Plasma Vitamin E Levels and Vitamin E/β-Lipoprotein Relationships in Small Preterm Infants During the Early Anemia of Prematurity

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Abstract. The vitamin E status of AGA preterm infants (birth weights ≤ 1500 g) was studied during the first 10 weeks of life. The total polyunsaturated fatty acid content of the diet was 12%, and medicinal iron was given from 4 weeks of age. Plasma vitamin E concentrations correlated significantly with β-lipoprotein levels in the infants not supplemented with tocopherol. The low plasma vitamin E levels observed in these infants thus reflect the low transport capacity of the plasma, and do not necessarily signify vitamin E deficiency. Erythrocytes from the preterm infants showed increased hemolysis in the hydrogen peroxide hemolysis test, also when the plasma tocopherol levels were above 11.6 μmol/l; and for the same level of tocopherol, the degree of hemolysis varied considerably. This indicates that factors other than the tocopherol concentrations influence this test. When glucose was added to the cells during the test the hemolysis decreased. A group of infants supplemented with 7.5 IU water-soluble tocopherol/day showed satisfactory median levels of the vitamin, both when assessed as plasma vitamin E concentrations and as vitamin E/β-lipoprotein ratios. However, some infants had low plasma concentrations and ratios, particularly at 4 weeks of age. This indicates that this supplementary dose is insufficient when a water-soluble preparation is used. A very wide range of plasma vitamin E concentrations was observed in the supplemented infants. A word of caution against too vigorous supplementation in small preterm infants is therefore warranted.

Key words: Infant, premature – Vitamin E – Lipoproteins, LDL.

Introduction

The consensus today is that vitamin E functions as part of the defense against the peroxidative effects of free radicals produced through the reduction of molecular oxygen [14]. Polyunsaturated fatty acids (PUFA) are prone to take part in these reactions and as cell and organelle membranes are rich in PUFA, this defense is vital to normal function of the cells. The PUFA content of the diet is important, since it will influence the PUFA content of the tissues. This is particularly true during rapid growth.

In adults and older children vitamin E deficiency causes a moderate decrease in the erythrocyte survival rate. However, the decrease is not large enough to influence hemoglobin concentrations or reticulocyte counts [5]. In very low-birth-weight infants (VLBW), vitamin E deficiency seems to cause hemolysis—which contributes to the early anemia of prematurity—when the PUFA content of the diet is high and iron supplementation is started early [13,18]. Vitamin E deficiency causes ineffective erythropoiesis in monkeys and pigs, and some evidence suggests that this may also be so in preterm infants [15]. Vitamin E supplementation has, in addition, been claimed to reduce the incidence of bronchopulmonary dysplasia [3], and retrolental fibroplasia in low-birth-weight infants [12]. The best mode of administration and optimal dose of the vitamin for these infants is not settled, but water-soluble tocopherol preparations are absorbed better from the gastrointestinal tract than lipid-soluble preparations [10]. In his major review, Dallman [2] suggested that a dose of 5–10 IU/day would be adequate when a water-soluble vitamin E preparation is given.

Relating the plasma vitamin E concentration to the tocopherol transport capacity (β-lipoprotein level)—
or alternatively plasma lipid level (cholesterol, total lipids)—is considered to be the most reliable index of vitamin E status routinely available [1, 5]. This has not been done in previous studies on vitamin E in VLBW infants. We have found that the low serum vitamin E levels in cord blood and the high maternal values at birth show a highly significant positive correlation with β-lipoprotein levels [11]. This indicates that the low levels of the vitamin in cord blood reflect the low transport capacity in serum, and thus do not necessarily imply a deficiency state. In the present study we sought to answer the following questions: (a) Do plasma levels of vitamin E correlate with β-lipoprotein levels in VLBW appropriate for gestational age (AGA) infants during the first weeks of life? (b) Is the hydrogen peroxide hemolysis (HPH) test a reliable index of vitamin E deficiency in VLBW AGA infants? (c) Is a dose of 7.5 IU/day of water-soluble α-tocopheryl acetate sufficient to ensure vitamin E adequacy during the early anemia of prematurity?

Materials and Methods

Twenty-five preterm infants (birth weights 870–1500 g) were studied at weekly intervals during their stay in the neonatal intensive care unit. They were scored as appropriate for gestational age according to the charts prepared by Gairdner et al. [8]. Except for short apnoeic spells, all infants experienced uneventful neonatal periods. They were fed a commercial formula (Nan, Nestlé): eight of them also received expressed milk from their mothers. The formula contains 6.6 mg iron/l and the vitamin E content is 2.6 mg/l. Polyunsaturated fatty acids account for approximately 12% of the fat in both maternal milk and the formula. From the fourth day of feeding the infants received vitamin A, C, D, folic acid, and vitamin E. Vitamin E was given as water-soluble α-tocopheryl acetate (AFI-E Vet, AFI, Oslo, Norway) in a dose of 7.5 I.U./day. Iron (ferrous carbonate equivalent to 30 mg Fe/’’day’’) was given from the fourth week of life. This amount of iron has been the recommended dose in Norway for the last 25 years. It is very similar to that used by Gairdner et al. [7], who used 36 mg Fe/’’day’’ in the only study that has shown any effect of iron on the early anemia of prematurity.

To investigate the relationship between transport capacity (β-lipoprotein) and the plasma vitamin E levels, 10 additional preterm infants (birth weights 740–1430 g) were followed without vitamin E supplementation, but otherwise on the same regimen.

Blood was sampled weekly, in conjunction with the routine hematologic assessment of the preterm infants and before the administration of the daily tocopherol dose. The blood samples were collected by venipuncture at the age of one and four weeks and then every second week. Samples taken at other times were obtained by heel prick. The blood was drawn into heparinized plastic tubes. Vitamin E was measured fluorometrically on 100 μl samples in duplicate as previously described [11], while β-lipoprotein concentrations were determined by single radial immunodiffusion.

The hydrogen peroxide hemolysis (HPH) test was performed by a modification of the method of Gordon et al. [9].

The red blood cells (RBC) were washed once in saline after separation from the plasma. A 2.5% suspension of the RBC was made in buffered saline (pH 7.4), and incubated at 37°C for 15 min. After centrifugation (2000 rpm/10 min), the RBC were resuspended at 5% in 0.9% NaCl: from this suspension, 250 μl was added to each of 5 test tubes (precleaned, disposable). To four of the glasses 250 μl of freshly made 2.4% H2O2 in phosphate buffer (pH 7.4) was added, and the glasses quickly covered with parafilm. To the fifth tube 250 μl of buffer was added as a control. The tubes were then incubated for 3 h at 37°C on a shaking water bath. To the control and 3 of the other tubes 4.5 ml of buffered saline was then added, while 4.5 ml of aqua dest. was added to the last tube. After mixing and centrifugation, the degree of hemolysis was determined photometrically at 540 nm. In some tests the 5% suspension of RBC was made in 0.9% NaCl to which was added 5.6 mmol/l of glucose.

Blood from a normal adult was assayed in every run of the test. In 51 consecutive assays, adults showed a median HPH value of 1%, with 3/4 of the values being 1% or less. The upper limit observed in adults was 3%.

Cord blood samples from normal term infants, and venous samples from healthy young adults were analyzed to establish normal values for vitamin E/β-lipoprotein ratio.

Correlation was tested by the Pearson correlation coefficient.

Results

Figure 1 shows the relationship between vitamin E levels and levels of β-lipoprotein in 10 AGA preterm infants (birth weights 740–1430 g). The infants were not given vitamin E supplementation and blood was collected from the first to the eighth week of life. The correlation is highly significant (r = 0.67, P < 0.001).

The results of the HPH test are shown in Fig. 2. In contrast to what is normal later in life, RBC from some of the infants showed increased hemolysis even when the plasma tocopherol concentrations were higher than 11.6 μmol/l (0.5 mg/dl). When glucose was added to the erythrocytes during the exposure to H2O2, the hemolysis invariably decreased (Fig. 3).

Figure 4 depicts the plasma vitamin E levels of tocopherol supplemented AGA preterm infants (birth weights 870–1500 g) during the first 10 weeks of life. Plasma concentrations of less than 11.6 μmol/l are usually considered to represent a deficiency. This value is, however, based on studies in adults. A recent study on plasma tocopherol levels in normal children 1–12 years of age indicate that the normal levels in children are lower than in adults, also when plasma lipid levels are taken into account, and that the lower normal limit in children is 7.0 μmol/l [6]. Figure 4 shows that after initiation of vitamin E supplementation the median plasma tocopherol levels rose rapidly to normal adult concentrations (we have previously found similar cord blood tocopherol levels in preterm and term infants [11]). However, from 4 weeks of age, some of the infants had low levels considered to represent defi-