been dialysed for 5 to 126 months and were on 5 to 5.5 hours thrice weekly
haemodialysis using a hollow Cuproammonium rayon fibre kidney. None of the patients were receiving preparations containing vitamin A or E. The patients were randomly divided into two groups: 15 patients received oral supplementation of 600 mg vitamin E daily for 30 days in addition to routine medication. The remaining 15 patients were not given vitamin E and served as controls. For the purpose of comparison, 10 healthy subjects were also studied. Blood samples were taken from the arterial line immediately before and after dialysis in all 30 patients on two occasions, just before vitamin E supplementation was started (day 0) and 30 days later (day 30). Plasma vitamin E concentrations were measured by high performance liquid chromatography after heparinised plasma was separated from red cells by centrifugation and storage at −20°C. The measurement of triple washed red cell vitamin E was carried out using a slight modification of Abe’s method [4]. The RBC vitamin E results were expressed as micrograms of alpha-tocopherol per ml of packed cells. Since only alpha-tocopherol is biologically active and accounts for about 85 per cent of total tocopherol in both plasma and RBC, the term ‘vitamin E’ used in this paper represents alpha-tocopherol. The osmotic fragility of RBCs was examined by the coil planet centrifuge method [5]. In this technique a stable linear gradient is created between two different concentrations of sodium chloride solution. Red cells pipetted into the higher concentration (150 mOsm) side of the tubes are forced to travel through the gradient to the lower osmotic points where haemolysis occurs. The distribution of haemoglobin indicates the start and end of osmotic fragility of the samples. Red and white cell counts, Hct, reticulocytes, plasma iron, ferritin, total protein, GOT, GPT, LDH, total lipids, BUN, creatinine, uric acid, Na, K, Ca, Cl, and P were determined using routine methods. Each subject served as his or her own control. The statistical analysis of results between the two groups was made using the paired ‘t’ test. Different groups of individuals were assessed by the unpaired ‘t’ test.

Results

The pre-supplemented mean plasma concentrations of vitamin E were 10.67 ± 0.85 and 9.73 ± 0.77 µg/ml in the two groups. These values were not lower than the normal range previously reported in haemodialysis patients [6]. Plasma vitamin E was significantly increased by oral vitamin E supplementation in 14 of 15 patients and the mean value of vitamin E almost doubled after 30 days treatment (20.37 ± 1.61 µg/ml, p<0.01). In contrast RBC vitamin E in regular dialysis patients ranged from 0.23 to 1.10 µg of vitamin E per ml of packed red blood with a mean of 0.57 ± 0.05 and 0.45 ± 0.07 µg/ml in two groups. These results are significantly lower than the mean for 10 normal control subjects (1.34 ± 0.12 µg/ml packed RBC, p<0.001). However, vitamin E oral supplementation increased the mean vitamin E concentration in RBC to 1.56 ± 0.11 µg/ml packed cells, which is almost triple the base-line value. RBC vitamin E in unsupplemented patients remained low. Twelve of 15 supplemented patients had a rise in Hct (mean from 26.1 ± 1.0 to 28.1 ± 1.2 per cent) and no subject showed a decrease in Hct after vitamin E administration, while an increase in Hct was
not observed in unsupplemented patients. The mean change in Hct of two per cent was significant using the paired 't' test. Mean osmolarities at which in vitro haemolysis occurs at the start and end of haemodialysis decreased from 102.8±0.9 to 98.0±0.7 and 72.1±1.1 to 67.4±0.8 mOsm/L, respectively after 30 days of treatment with vitamin E. These differences are statistically significant (p<0.05). Unsupplemented patients showed no improvement in osmotic fragility of RBC. There were no other biological or haematological changes. None of the patients received a blood transfusion during the 30 days treatment period.

Discussion

Since the observation by Rose and György that vitamin E can act as antioxidant which can reduce haemolysis of RBC caused by oxidant stress [7], additional evidence of a wide variety of roles in membrane-related activities and pathological processes secondary to a deficiency have been reported [8]. Polyunsaturated fatty acids represent 40 per cent of the total fatty acids in the human erythrocyte membrane. The fact that these polyunsaturated fatty acids do not normally auto-oxidise in cells implies the presence of a highly efficient protective mechanism. However, impairment of this mechanism, or conversely, susceptibility to peroxidation has been found in a variety of haemolytic states. Because of these observations we became interested in evaluating the effect of vitamin E on anaemia in regular haemodialysis patients. There is a paucity of information about the importance of vitamin E in haemodialysis patients, and investigations on the metabolism of vitamin E in such patients is lacking. Because vitamin E is thought not to be lost during dialysis most authors have stated that there does not appear to be a need for supplementation [9]. Vitamin E status has traditionally been determined by measuring plasma vitamin E but there have been relatively few measurements of alpha-tocopherol in red cells owing to technical difficulties of the assay. In contrast to the original report on plasma vitamin E concentration in dialysis patients by Ito who found low values [10], succeeding papers have concluded that the plasma concentration of vitamin E was normal or rather high in dialysis or CAPD patients [9]. On the basis of these findings it has been suggested that the vitamin E requirement of haemodialysis or CAPD patients can be met by the usual dietary intake without the use of supplementation [6]. Our results agree with previous publications indicating that plasma vitamin E is normal in such patients. Although normal, because of the importance of vitamin E on cell membranes, the red cell concentrations need to be directly measured rather than continuing to rely on plasma values. This is particularly important as haemolysis may be more directly related to the tocopherol in RBC than the plasma. In a recent study of vitamin E in regular dialysis patients, it has been stated that since the serum vitamin E was normal and since serum values reflected the concentration of the vitamin E within the erythrocyte membrane, it is reasonable to assume that the concentration of vitamin E in the RBC membrane is normal and that the antioxidant effect would likewise be normal. Our results do not confirm this assumption as the content of vitamin E in RBC is markedly below normal despite the normal plasma values: the reason for this is not clear. Increased utilisation of vitamin E
by RBC membranes or a low transfer rate of vitamin E from plasma to RBCs in particular pathological situations, such as uraemia, might be an explanation for the discrepancy in vitamin E in the two compartments. The results of this present study indicate that the administration of oral vitamin E appears to increase both plasma and RBC values. Twelve of 15 supplemented patients showed an increase in Hct and all patients showed a reduced susceptibility to osmotic haemolysis as measured by colloid planet centrifuge. It could be argued that the erythrocyte haemolysis test may not be a valid assessment of vitamin E status in haemodialysis patients. Uraemic toxins could interfere with the test particularly as slightly improved osmotic haemolysis can be seen in post-dialysis blood samples. Our results, however, show a very significant improvement after vitamin E administration, suggesting an important role for this substance in RBC fragility. This finding is similar to the observation that the impaired osmotic fragility of thalassaemic erythrocytes returned to near normal following administration of vitamin E. Increasing plasma vitamin E by supplementation could provide an excess of alpha-tocopherol to RBC membranes which may improve the ability of the cells to withstand osmotic stress. A similar conclusion was recently reported after intramuscular administration of vitamin E which decreased the content of malonyldialdehyde in RBCs. As this is an intermediate product of polyunsaturated fatty acid oxidation a decrease could mean an increase in the alpha-tocopherol in RBCs leading to a higher Hct in regular dialysis patients. In conclusion, our study suggests that oral supplementation of vitamin E in regular dialysis patients could be of clinical importance by reducing anaemia and the efficacy of long-term vitamin administration in such patients should be seriously considered.

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