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Alpha-Tocopherol Concentration in Fetal and Maternal Tissues of Pregnant Rats Supplemented with Alpha-Tocopherol

M. Viana C. Barbas M. Castro E. Herrera B. Bonet

Facultad de Ciencias Experimentales y Técnicas, Universidad San Pablo-CEU, Division of Pediatrics, Fundación Hospital Alcorcón, Madrid, Spain

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Alpha-tocopherol · Pregnancy · Newborns

Abstract
We wanted to determine whether alpha-tocopherol supplementation to pregnant rats could increase the concentration of alpha-tocopherol in maternal and fetal plasma and tissues. Pregnant rats were treated with alpha-tocopherol on days 18 and 19 gestation and studied at day 20. A control group was studied in parallel. Treatment of pregnant rats with alpha-tocopherol increased its concentration in maternal and fetal plasma, in all maternal plasma lipoprotein fractions, in maternal and fetal liver and in the placenta. The fetal and maternal concentration of alpha-tocopherol were positively correlated.

Introduction

Oxygen radical injury may be a common mechanism for several neonatal diseases. Retinopathy of prematurity, bronchopulmonary dysplasia, intraventricular hemorrhage and congenital malformations related to diabetic pregnancy have been linked to an increased generation of free radicals and lipid peroxidation [1–4]. Some studies have shown a reduction in the severity of these diseases with the administration of vitamin E during early pregnancy, prior organogenesis in the case of fetal malformations [4] or early after birth [2, 3]. The damage induced by episodes of hypoxia and ischemia/reperfusion during gestation, delivery and early postnatal life, are partially due to an increased production of free radicals [5]; therefore, they may also be prevented with the administration of alpha-tocopherol.

Of all known antioxidants, alpha-tocopherol seems to be best suited for use during pregnancy. No side effects have been described when administered in high doses [6] and only

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Dr. B. Bonet
Department of Pediatrics, Fundación Hospital Alcorcón
C/Budapest 1
E–28922 Alcorcón, Madrid (Spain)
E-Mail bbjbonet@fhalcorcon.es

one study has raised the possibility of increasing the risk of hemorrhages [7]. Alpha-tocopherol lipid solubility allows its broad distribution among the different tissues [8] and it is one of the most efficient chain-breaking antioxidants [8].

Because of these unique characteristics, we wanted to determine how the oral administration of alpha-tocopherol during late pregnancy in the rat modified its concentration in both maternal and fetal tissues.

Subjects and Methods

Animals and Experimental Design

Virgin female Wistar rats from our own colony, weighing 200–220 g, were housed in a temperature-controlled room (22 ± 1°C). The care and handling of the animals throughout the study followed the current law for Animal Care of the European Community (Strasburg March 18, 1986). Rats were mated and the day that sperm appeared in the vaginal smears was considered day 0 of gestation. The rats were divided into two groups, one receiving 150 mg of alpha-tocopherol (Sigma, St. Louis, Mo., USA), administered by gavage, on days 18 and 19 of gestation, and the second group not receiving any treatment. On day 20 of pregnancy the animals were decapitated and their blood was collected from the neck wound in EDTA (1 mg/ml). The two uterine horns were immediately dissected and the fetuses decapitated for bleeding into EDTA (1 mg/ml). The plasma of all fetuses from the same dam was pooled and processed as a single sample. The placenta and maternal and fetal liver were removed, immediately placed into liquid N_2 and stored at –80°C until the assay for alpha-tocopherol.

Lipid Analysis

To isolate the different lipoprotein fractions maternal plasma was subjected to ultracentrifugation in a TLA 100.2 Beckman rotor (Palo Alto, Calif., USA) using a TL-100 Beckman ultracentrifuge. Briefly, the plasma (density 1.006 g/ml) was centrifuged at 100,000 rpm for 3 h and the floating supernatant containing the VLDL fraction was stored at –80°C. Density in the infranatant was raised to 1.063 with KBr and centrifuged at 100,000 rpm for 3 h. The floating supernatant containing the IDL-LDL fraction was removed and stored at –80°C. The procedure was repeated after raising the infranatant density to 1.21 g/ml with KBr, but centrifuged for 6 h, in order to obtain the HDL fraction in the floating supernatant. All the floating supernatants were obtained by tube slicing. Triacylglycerol and cholesterol were measured in total plasma and in the lipoprotein fractions with a Hitachi 705 autoanalyzer (Boehringer Mannheim, Mannheim, Germany) and alpha-tocopherol was determined by HPLC following the method previously described [9, 10].

Alpha-Tocopherol Determination in Tissues

Placenta aliquots (approximately 50 mg) were sonicated in the presence of 200 µl of phosphate buffer (5 m*M*, pH: 7.4), 50 µl of vitamin K (0.56 mg/ml, as internal standard) and 1 ml of hexane. The homogenates were centrifuged at 1,500 *g* for 15 min and the supernatant collected. The pellet was submitted to the same procedure with another ml of hexane and the supernatant pooled with the previous one. The extract was dried with a centrifuge concentrator and redissolved in chloroform:methanol (1:1). 50 µl of alpha-tocopherol (8 µg/ml) was subjected to the same procedure as the samples and used as external standard. Alpha-tocopherol was quantified following the procedure previously described for plasma [9].

Since the liver was more easily homogenized than the placenta, no addition of phosphate buffer was needed during the extraction procedure.

The inter- and intraassay variabilities for alpha-tocopherol were, respectively, 5.7 and 7.2% in placenta and 5.4 and 2.95% in liver.

Statistical Analysis

The means ± SEM are given. The significance of the difference between the means of the two groups was obtained with the Student's t test and the correlation between samples was estimated by linear regression analysis, using the Systat program (Systat, Inc., Evanston, Ill., USA).

Results

On day 20 of gestation, pregnant rats receiving vitamin E during days 18 and 19 of gestation showed a higher concentration of plasma alpha-tocopherol than the nontreated rats, either expressed in absolute values or per

Table 1. Alpha-tocopherol concentration in maternal plasma, lipoprotein fractions, liver and placenta of pregnant rats supplemented with vitamin E

	Treated (n = 13)	Control (n = 11)
Total plasma		
μg/dl	3,344.1 ± 417.3	1,649.8 ± 422.7**
μg/mg cholesterol	42.0 ± 5.2	15.6 ± 4.1***
VLDL		
μg/dl	2,803.3 ± 694.8	1,755.9 ± 516.9
μg/mg cholesterol	118.4 ± 29.3	49.2 ± 14.5*
IDL-LDL		
μg/dl	337.9 ± 51.8	120.9 ± 18.0**
μg/mg cholesterol	27.3 ± 4.2	8.2 ± 1.2***
HDL		
μg/dl	921.6 ± 119.6	446.0 ± 121.9*
μg/mg cholesterol	20.8 ± 2.7	8.5 ± 2.3**
Liver, μg/g tissue	150.8 ± 32.6	25.2 ± 4.3**
Placenta, μg/g tissue	30.5 ± 2.6	14.3 ± 2.1***

Treated vs. control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

mg of cholesterol (table 1). Alpha-tocopherol was found in all the lipoprotein fractions (VLDL, IDL-LDL and HDL), although the greatest amount appeared in the VLDL fraction (table 1). As would be expected, the alpha-tocopherol concentration in the different lipoprotein fractions of the supplemented animals was significantly higher than in the non-treated rats (table 1).

A higher concentration of alpha-tocopherol was found in the liver and placenta of treated rats compared to the non-treated rats (table 1) ($p < 0.001$). When individual values of alpha-tocopherol concentration in maternal plasma, LDL and HDL were plotted against placental concentration, a highly positive correlation was seen (fig. 1A). In contrast, only a slight correlation was found between the alpha-tocopherol content in VLDL and placenta (fig. 1A).

Alpha-tocopherol concentration in fetal plasma and liver always appeared lower than in their dams. The fetuses of dams supplemented with alpha-tocopherol showed higher levels of alpha-tocopherol, both in plasma and liver, than the fetuses of nontreated rats (table 2; $p < 0.01$). In contrast, no difference in the fetal brain alpha-tocopherol content was found between the supplemented and the nonsupplemented fetuses (table 2). When all

Table 2. Alpha-tocopherol concentration in plasma and liver of fetuses from pregnant rats treated with vitamin E

	Treated (n = 11)	Control (n = 9)
Total plasma		
μg/dl	322.6 ± 40.8	148.1 ± 36.6**
μg/mg cholesterol	6.1 ± 0.8	2.8 ± 0.7**
Liver, μg/g tissue	5.7 ± 0.8	2.4 ± 0.3**
Brain, μg/g tissue	3.9 ± 0.4	3.7 ± 0.2

Treated vs. control: ** $p < 0.01$.

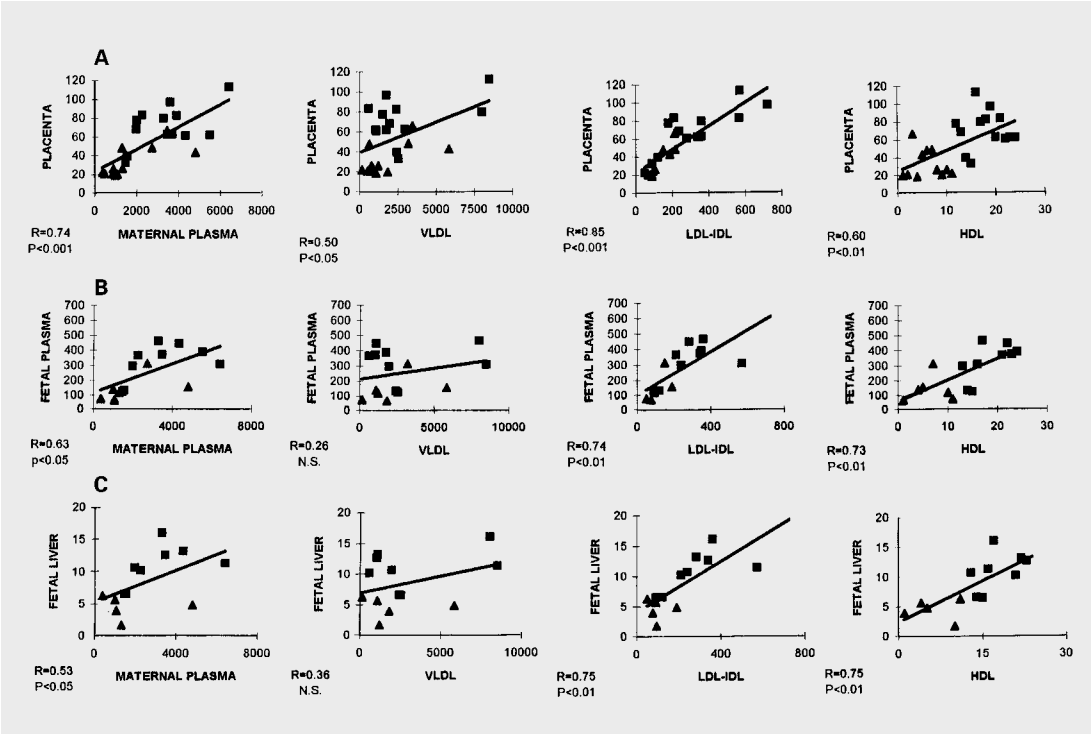

Fig. 1. Correlation of alpha-tocopherol concentration between maternal plasma and the different lipoprotein fractions with the placenta (**A**), fetal plasma (**B**) and fetal liver (**C**). For these correlations all the animals were used. ■ = Values of rats supplemented with alpha-tocopherol; ▲ = nonsupplemented rats. Both in plasma and all the lipoprotein fractions, the alpha-tocopherol values are shown as μg/dl. In the tissues the values are expressed as μg/g tissue.

the animals were plotted together, a linear and positive correlation was found between the alpha-tocopherol content in maternal plasma, HDL and LDL and the fetal plasma and no correlation was observed between the alpha-tocopherol content in the VLDL and the fetal plasma (fig. 1B). A similar correlation was found with the alpha-tocopherol content of fetal liver (fig. 1C). Finally, as expected a lineal and positive correlation was observed between placenta and fetal plasma ($r = 0.7$; $p < 0.01$) (fig. 2) vitamin E content.

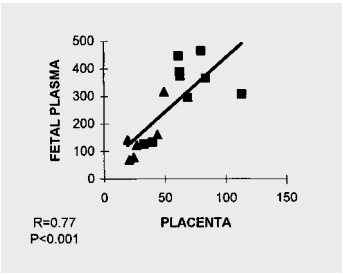

Fig. 2. Correlation of the alpha-tocopherol concentration between the placenta and the fetal plasma. As in figure 1, for this correlation, all animals were used.

Discussion

Present findings show that in the rat, the administration of alpha-tocopherol during late pregnancy leads to an enrichment of alpha-tocopherol in both maternal and fetal tissues. A greater store of vitamin E in the fetus at late gestation could facilitate a better adaptation to the extrauterine life, when the newborn is submitted to an increased oxygen concentration and the free radical scavenger enzymes activity and the concentration of most antioxidants are lower than in adult animals [11–13]. This could be specially relevant in those situations with increased risk for free-radical-induced injuries, as it occurs in the event of hypoxia and ischemic/reperfusion injury or in the premature newborns, which usually are submitted to higher O_2 concentrations and their antioxidant systems have lower activity than the term newborns or in the adult life [11–13]. Therefore, the capabilities of the fetus to increase its antioxidant stores when given to the mother, as was found here for alpha-tocopherol, could constitute an efficient prevention therapy under risk conditions such as those described above.

The higher levels of vitamin E found in the placenta of treated animals may also protect against some diseases associated with increased lipid peroxidation in the placenta as it is thought to occur in preeclampsia [14, 15]. In this situation, the release of lipid peroxides from the placenta seems to play a role in the altered endothelial relaxation found [14, 15].

As would be expected, the alpha-tocopherol administration to pregnant rats led to an alpha-tocopherol enrichment in all the lipoprotein fractions. The highest concentration appeared always in the VLDL fraction, which fits with the fact that in the pregnant rat this is the most abundant lipoprotein in plasma [16]. However, despite VLDL being the main carrier of vitamin E, no correlation was found between the alpha-tocopherol content in this lipoprotein and the content in fetal plasma and liver. This could be related to the fact that the rat placenta has very little LPL activity [17] and that the transfer of alpha-tocopherol from VLDL to the tissues seems to be associated with VLDL triglyceride hydrolysis [18, 19], a process that does not seem to occur in rat placenta [17]. In contrast, a highly positive correlation was found between the alpha-tocopherol content in LDL and HDL and fetal plasma and liver. Both lipoproteins, through different mechanisms, are able to transfer alpha-tocopherol to the tissues, while LDL transfers vitamin E through the apo B receptor present in the placenta [18, 19]. HDL could transfer alpha-tocopherol to the placenta by passive transfer as has been shown for cholesterol [20], by a gradient mechanism, and, therefore, higher levels of alpha-tocopherol in HDL would lead to transfer of this vitamin to the placenta cell membrane with lower alpha-tocopherol content. A similar mechanism is thought to occur between HDL and red blood cells [18, 19].

Despite 2 days' supplementation with alpha-tocopherol no changes in alpha-tocopherol concentration were found in the brain. This lack of enrichment of alpha-tocopherol in fetal brain may be related to the low turnover of alpha-tocopherol in the brain, as has been shown in adult animals [21]. Therefore, a longer period of supplementation may be needed to attain alpha-tocopherol enrichment in this organ. This would be in agreement with the fact that both brain depletion of alpha-tocopherol with deficiency and brain enrichment with supplementation takes longer than in other organs [21].

In summary, the present results show that alpha-tocopherol administration to pregnant rats leads to an alpha-tocopherol enrichment of both maternal and fetal tissues, which may constitute a mechanism for prevention of

some of the diseases associated with free radical generation during pregnancy and the newborn period. The main source of alpha-tocopherol for the placenta and fetal tissues seems to be mediated by LDL and HDL.

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