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Effect of Different Doses of Vitamin E on the Incidence of Malformations in Pregnant Diabetic Rats

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Key Words
Teratogenesis · Vitamin E · Pregnancy · Free radicals · Diabetes

Abstract
Background/Aims: Previously we have shown that administration of 150 mg of vitamin E (α-tocopherol) per day to rats having diabetes decreases the rate of embryo malformations and increases their maturation and size. The present study was addressed to determine the effects of different doses of vitamin E upon these parameters. ***Methods:*** Female rats were made diabetic (D) with streptozotocin, and from day 0 of gestation they were treated daily with 25 (D+25), 50 (D+50), 100 (D+100), 150 (D+150), and 500 (D+500) mg of vitamin E administered orally and were compared with control (C) animals. ***Results:*** On day 11.5 of gestation, crown-rump length, somite number, and protein and DNA levels were lower in D than in C embryos. Crown-rump length and somite number increased with 100 mg or higher doses of vitamin E, although the values observed in C embryos were not reached. The proportions of reabsorption and malformations were 24.7 and 50%, respectively, in D rats, and in the rats supplemented with vitamin E they decreased to 22.7 and 19% in D+25, 16.4 and 21.3% in D+50, 16.2 and 12% in D+100, 12.9 and 13.9% in D+150, and to 43.9 and 10.8% in D+500 rats, whereas the values were 6.8 and 4.9% in C animals. ***Conclusions:*** Administration of vitamin E to D rats decreases the rate of embryo malformations, dependent on the dose administered. However, high doses have a negative effect in the conceptus, as shown by the increased rate of reabsorptions in the D+500 group.

Introduction

In women having diabetes, improvement of the metabolic control prior to conception and, in pregnancy, during the early phases of embryonal development decreases the rate of fetal malformations [1–4]. Nevertheless, in the clinical setting, the achievement of optimal metabolic control prior to conception is not always possible; therefore, any method able to decrease the incidence of malformations, not related to the degree of metabolic control, may have a major impact in clinical practice.

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Over the past few years, we and others have shown in experimental animal models that administration of vitamin E as well as other antioxidants decreases the rate of embryonal malformations [5–7], despite no improvement in the metabolic control. Administration of vitamin E to pregnant rats having diabetes decreases the liver lipid peroxide concentrations and the number of DNA base alterations [8], both a cause of the reaction of free radicals with lipids and DNA. Therefore, the above results link the diabetic teratogenic effects with increased oxidative stress and free radical damage.

Among the antioxidants used to prevent the teratogenic effects of diabetes in the experimental animal model, vitamin E seems the most suitable to be used in human pregnancy. In humans, although no major side effects have been described when vitamin E is administered at high doses [9], some studies have raised the possibility of an increased risk of cerebral hemorrhages [10, 11]. Therefore, before considering its use in human diabetic pregnancies, there is a need of more experimental studies regarding the minimal effective dose and the potential toxic effects to the developing embryo of high doses of vitamin E. In fact, in a previous study performed by Siman and Eriksson [7], the administration of low doses of vitamin E to pregnant rats having diabetes did not decrease the rate of embryo malformations, being only effective when it was administered at a high dose.

The present study was addressed to determine whether the prevention of the teratogenic effects of diabetes by vitamin E is dose dependent.

Material and Methods

Animals and Experimental Design

Female virgin Wistar rats from our own colony, weighing 190–220 g, were housed in a temperature-controlled room (22 ± 1°C) with 12-hour light-dark cycles and fed a commercially available diet (Purina Rat and Mouse Standard Diet). The care and handling of the animals throughout the study followed the current animal care law of the European Community (Strassburg, France, March 18, 1986). Diabetes was induced by the administration of a single intravenous dose of streptozotocin (45 mg/kg; Sigma Chemical, St. Louis, Mo., USA) in citrate buffer (0.05 mol/l; pH 4.5). After 5 days, the state of insulin deficiency was confirmed by the presence of a positive urine reaction by Chemstrip for both glucose and ketone bodies, and insulin replacement was started. The insulin dose was chosen on the basis of our previous laboratory experience [5]. 1.5 IU/100 g body weight of MC Lente Novo insulin (90% bovine, 10% porcine; Novo Nordisk, Bagsværd, Denmark) was administered subcutaneously between 09.00 and 10.00 h.

The animals were mated with untreated control animals, and on the same day that sperm appeared in vaginal smears (day 0 of gestation), they were divided into six groups: one group of diabetic (D) animals without any supplementation and five groups of D rats supplemented with increasing doses of vitamin E: 25 (D+25), 50 (D+50), 100 (D+100), 150 (D+150), and 500 (D+500) mg/day, administered orally by gavage. A group of normal rats was also studied in parallel (controls, C).

The rats were decapitated on day 11.5 of gestation which corresponds to the end of the embryonal period. Blood was collected in EDTA (1 mg/ml) and plasma separated by centrifugation at 4°C and kept at –20°C until processed. The two uterine horns were immediately dissected and immersed at room temperature in saline contained in a Petri dish (100 mm). Embryos and investing membranes were teased apart with fine jeweler's forceps, during visualization through a dissecting microscope (model 212T OPM; Carl Zeiss, Jena, Germany). The yolk sac was isolated from the surrounding decidua and the embryo removed. In all embryos the crown-rump length and the number of somites were determined. The embryos were inspected under the microscope to determine whether the morphology of brain spheres, neural tube, heart, optic and otic vesicles, limb buds, and axial curvature conformed to that expected on day 11.5 of gestation. Embryos not conforming to normal morphology in any of the above structures were considered dysmorphic. When the decidua was present but the yolk sac or the embryo was not, it was considered a reabsorption. After visual inspection, the embryos were divided into two groups, in one of them the embryos were introduced into 0.1 *N* NaOH and analyzed for total protein [12] and DNA [13]; in the other, embryos were stored in phosphate-buffered saline at –70°C for vitamin E determination.

Processing and Analysis of Samples

An aliquot of plasma was used to determine the glucose level (God-Pad enzymatic colorimetric test; Boehringer Mannheim, Germany), whereas another aliquot was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and used for β-hydroxybutyrate determination [14]. Vitamin E was determined by high-performance liquid chromatography following methods previously described [15], in both plasma and embryos.

Statistics

Mean values ± SEM are given. The significance of the differences between the mean values of the two groups was obtained by analysis of variance and the Tukey HSD test for multiple comparisons, using the Systat program (Systat, Evanston, Ill., USA). The χ^2 test was used to detect differences in the rates of malformations and reabsorptions between the different experimental groups.

Results

Metabolic Parameters

Plasma glucose and β-hydroxybutyrate were measured as indices of the metabolic condition of the animals. As shown in table 1, the plasma concentrations of these two variables were higher in the D animals than in the C group, independently of the administration of vitamin E. Administration of different doses of vitamin E had only small effects on plasma levels: variable, slightly reducing plasma glucose in D rats receiving the 500-mg dose and

Table 1. Metabolic parameters in D rats supplemented with vitamin E

Group	Number of animals	Glucose mmol/l	β-Hydroxybutyrate µmol/l	Vitamin E µmol/l
C	17	6.8 ± 0.2	85.0 ± 5.9	16.4 ± 22.9
D	16	28.5 ± 1.7***	329.1 ± 55.0***	20.4 ± 2.3
D+25	6	21.5 ± 4.3**	167.5 ± 29.8	58.9 ± 20.0
D+50	12	26.2 ± 3.0***	374.2 ± 79.2***	70.3 ± 13.6***, †††
D+100	9	26.3 ± 3.0***	287.7 ± 37.0**	81.7 ± 17.2***, †††
D+150	11	25.3 ± 3.2	301.8 ± 61.2	75.5 ± 6.6***, †††
D+500	15	19.8 ± 3.6	244.8 ± 13.4	117.6 ± 33.8***, †††

* C versus D rats with and without treatment: ** $p < 0.01$; *** $p < 0.05$.
† D rats without versus with treatment: ††† $p < 0.05$.

Table 2. Number of somites, crown-rump length, and total protein and DNA contents in 11.5-day-old rat embryos

Group	Number of embryos	Number of somites	Crown-rump length, mm	Protein, µg	DNA, µg	Vitamin E, ng
C	164	29.0 ± 0.1	4.0 ± 0.03	340.3 ± 13.8	55.2 ± 4.2	103.3 ± 4.2
D	134	26.2 ± 0.2***	3.3 ± 0.04***	254.7 ± 15.2**	45.6 ± 3.7	70.2 ± 2.4
D+25	58	27.2 ± 0.2***	3.6 ± 0.04***	273.7 ± 15.1	44.7 ± 4.3	131.8 ± 6.9†
D+50	122	27.0 ± 0.3***	3.5 ± 0.04***	278.1 ± 16.7	48.1 ± 4.7	148.9 ± 12.0†††
D+100	150	27.2 ± 0.2**, ††	3.6 ± 0.03***, †††	263.1 ± 16.6**	45.2 ± 4.0	276.4 ± 14.7***, †††
D+150	115	27.1 ± 0.2***, †††	3.6 ± 0.04***, †††	266.6 ± 20.4*	44.1 ± 5.7	242.8 ± 26.3***, †††
D+500	74	27.6 ± 0.2***, †††	3.7 ± 0.03***, †††	274.0 ± 9.8	49.4 ± 3.1	482.5 ± 61.4***, †††

Protein DNA, and vitamin E contents were determined only in 20 embryos of each group.
* C versus D rats with and without treatment: * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$.
† D rats without versus with treatment: † $p < 0.001$; †† $p < 0.01$; ††† $p < 0.05$.

β-hydroxybutyrate in those receiving the 25- or the 500-mg dose. The vitamin E levels in plasma were similar in D and C animals (table 1), and, as expected, higher levels were observed in D animals supplemented with vitamin E when compared with either C or untreated D animals, the differences being statistically significant only when the rats were supplemented with doses of vitamin E of 50 mg or higher doses (table 1).

Embryos

The embryos of D rats had significantly lower crown-rump lengths, somite numbers, and DNA and protein concentrations than C embryos (table 2). When vitamin E was administered to D rats, no effects were observed upon the DNA and protein contents (table 2). Somite number and crown-rump length improved by treatment with vitamin E, although these differences only reached statistically significant values in the D groups supplemented with 100, 150, and 500 mg of vitamin E. The amount of vitamin E in embryos from D rats was slightly lower than in C embryos, although not significant, and the administration of vitamin E increased in a dose-dependent manner the total content of vitamin E in embryos (table 2). The incidences of malformations and reabsorptions were 4.9 and 6.8%, respectively, in C animals (table 3). As expected, the incidences of both malformations and reabsorptions raised dramatically in D animals, reaching values of 24.7 and 50%, respectively (table 3). The rate of malformations decreased to 19.0 and 21.3%, respectively, in D+25 and D+50 rats; a further decrease was observed with higher doses, reaching a plateau of approximately 12% from 100 mg of vitamin E (table 3). The rate of reabsorptions

Table 3. Rates of malformations and reabsorptions

Group	Number of sacs	Yolk sacs with reabsorptions		Embryos			Malformations and reabsorptions	
				total number	malformed			
		n	%		n	%	n	%
C	176	12	6.8	164	8	4.9	20	11.4
D	178	44	24.7***	134	67	50.0***	111	62.4
D+25	75	17	22.7***	58	11	19.0***, †††	28	37.3
D+50	146	24	16.4**	122	26	21.3***, †††	50	34.2
D+100	179	29	16.2**, †	150	18	12.0***, †††	47	26.3
D+150	132	17	12.9††	115	16	13.9***, †††	33	25.0
D+500	132	58	43.9***, †††	74	8	10.8***, †††	66	50

* C versus D rats with and without treatment: ** $p < 0.01$; *** $p < 0.05$.
D rats without versus with treatment: † $p < 0.001$; †† $p < 0.01$; ††† $p < 0.05$.

also decreased with the administration of vitamin E, from 24.7% in the D group to 22.7, 16.4, 16.2, and 12.9%, respectively, in D+25, D+50, D+100, and D+150 rats, but doses of 500 mg increased the rate of reabsorptions, reaching a value of 43.9% which is even higher than that found in non-supplemented D rats (table 3).

Discussion

In the present study, we have demonstrated that the prevention of the diabetic teratogenic effects by vitamin E is dependent on the dose administered. These results confirm and expand previous data showing a reduction in the teratogenic effects of diabetes with the administration of antioxidants [5–8] and support the role of free radical mediated teratogenesis in the diabetic rat.

Although an improvement in the rates of reabsorption and malformations was observed with doses of 25 and 50 mg, the maximal benefit in both parameters was found with doses of 100 or 150 mg. These results agree with those of previous studies performed by other authors, showing that the prevention of teratogenic effects of diabetes by vitamin E is dose related [6, 7]. In these studies the optimal dose found was higher (300–400 mg/day), and in one of them [7], the optimal benefit was reached when vitamin E was administered continuously in the diet rather than as a bolus. The differences between us and the other groups in the optimal dose needed to obtain the maximal benefit from vitamin E administration may be related to the fact that different species and strains of rats were used. Since it is a fact that the teratogenic effects of diabetes vary among different strains of rats [16], interspecies differences in the doses of vitamin E needed to obtain maximal benefits for the reversion of malformations and reabsorptions caused by diabetes could be expected. In the present study 500-mg doses of vitamin E not only did not improve the malformation rate, but significantly increased the rate of embryo reabsorptions, suggesting that in the pregnant rat having diabetes high doses of vitamin E may be harmful. This effect has not been demonstrated before, because the vitamin E doses tested previously [6, 7] were always below the 500 mg used in the present study. Nevertheless, Siman and Eriksson [7] already found a slight tendency toward an increasing rate of reabsorptions when high doses of vitamin E were administered to nondiabetic rats.

The nature of this toxic effect is as yet unknown; nowadays in humans some studies have raised the possibility of an increased risk of hemorrhages when vitamin E was used in clinical trials [10, 11]. Furthermore, several studies [17, 18] have shown a decreased platelet aggregation in diabetic and nondiabetic subjects supplemented with high doses of vitamin E. In fact, in our D-500 group, of 15 animals studied, 6 were found to have hemorrhaged tissue in the uterine horn, and this effect should be considered before using vitamin E as prophylactic treatment for embryonal malformations in human diabetic pregnancies.

Despite the different doses of vitamin E used, the incidence of embryo malformations in D rats always remained higher than in the C animals. This is not surprising, since it is well established that the origin of the terato-

genic effects of diabetes is multifactorial [19, 20], and, therefore, free radical scavengers may not prevent all of them.

In summary, the present study shows that although oral administration of vitamin E to pregnant rats having diabetes decreases the rates of embryo malformations and reabsorptions, this effect is dependent on the dose administered, and high doses appear to be toxic to the conceptus. This finding obliges to introduce caution in clinical trials with different doses of vitamin E, if vitamin E is going to be administered to pregnant diabetic women, since small doses may not be effective and high doses toxic.

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