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Oral Supplements of Vitamin E Improve Measures of Oxidative Stress in Plasma and Reduce Oxidative Damage to LDL and Erythrocytes in β-Thalassemia Intermedia Patients

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Fifteen β-thalassemia intermedia patients, not requiring chronic transfusional therapy, were monitored in order to check their antioxidant status, and the lipid oxidation products in plasma, LDL, and erythrocytes before and during a 9-month oral treatment with 600 mg/day vitamin E. The low level of vitamin E, and high level of malondialdehyde in plasma clearly tended to normalize after three months ($P<.001$), and were quite similar to control after six months. The abnormally low level of vitamin E in LDL and the four times higher than control basal level of conjugated dienes (LDL-CD), were not modified after three months of treatment. Significant changes of LDL-VE ($P<.05$) and of the basal LDL-CD ($P<.001$) were evident after six months. LDL-VE was within the normal range after nine months, whereas LDL-CD still appeared twice as higher than control.

Plasma vitamin A, ascorbate, β-carotene, and lycopene increased markedly at the end of the trial ($P<.005$).

The level of vitamin E in red blood cells was normalized after six months of supplementation. A decrease of the baseline value of conjugated dienes was observed after nine months, although it remained 1.4-fold higher than control. The RBC count and hematocrit appeared higher at the end of the trial ($P<.05$ and $P<.001$, respectively). The hemoglobin value did not show variations. A shift to normal of the resistance of erythrocytes to osmotic lysis was observed.

Our findings provide evidence that an oral treatment with vitamin E improves the antioxidant/oxidant balance in plasma, LDL particles, and red blood cells, and counteracts lipid peroxidation processes in β-thalassemia intermedia patients.

Keywords: antioxidants; atherogenesis; erythrocytes; LDL; oxidative stress; thalassemia; vitamin E

INTRODUCTION

Due to the impaired synthesis of hemoglobin chains, β-thalassemia patients undergo a chronic

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redox unbalance in red blood cells (RBC). Precipitation of excess α-chains causes the formation of free radicals and reactive iron, which impair antioxidant defence systems and promote oxidation of various RBC components leading to hemolysis [1–9]. Beta-thalassemia intermedia is a clinical definition applied to thalassemia patients exhibiting a milder clinical course than those with the severe transfusion-dependent thalassemia major, with a more or less severe anemia which does not require treatment with regular blood transfusions. Recent data from this laboratory showed that such a condition is accompanied by a marked depletion of plasma antioxidants, and an alteration of parameters of oxidative damage to lipids [10]. In addition, evidence has been provided of oxidized and cytotoxic circulating low-density lipoproteins (LDL) in β-thalassemia intermedia patients [10].

In spite of the uncertainties about the role of antioxidants such as vitamin E (VE) in the protection of LDL under physiological *in vivo* conditions [11–13], depletion of vitamin E has appeared crucial for the oxidative status of LDL in thalassemia intermedia patients. Indeed a strong inverse correlation was found between the conjugated dienes in LDL (LDL-CD) and the level of vitamin E in plasma and LDL [10]. Administration of a pharmacological dosage of vitamin E to humans leads to an increasing of VE in plasma and in LDL [12, 13], which protects LDL towards oxidation [14] and prevents LDL-mediated vascular injury *in vitro* [15]. Since vitamin E does not seem to have serious toxicity nor adverse effects at oral doses as high as 60 to 800 IU/day [16–18], a small-scale therapeutical trial was started in order to investigate if a long term oral treatment with vitamin E could improve the oxidant/antioxidant balance in plasma and LDL of thalassemia intermedia patients. In addition, the present study also focused on the oxidative status and some of the parameters characterizing red blood cells. Data collected at three month intervals, for nine months, are reported.

MATERIALS AND METHODS

Subjects

Thalassemia intermedia patients, previously characterized for β-globin gene mutation, 10 females and 5 males, aged 10 to 51 (mean 30±13), not smokers, were recruited, with consent, for this study, and were under observation for 9 months. With the exception of four patients, who were occasionally transfused, the patients had never been transfused (n=7) or they had received blood transfusions at least two years before entering the trial (n=4). All patients were regularly interviewed and examined by a staff of physicians, at intervals of 15 days to one month. No patient was diabetic, nor showed abnormal levels of alanine or aspartate aminotransferases. Two patients were HCV positive. Patients were not under lipid altering medications. All of them were under allopurinol treatment, to control plasma levels of urate. Hemoglobin levels were 6.5 to 11.3 g/dl (mean 8.6 ± 1.2). Ferritin was measured every 4 months, and cardiac, endocrinologic and hepatologic evaluations were performed regularly. Blood from healthy individuals, 10 females and 5 males, aged 20 to 44 (33±13), not smokers, nor under any medical treatment, was used to obtain the reference parameters reported as control. They did not receive any vitamin supplementation and their and the patients food intake was that of the Mediterranean diet. Patients were given two oral doses of 300 mg/day of vitamin E (*dl*-α-tocopherolyl acetate, Ephynal, Roche). They were instructed to take the supplements with meals to enhance absorption.

Blood from patients and controls was collected in EDTA (1 mg ml^{-1}) after an overnight fasting. Red blood cells were sedimented at 1000 g for 10 min and washed three times with phosphate buffer saline (PBS), pH 7.4. Plasma was divided in suitable aliquots, to prepare low density lipoprotein and perform the analytical determinations described below.

Clinical chemistry determinations

Triglycerides, total and HDL-cholesterol, bilirubin, and urate were measured with commercial analytical kits (Sigma, St. Louis, Mo) Concentrations of LDL-cholesterol were calculated using the Friedwald formula [19]. Ferritin was determined by an enzyme-immuno assay (Abbott Labs, North Chicago, IL). Hemoglobin and hematocrit were measured by Cobas Micros O.T. (Roche, France).

Preparation of LDL

LDL (*d* 1.019–1.063 g/ml) was isolated from EDTA plasma by stepwise ultracentrifugation at 4°C in a Beckman L8–70 M ultracentrifuge fitted with a 50 Ti rotor using potassium bromide for density adjustments, according to Kleinveld *et al.* [20]. LDL fraction was shown to be free of other lipoproteins by electrophoresis on agarose gel. EDTA and salts were removed from LDL by gel filtration on Sephadex G-25 Medium (Pharmacia). Proteins were determined by the Bio Rad colorimetric method [21]. In typical preparations, 0.6 mg apo B-100 was obtained from 1 ml plasma. To prevent autoxidation reactions, LDL was used immediately or after an overnight storage at –70 °C.

Biochemical analyses

Malondialdehyde (MDA) was evaluated in 50 µl plasma samples by a colorimetric reaction with thiobarbituric acid (TBA, Sigma, St. Louis, MO) [22], followed by neutralization of samples with equivalent volumes of a mixture consisting of 4.5 ml 1.0 mol/l NaOH and 45.5 ml methanol. Isocratic high performance liquid chromatography (HPLC) separation of the MDA adduct was performed by a Supelco Supelcosil™ (Bellefonte, PA) LC-18 column (0.46 × 25 cm), eluted with 40% methanol in 50 mmol/l potassium phosphate buffer, pH 6.8, at 1.5 ml min^{-1}. The MDA-TBA adduct was revealed at 532 nm and quantified by reference to a calibration curve of tetraethoxypropane (Sigma, St. Louis, MO), submitted to the TBA colorimetric procedure. Butylated hydroxytoluene (0.03%) was added to the thiobarbituric acid reagent to prevent artifactual lipid peroxidation during the assay procedure. The conjugated dienes in the lipid fraction of LDL (LDL-CD), or RBC (RBC-CD) were extracted from LDL samples (200 mg protein in 1.0 ml 0.15 mol/l NaCl), and from 1.0 ml of 2% RBC suspension, with 3.0 ml $CHCl_3$:MeOH (2:1. v:v). The organic extract was dried under a nitrogen stream, resuspended in cyclohexane and quantitated spectrophotometrically at 234 nm, using a molar absorption coefficient of 27,000 [23].

All-*trans* retinol and α-tocopherol were extracted from 200 µl of plasma samples, diluted to 1.0 ml with 0.15 mol/l NaCl, by mixing with two volumes of absolute ethanol, followed by two successive extractions with six and two volumes of petroleum ether. The organic extracts were gathered, dried under nitrogen, resuspended in several microliters of suitable solvent and injected on top of a LC-18 HPLC column (see above). Analysis was carried out by eluting with methanol at 1.0 ml min^{-1} Detection of all-*trans* retinol and α-tocopherol was at a wavelength of 320 nm and 290 nm, respectively. Under the conditions described, all-*trans* retinol eluted after 5.2 min and α-tocopherol after 12.8 min. Automatic wavelength change after 9 min allowed the detection of both compounds in the same sample.

Alpha-tocopherol was extracted from LDL samples (50 mg protein in 1.0 ml PBS), or from 500 µl of a 10% RBC suspension, and analyzed by HPLC as described above.

Beta-carotene and lycopene were extracted from 500 µl plasma, after mixing with one volume of methanol and three volumes of hexane:diethyl ether (1:1, v:v). The extracts were then dried under nitrogen, resuspended with several microliters of a mixture of acetonitrile:methanol: tetrahydrofurane (58.5:35:6.5,

v:v:v) and analyzed with the same solvent [24] by a LC-18 Supelco column as above, at a flow rate of 2.5 ml min^{-1}. Under these conditions lycopene eluted at 8.2 min, and β-carotene eluted at 13.8 min. Revelation was at 450 nm.

Ascorbate was determined in 500 μl serum from blood collected in 1.0 mmol/l dithiothreitol. Extraction, HPLC separation, and spectrophotometric revelation at 266 nm were as reported by Lazzarino et al [25] with minor changes, which included length of the column (25 × 0.46 cm), and isocratic elution with 10 mmol/l KH_2PO_4 buffer, pH 7.0, in 10% methanol in water, containing 10 mmol/l tetrabutylammonium bromide, at 1.2 ml min^{-1}. Retention time was 5.3 minutes.

All compounds evaluated by HPLC were quantified with reference to standard curves constructed with 5 to 100 ng of each compound, and by relating the amount of the compound under analysis to the peak area. All procedures were performed under dim red light to avoid artifactual photooxidation of lipids and to preserve light sensitive vitamins.

Osmotic fragility

Aliquots (200 μl) of 1% RBC suspensions were added to a series of test tubes containing 1.8 ml of diluted PBS in water (30–44%, v:v), pH 7.4, and centrifuged at 1,000 g for 10 min. Then, the supernatants were assayed for the degree of hemolysis by comparing the absorbance at 540 nm with that of 200 μl of RBC suspensions treated with 1.8 ml hypotonic phosphate buffer. The percentage of hemolysis was calculated from the ratio of the absorbances.

Statistical analysis

Conventional methods were used for calculation of means and standard deviations. Comparison between controls and thalassemia patients was performed by the unpaired Student's *t*-test.

RESULTS

Hematological data concerning hemolysis, iron status, and plasma lipid pattern are markedly varied in β-thalassemia. Data relevant to our patients are reported in Table I. With the exception of a small increase of urate, the supplementation of vitamin E for nine months did not affect any of the parameters (Table I).

TABLE I Hematologic data in plasma from β-thalassemia intermedia patients before and after nine months of oral supplementation with vitamin E and controls

	β-thalassemia intermedia patients		*Controls*
	before supplementation n=15	*after supplementation n=15*	
Ferritin (ng/ml)	900±390[§]	1020±420[*]	78±10
Bilirubin (mg/dl)	2.1±1.51[§]	1.89±1.61[*]	0.70±0.19
Urate (mg/dl)	5.1±0.9[§§]	5.75±0.7[**,§]	4.76±0.5
Total cholesterol (mmol/l)	2.44±0.62[§]	2.60±0.55[*]	5.22±0.25
HDL cholesterol (mmol/l)	0.71±0.25[§]	0.69±0.31[*]	1.37±0.16
LDL cholesterol (mmol/l)	1.16±0.35[§]	1.31±043[*]	3.59±0.51
Triglycerides (mmol/l)	1.35±0.51[§]	1.43±0.6[*]	1.22±0.55

Values are the mean ± SD of (n) determinations performed in duplicate on blood samples from different subjects. With respect to the relevant control, values were: [§]significant with P < .001; [§§]not significant; with respect to the relevant value before supplementation, values were: [*]not significant; [**]significant with *P*<.05 (Student's *t*-test)

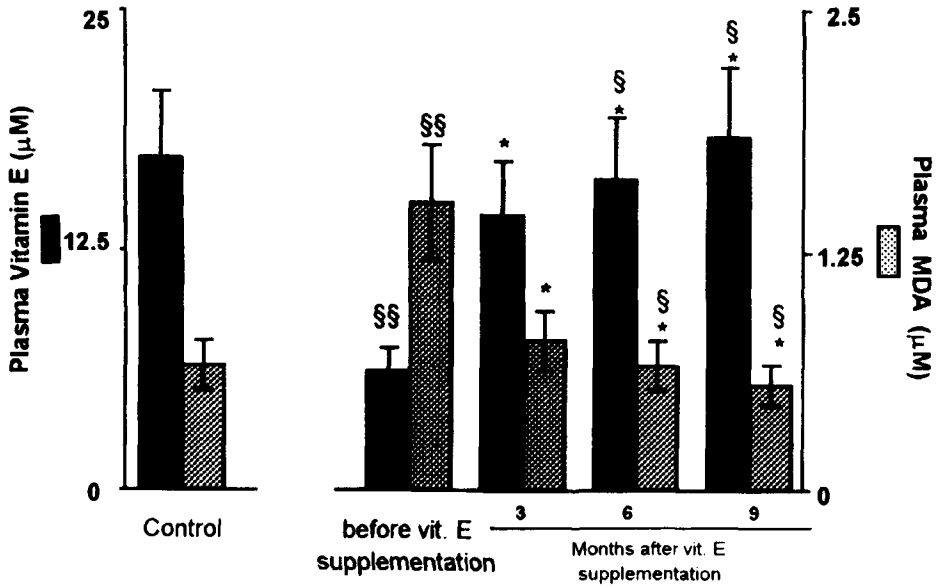

FIGURE 1 Vitamin E and malondialdehyde (MDA) in plasma from β-thalassemia intermedia patients before and during nine months of oral supplementation with vitamin E. Each sample was simultaneously analyzed for vitamin E and MDA. Values are the mean ± SD of 15 determinations performed in duplicate on plasma samples from different subjects. With respect to the relevant control, value were: §not significant; §§significant with $P<.001$; with respect to the relevant value before vitamin E supplementation, values were significant with *$P<.001$ (Student's t-test)

Depletion of vitamin E in β-thalassemia intermedia patients is accompanied by a marked elevation of products from lipid peroxidation such as malondialdehyde in plasma, and conjugated dienes in LDL [10]. A time-course investigation was performed to monitor the variations of vitamin E in plasma and LDL, as well as the plasma level of MDA and the baseline level of conjugated dienes in LDL, following vitamin E supplementation. The measurements were carried out every three months. An increase of the plasma concentration of vitamin E is soon evident after a 3-month administration, while the level of plasma MDA decreases (Figure 1). Both vitamin E and MDA achieve the level of healthy subjects after six months (Figure 1). No further modification occurs thereafter.

The daily intake of 600 mg vitamin E brings about a significant rise of vitamin E in LDL only after six months. The vitamin E levels were not significantly different from control at the end of the 9-month treatment (Figure 2). On the other hand, the oxidative damage to LDL, measured as conjugated dienes, is only partially reversed by the vitamin E supplementation. Though their level is half respect to the value before the treatment, conjugated dienes remain two-fold higher than control (Figure 2).

The severe oxidative stress, which is a consequence of the disease process, markedly affects the whole plasma antioxidant status in β-thalassemia. Table II reports on the concentration of the major plasma antioxidants in our patients before and after vitamin E treatment. A substantial increase was observed for all the antioxidants. The level of vitamin A and β-carotene are comparable to those of healthy controls, while depletion of lycopene and ascorbate appears significantly less severe (Table II).

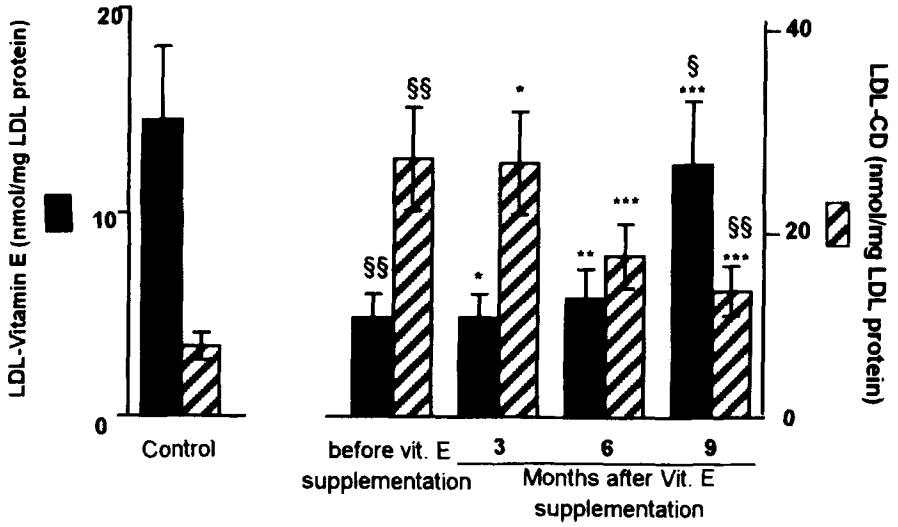

FIGURE 2 Levels of vitamin E and conjugated dienes (CD) in LDL from β-thalassemia intermedia patients before and during nine months of oral supplementation with vitamin E. Each sample was simultaneously analyzed for vitamin E and CD. Values are the mean ± SD of 15 determinations performed in duplicate on LDL samples from different subjects. With respect to the relevant control, value were: §not significant; §§significant with $P<.001$; with respect to the relevant value before vitamin E supplementation, values were: *not significant; significant with $^{**}P<.05$ and $^{***}P<.001$ (Student's *t*-test)

TABLE II Major antioxidant vitamins in plasma from β-thalassemia intermedia patients before and after nine months of oral supplementation with vitamin E and controls

	β-thalassemia intermedia patients		*Controls*
	before supplementation n=15	*after supplementation n=15*	
Vitamin E (μM)	6.18±1.21§	18.4±3.00*,§§	17.3±3.8
Vitamin A (μM)	1.12±0.51§	1.66±0.40**,§§	1.81±0.2
Ascorbate (μM)	28.3±8.7§	38±7.2**,§	50.1±7.5
β-carotene (μM)	0.31±0.08§	0.39±0.05**,§§	0.41±0.05
Lycopene (μM)	0.15±0.08§	0.25±0.10**,§	0.54±0.05

Values are the mean ± SD of (n) determinations performed in duplicate on blood samples from different subjects. With respect to the relevant control, values were: §significant with $P<.001$; §§not significant; with respect to the relevant value before supplementation, values were significant with $^{*}P<.001$ and $^{**}P<.005$ (Student's *t*-test).

Vitamin E supplementation rapidly increases the vitamin E level in red blood cells, which is comparable to control after six months, and is higher than in control erythrocytes at the end of the trial (Figure 3). On the other hand, the basal level of conjugated dienes in red blood cells from patients show a significant decrease after six months, although it remains 1.4 times the control value at the end of the trial (Figure 3). Taking into account the hematocrit and the cell count, the results reported in Figure 3 are expressed per cell number. This appears appropriate as vitamin E supplementation brings about a significant increase of both the RBC count and the

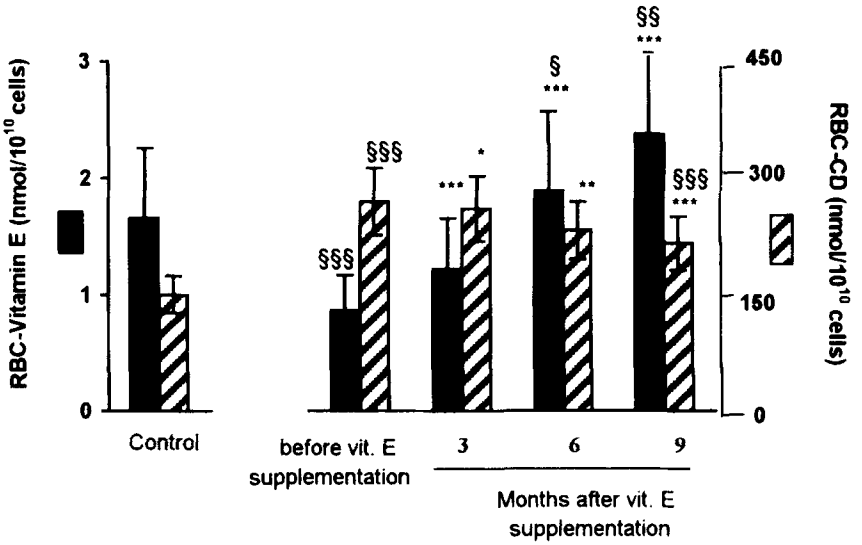

FIGURE 3 Levels of vitamin E and conjugated dienes (CD) in red blood cells (RBC) from β-thalassemia intermedia patients before and during nine months of oral supplementation with vitamin E. Each sample was simultaneously analyzed for vitamin E and CD. Values are the mean ± SD of 15 determinations performed in duplicate on RBC samples from different subjects. With respect to the relevant control, value were: §not significant; significant with $^{\S\S}P$=.01 and §§§ P<.001; with respect to the relevant value before vitamin E supplementation, values were: *not significant; significant with $^{**}P$<.05 and $^{***}P$<.001; (Student's t-test)s

hematocrit (Table III). The increase of RBC count is not accompanied by an increase of the hemoglobin level (Table III). Total hemoglobin had not varied, and the mean cell hemoglobin had decreased at the end of the trial (Table III).

Impairment of the membrane transport systems in thalassemia erythrocytes determines variation of the cell volume and an increased resistance to osmotic lysis. The osmotic fragility of thalassemia red blood cells, before and after nine months of vitamin E supplementation is shown in Figure 4. The higher percentage of PBS in water which is necessary to bring about the hemolysis of 50% of RBC suspensions is indicative of a decreased resistance after the treatment with vitamin E.

TABLE III Total Hb, erythrocyte count, hematocrit and MCH (mean cell hemoglobin) in β-thalassemia intermedia patients before and after nine months of oral supplementation with vitamin E and controls

	β-thalassemia intermedia patients		*Controls*
	before supplementation n=15	*after supplementation n=15*	
Hemoglobin (g/dl)	8.6±1.2§	8.9±1.1*,§	13.5±0.5
Erythrocyte count (x10^{12}cells/l)	3.4±0.64§	3.98±0.78**,§§	4.7±0.3
Hematocrit (%)	24.8±0.9§	26.7±1.6***,§	40±1.0
MCH (pg)	25.79±2.78§	22.74±2.99****,§	29±2

Values are the mean ± SD of (n) determinations performed in duplicate on blood samples from different subjects. With respect to the relevant control, values were significant with $^{\S}P$<.001 and $^{\S\S}P$=.002; with respect to the relevant value before supplementation, values were: *not significant; significant with $^{**}P$<.05, $^{***}P$<.0001, and $^{****}P$=.007 (Student's t-test).

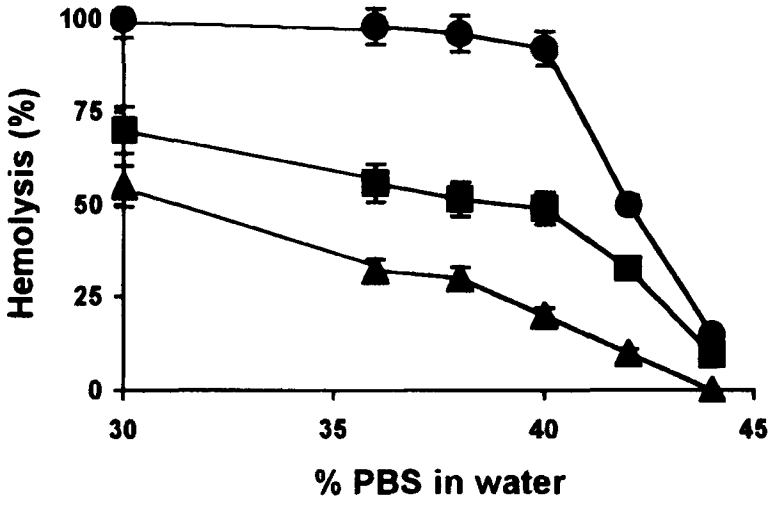

FIGURE 4 Susceptibility to osmotic lysis of red blood cells (RBC) from β-thalassemia intermedia patients before (▲) and after (■) nine months of oral vitamin E supplementation as compared to control RBC (●). Values are the mean ± SD of 15 determinations performed in duplicate on RBC samples from different subjects

DISCUSSION

Genetic disorders such as thalassemias encompass a variety of clinical phenotypes, from the clinically silent heterozigous β-thalassemia to the severe transfusion-dependent thalassemia major. It has been long since known that a severe depletion of plasma and RBC vitamin E [26–35], accompanied by the increase of lipid oxidation products such as MDA in plasma [26, 28. 30, 34, 36], occurs in β-thalassemia patients. This led various physicians to test vitamin E administration in order to reduce deleterious effects, and to improve clinical parameters of the disease. However, attempts carried out with thalassemia major patients (seven trials from 1974 to 1986 [26–32]) were disappointing, in that no substantial benefit was observed in the requirement of transfusions, nor in the Hb levels, although the plasma level of vitamin E was corrected [26, 28, 29, 31]. Thus, vitamin E cannot prevent the massive oxidative injury to transfused RBC which, in thalassemia major, is primarily caused by the secondary iron overload due to the transfusional treatment. These results possibly discouraged other attempts. We started a therapeutical trial with selected β-thalassemia intermedia patients, not requiring chronic transfusional therapy. These patients exhibited a severe depletion of all plasma antioxidants, including vitamin E, and an increased plasma level of MDA, with marked oxidative modifications to circulating LDL which were correlated with the level of LDL cytotoxicity, and with vitamin E depletion [10]. We explored if an oral administration of 600 mg vitamin E per day, could result in some correction of the plasma redox balance and of the oxidative status of lipids and LDL. This paper reports on the data obtained during a 9-month trial which had total compliance.

A marked increase of plasma vitamin E was soon achieved after a 3-month supplementation, and the level was corrected after six months. In addition, because of the interdependence among antioxidants [37, 38], the increase of vitamin E also improved the entire antioxidant pattern.

The level of vitamin A and β-carotene was within the control range, while ascorbate and lycopene showed a marked increase, at the end of the trial. Urate, another molecule which participates in the plasma antioxidant defence [37], also increased after vitamin E supplementation.

In spite of the rapid increase of vitamin E in plasma, the abnormally low level of vitamin E in LDL from patients did not show any variation before six months of supplementation, and LDL-VE reached a level almost comparable to control only at the end of the trial. These observations may reveal an abnormal distribution of the vitamin among plasma lipoproteins, at least for a certain time during which tissues become enriched with vitamin E. Because of the major role of LDL in delivering and releasing vitamin E into tissues [39, 40], the delay in accumulation may reflect the time required to attain a steady-state. All this appears consistent with the finding that a sharp increase of vitamin E is monitored in red blood cells after three months of supplementation, and a level quite comparable to control is achieved after six months.

The enrichment of red blood cells with vitamin E is accompanied by a clear decrease of the basal level of conjugated dienes, in accordance with the antioxidant role of the molecule. However, conjugated dienes were still higher than control at the end of the trial, when and although the amount of vitamin E in erythrocytes was higher than control. The increased membrane surface area, and the higher amounts of lipids in thalassemia erythrocytes [4], may account for the observed level of basal dienes, and can also favour higher amounts of vitamin E to be arranged into the lipid structure. More importantly, the ratio RBC-VE/RBC-CD in both control and thalassemia RBC is quite similar (0.011) at the end of the trial, indicating that vitamin E supplementation may restore a normal antioxidant/oxidant balance into the membrane lipid compartment.

Because of the oxidative damage imposed by hemoglobin denaturation and precipitation [6–9], thalassemia erythrocytes bear marked structural modifications and transport abnormalities, which contribute to recognition and rapid clearance by the reticulo-endothelial system (RES), and to a decreased osmotic fragility [4, 5]. Nine months of vitamin E supplementation lead to an increased RBC count and hematocrit, and to a shift to normal of the resistance of RBC to osmotic lysis This suggests that the replenishment of erythrocytes with vitamin E, which results in protection of the membrane lipids, could also delay membrane structural and functional modifications. As a result, the erythrocyte survives for a longer time before being phagocytosed by the RES macrophages, and achieves an almost normal osmotic fragility. Vitamin E, however, by no means can affect precipitation and the endogenous oxidative destruction of hemoglobin. Total hemoglobin was unchanged, and the MCH value was decreased in response to the increased number of cells, as a further indication that only the membrane structure was somewhat protected by vitamin E. Consistent with our findings, a membrane protection following the oral administration of vitamin E has also appeared important in prolonging the survival of erythrocytes in three of seven thalassemia major patients [26]. A return to an almost recovered resistance of RBC to osmotic lysis, following three to six months of vitamin E administration, was also observed by Kahane *et al* [27] with thalassemia major and intermedia patients. Bilirubin level, as an expression of RBC and hemoglobin catabolism, did not fall despite the elongation of the RBC life. Possibly, the level of bilirubin, which participates in the body antioxidant system [37], undergoes a complex balance in these patients.

The decrease of the plasma level of MDA, which is currently considered as an expression of oxidation processes in the body, and of the baseline level of conjugated dienes in LDL and in red blood cells, also are a reflection of the improved oxidant/antioxidant balance due to the vitamin E treatment. We observed that

plasma MDA is in the normal range after VE administration, whereas LDL-CD, though markedly decreased, are still higher than normal. This discrepancy is only apparent. Conjugated dienes are produced only in early stages during the lipid oxidation process, then decompose to give final products such as reactive aldehydes [41]. Thus, prevention of the lipid breakdown by vitamin E may result, as it does, in a decrease of MDA, while the steady-state level of CD might eventually appear unmodified. In addition, in oxidized LDL, much of the CD content is derived from linoleate, which, having but two carbon-carbon double bonds, is not a source of MDA.

The fact that the treatment with vitamin E can restore the plasma level of MDA deserves attention. Reactive aldehydes such as MDA are cytotoxic [42, 43], and can damage collagen in the cardiovascular system [44], and modify LDL [45]. High level of MDA, oxidized LDL [46–49] and damaged RBC [50, 51] may concur to the development of the atherogenesis-related vascular alterations, which have been reported in β-thalassemia patients [52–56]. Thalassemia intermedia patients usually have a long life expectancy. Although our present findings are indicative of beneficial effects on the individual oxidant/antioxidant balance, a long-term response if chronic supplementation with high dosage vitamin E may provide a significant protection against atherogenesis-related complications, remains to be established. However, the evolving understanding of the nature of atherogenesis at levels both cellular and molecular, and the absence of toxicity of vitamin E [16–18], could suggest with some confiance to consider vitamin E as a potential adjuvant therapy in thalassemia intermedia, particularly if supplementation is initiated early [57]. Beyond its antioxidant effect, vitamin E could also act as an antiatherogenetic factor through its action on coagulation factors [58], platelets [59], smooth muscle cells [60], scavenger receptor activity in macrophages [61] and monocyte adhesion [62].

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