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Vitamin E and the hypercoagulability of neonatal blood

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Abstract

This study examines whether vitamin E deficiency has any role in the hypercoagulability of neonatal blood. Blood was collected from mothers and their full-term placental cords. Vitamin E was measured by high-pressure liquid chromatography and whole blood clotting time was measured by recalcification. Cord plasma had significantly lower vitamin E ($P < 0.0001$) compared with maternal plasma. Whole blood clotting time of cord blood was significantly ($P < 0.002$) shorter compared with the clotting time of maternal blood. There was a significant correlation between plasma vitamin E and whole blood clotting time ($r = 0.54$, $P < 0.04$) of cord blood. The addition of standard vitamin E to cord blood in vitro resulted in prolongation of whole blood clotting time. This suggests that a deficiency of plasma vitamin E can shorten whole blood clotting time in newborns, which may have a role in the disseminated intravascular coagulation frequently experienced by newborn infants.

Key words: Vitamin E; Newborn infants; Whole blood clotting time

1. Introduction

Numerous studies have reported that neonatal blood has a lower level of vitamin E than maternal blood [1,2]. On the other hand, although newborns are physiologically deficient in blood clotting factors, their whole blood clotting time is equal to or shorter than adult values [3–5]. This indicates that although the prothrombin time, partial thromboplastin time and thrombin time are normally pro-

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longed in infants, an unknown procoagulant effect is present in the whole blood clotting that could contribute to the derangement in coagulation and, consequently, to the hemostatic disorders found in newborns. This study was designed to determine if plasma vitamin E deficiency has any role in the hypercoagulability of neonatal blood.

2. Materials and methods

Blood was collected by the obstetrician or the obstetrician's nurse when the mother was venipuntured for other clinical tests; cord blood was collected from the fresh placenta of full-term newborns immediately after delivery. Blood was collected into silconized tubes containing ethylenediamine tetraacetate (EDTA) or acid-citrate dextrose (ACD). Blood collected into EDTA tubes was immediately processed for the plasma separation by centrifuging blood at 2,000 rev./min for 7 min in a Sorvall RC3B refrigerated centrifuge.

Vitamin E (alpha-tocopherol) in plasma was quantitated by high pressure liquid chromatography (HPLC) using the method of Hatam and Kayden [6]. Freshly obtained plasma was treated with pyrogallol. Vitamin E as alpha-tocopherol in pyrogallol-treated extract was separated with reverse phase C-18 column (Waters, Millipore, Milford, MA), 95% methanol solvent system and an HPLC system (Waters) attached to a multiwavelength UV/visible light detector set at 292 nm. Vitamin E values are expressed both as mg% and μmol total lipids in the plasma. Total lipids in the plasma were determined by adding together the triglyceride, the cholesterol and the phospholipid values. Plasma triglyceride and cholesterol measurements were obtained from the clinical chemistry laboratory of the Louisiana State University Medical Center; total phospholipids were determined from the plasma lipid extracts using chloroform-methanol (2:1) and measuring phospholipid-phosphorus [7].

Whole blood clotting time of ACD blood was determined by recalcification as described by Dacie and Lewis [8]. For this assay, 0.2 ml of blood was transferred to a plastic cup in the central well of the BBL Fibrosystem coagulometer at 37°C. After 30 s, 0.1 ml of 25 mM calcium chloride solution was added to initiate clotting. The addition of calcium chloride automatically starts the timer and releases the clot probe into the cup. To test the in vitro effect of vitamin E on whole blood clotting time, vitamin E was added to the blood before recalcification. Since vitamin E was dissolved in alcohol, a control for clotting time was established by using the same volume of alcohol for each blood sample and treatment.

The criteria for subject entry into this study included exclusion of those mothers with premature delivery (gestational age <38 weeks), with smoking habit (≥ 1 pack a day), with sickle cell trait or disease, jaundice, chlamydia, chorioamnionitis, or gonorrhea infection, or with a history of hypertension and drug abuse. Information about the patient's gestational age and any complications was obtained from the hospital records after completion of the analyses and were excluded from this study before data analysis. We have prior approval from the Institutional Human Experimentation Committee to use blood of mothers and placental cord for this study.

Statistical analyses were carried out using the non-paired Student's t-test and

Pearson correlation coefficient (r) using Sigma Plot 4.1 statistical software with an IBM computer.

3. Results

Figure 1 illustrates vitamin E levels in the plasma of mothers and their newborns. There was a significantly lower level of vitamin E in neonatal plasma compared with maternal plasma. Vitamin E levels were 0.26 ± 0.02 mg% or 1.91 ± 0.15 nmol/μmol total lipids in newborns and 1.13 ± 0.06 mg% or 2.67 ± 0.14 nmol/μmol total lipids in their mothers. The differences between mothers and newborns were significant irrespective of whether the values are expressed in mg% of plasma or after normalization with total lipids in plasma.

Figure 2 illustrates maternal and neonatal whole blood clotting time. Neonatal blood took 23% less time to clot compared with the maternal blood, suggesting that neonatal blood clots faster than maternal blood.

Figure 3 shows the relationship between vitamin E levels and whole blood clotting time of neonatal blood. The blood of newborns with higher levels of vitamin E took longer time to clot than the blood of newborns with lower vitamin E levels. This suggests that lower vitamin E levels may contribute to hypercoagulability of neonatal blood.

To determine whether low levels of vitamin E contribute to the hypercoagulability of blood in newborn infants, we examined the effect of an in vitro addition of vitamin E on whole blood clotting time of cord blood. Figure 4 shows that the addition of vitamin E prolonged whole blood clotting time.

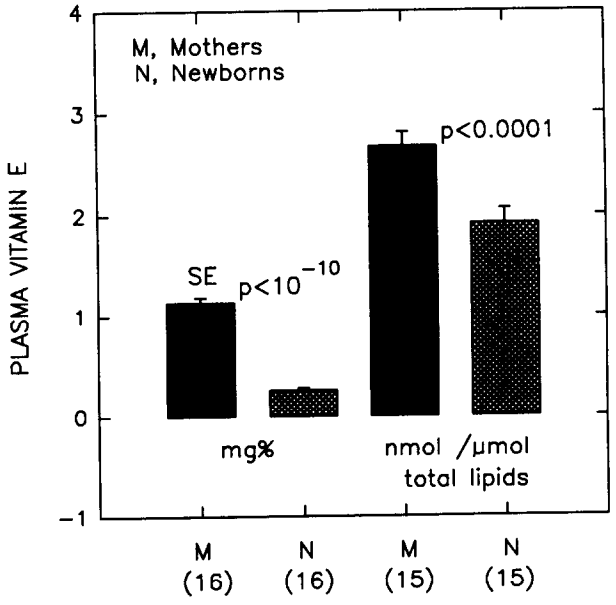

Fig. 1. Plasma vitamin E levels in blood of paired mothers and newborns. Values are mean ± S.E. Note that newborns have significantly lower vitamin E levels even after normalization with total lipids.

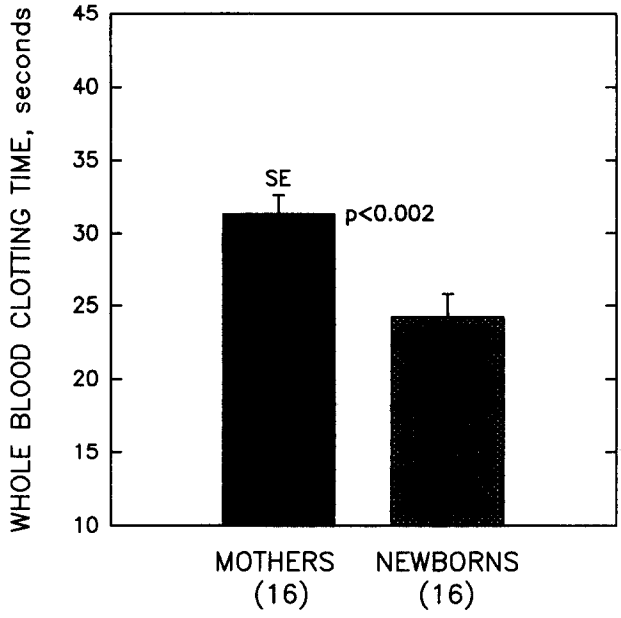

Fig. 2. Maternal and neonatal whole blood clotting time. Values are mean ± S.E. Note that neonatal blood took significantly less time to clot compared with maternal blood.

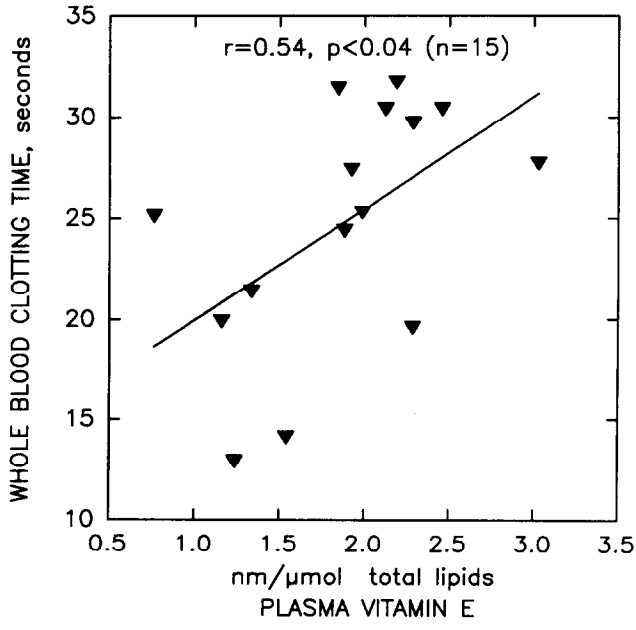

Fig. 3. Relationship between whole blood clotting time and plasma vitamin E levels in cord blood. Note a modest but significant relationship between higher plasma vitamin E levels and prolonged whole blood clotting time among newborn infants.

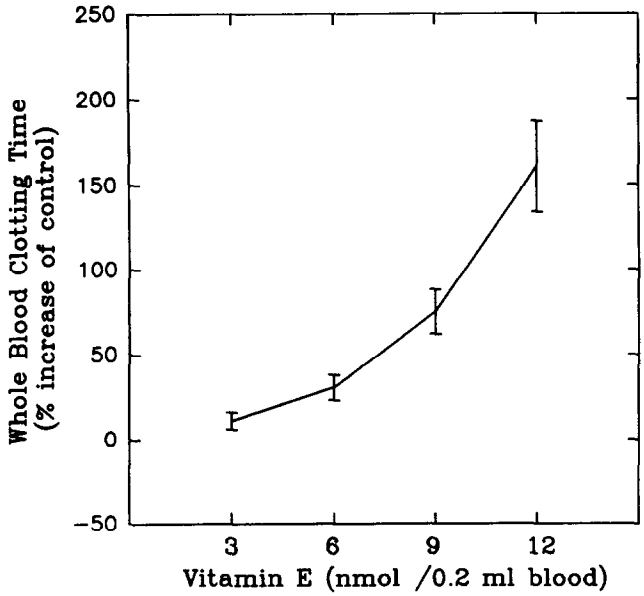

Fig. 4. Effect of in vitro vitamin E addition on whole blood clotting time of cord blood. Values are mean ± S.E. of separate experiments with six cord blood samples. Different concentrations of vitamin E in 10 μl alcohol were added to blood before recalcification in a coagulometer. Control clotting times were obtained by adding 10 μl alcohol alone to each blood sample.

4. Discussion

Vitamin E is a well-known physiological antioxidant and is structurally similar to vitamin K [9]. Adamstone [10] first reported the obstruction of blood vessels in the yolk sac of chicks from hens fed on vitamin E-deficient diets and suggested that vitamin E may have some effect on blood coagulation or thrombosis. In 1948, Zierler [11] demonstrated that alpha-tocopherol phosphate has an antithrombic activity both in vitro and in vivo. Kay et al. [12] confirmed this activity and reported that alpha-tocopherol appears to have antithrombic activity at normal concentrations in human plasma and might be an antithrombin and antimetabolite of vitamin K. Increased platelet aggregability and its reversal after oral vitamin E supplementation has been reported in vitamin E-deficient children [13] and in platelets of vitamin E-deficient older people [14]. In experimental models, thromboses were discovered in fetal rats whose dams were vitamin E deficient [15].

Studies of Diez Marques et al. [16] have shown that vitamin E supplementation significantly reduced platelet aggregation of normal rats. In addition, they found a highly significant prolongation of the plasma clotting time in vitamin E-supplemented rats and concluded that vitamin E supplementation can induce a hypocoagulable situation [16]. On the other hand, in vitro treatment of rabbit platelets with *t*-butylhydroperoxide resulted in membrane lipid peroxidation and hyperaggregability that was prevented by vitamin E [17]. Similarly, Yoshikawa et al. [18]

found that vitamin E blocked endotoxin-induced disseminated intravascular coagulation in rats.

This study documents a relationship between lower vitamin E levels and hypercoagulability of blood in newborn infants. Additionally, this study shows that the in vitro addition of vitamin E to cord blood can prolong clotting time. This suggests that lower vitamin E levels contribute to the increased coagulability of neonatal blood. The low level of vitamin E in newborns may be due to the relative impermeability of the placenta to vitamin E or to the low level of lipoproteins that transport vitamin E in newborns compared with mothers [1]. Vitamin E is structurally similar to vitamin K [9]. The anticoagulant effect of vitamin E probably occurs because it can act as an antimetabolite of vitamin K and therby act as an antithrombin and prolong the clotting time. In vitro, vitamin E has also been shown to inhibit platelet adhesion, which can also inhibit coagulability [19]. In vivo, vitamin E can also scavenge lipid peroxidation and decrease oxidative damage to proteins and lipids, which otherwise could participate in the activation of the coagulation cascade [20,21].

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