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Antioxidant therapy and streptozotocin-induced diabetes in pregnant rats

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Abstract The aim of our study was to analyse the effect of chronic hyperglycaemia on lipid peroxidation and scavenging enzyme activity in pregnant animals and their offspring supplemented and not supplemented with vitamin E – a natural antioxidant. Thirty pregnant female Wistar rats were used in our experiments. Diabetes was induced on day 7 of pregnancy using a single dose of streptozotocin (40 mg/kg). Diabetic animals were divided into two equal groups: vitamin E supplemented and those fed with standard diet. Our controls consisted of 15 healthy rats. On day 1 after delivery homogenates of maternal liver and uterus as well as neonatal lungs and liver were prepared. Then the following parameters were measured: malondialdehyde (MDA) concentrations in the homogenates and blood serum, glutathione (GSH) levels, the activity of CuZn superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Bioxytech, France). Statistical analysis was performed using Mann-Whitney U test. The neonates of diabetic rats were smaller than those from healthy rats and serum glucose concentration was markedly higher in diabetic animals, both in mothers and neonates. MDA levels increased significantly, whereas GSH content and SOD as well as GPx activities were markedly diminished in diabetic pregnant rats and their offspring in comparison with the control group. In animals supplemented with tocopherol, MDA concentrations declined significantly, GSH contents and SOD activities were markedly elevated in almost all types of tissues studied, whereas glutathione peroxidase remained suppressed. Our results suggest that diabetic pregnant rats and their neonates are exposed to oxidative stress (OS), but vitamin E supplementation could in part reduce the imbalance between uncontrolled reactive oxygen species generation and scavenging enzyme activity, and may potentially serve as a useful prophylactic factor against OS development.

Key words Oxidative stress • Hyperglycaemia • Pregnancy • Diabetes mellitus • Vitamin E

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Introduction

Pregnancy complicated by poorly controlled diabetes is associated with an increased risk of abortion, congenital malformations and perinatal mortality. The mechanism of the teratogenic effect is not completely understood, but it seems to be multifactorial and associated directly with hyperglycaemia [1, 2]. The excess availability of glucose causes nonenzymatic glycation and increased mitochondrial oxidation activity, resulting in an oxidative stress state (OSS) [3]. There is also a hypothesis that decreased levels of natural antioxidants – such as vitamin E – and diminished scavenging enzyme capacity may be responsible for the excess of free radicals observed in diabetic animals [4-6]. Such conditions increase an intracellular level of hydroperoxide and promote lipid peroxidation, which inhibit prostaglandin biosynthesis – a crucial factor for normal fetal development [7, 8].

A disturbed balance between generation of reactive

oxygen species (ROS) and antioxidant defence seems to be most important during organogenesis, resulting in diabetic embryopathy, but ROS may be also involved in the pathogenesis of toxemia in pregnancy, intrauterine growth retardation, retinopathy of prematurity, bronchopulmonary dysplasia and several other conditions of human pathology [9]. Moreover, some authors suggest ROS involvement in complications of diabetic pregnancy [10, 11].

Therefore, the aim of our study was to analyse the effect of chronic hyperglycaemia on lipid peroxidation and scavenging enzyme activity in pregnant animals and their offspring supplemented and not supplemented with a natural antioxidant – vitamin E.

Materials and methods

Experimental animals

Thirty female Wistar rats weighing 180-200 g, housed in a temperature-controlled room (22 ± 1°C) with 12-h light and dark cycles, were used in our experiments. The care and handling of our animals followed European Union legislation throughout the study. The animals were fed on Rat and Mouse Standard diet. They were mated, and on the same day vaginal smears were checked for sperm (day 0 of gestation). Diabetes was induced on day 7 of pregnancy using a single dose of streptozotocin (40 mg/kg, Calbiochem Biochemicals) given intraperitoneally in citrate buffer (0.05 mol/L, pH 4.5). Then rats were divided into two groups of 15 animals each. Supplements of 30 mg of vitamin E (alpha-tocopherol acetate) were added to each 100 g of diet in the first group. The second group of animals was fed with standard, yet vitamin E-deficient diet. Our controls consisted of 15 normal rats fed with the standard diet.

On the first day after delivery the following tests were carried out: plasma glucose concentration was measured in all groups using the oxidase method (Cormay, Poland). Subsequently rats were decapitated and homogenates of maternal liver and uterus as well as neonatal lungs and liver were prepared.

Preparation of homogenates

Liver or pulmonary tissue was dissected, chopped into small pieces on ice, weighed and immediately immersed in ice- cold normal saline (0.9% NaCl). Liver and pulmonary slices were rinsed with the saline, clots washed off and homogenised in a glass piston homogenizer (MPW - 306) for 1 min. Tissue samples of 1 g each and 9 ml of ice-cold saline were used for homogenisation (10% w/v). The suspended mixture was then centrifuged at 9000 x *g* for 30 min at 4° C. The resulting supernatant served for immediate malondialdehyde (MDA) evaluation. Samples (3 mL) of adult animal blood were drawn and added to 48 μL of 0.17 mol/L ethylenediaminetetraacetic acid (EDTA), centrifuged at 2500 x *g* for 10 min at 4° C [12]. All specimens were freshly prepared.

Sample analysis

The activities of CuZn superoxide dismutase (SOD) and glutathione peroxidase (GPx) were evaluated in maternal and neonatal homogenates using the spectrophotometric method (Bioxytech SOD - 525; Oxis International S.A., France) and immunoenzyme technique (Bioxytech, pl. GPx - EIA), respectively. Enzymatic activities were expressed per mg of protein (Lowry's method) [13]. Glutathione activity (GSH) in our homogenates was assessed by the colorimetric test (Bioxytech, GSH - 400). MDA concentrations were determined in the homogenates and blood serum using colorimetric assay of lipid peroxidation (Bioxytech S.A., France, LPO - 586).

Statistical analysis

Values are expressed as mean ± SD. Statistical significance of differences was assessed by Mann-Whitney U test ($P < 0.05$ was considered significant).

Results

As shown in Table 1, the mean weight of pregnant rats before delivery did not essentially differ between the groups studied. The neonates from diabetic rats were smaller than those of healthy rats, but the difference did not achieve statistical significance. Serum glucose concentration was markedly higher in diabetic animals – both in mothers and neonates ($P < 0.05$). However, all studied groups of neonates had glucose levels within the normal range.

Serum and tissue concentrations of MDA increased markedly in diabetic pregnant rats and their neonates in com-

Table 1 Weight and blood glucose in all groups of studied animals

Animal group	Body weight (g)	Blood glucose (mmol/L)
Control (mothers)	255.81 ± 30.33	6.15 ± 1.39
Diabetes (mothers)	271.86 ± 25.54	10.67 ± 6.77*
Diabetes + vitamin E (mothers)	285.42 ± 24.67	11.87 ± 6.88*
Control (neonates)	7.06 ± 1.49	4.28 ± 0.67
Diabetes (neonates)	6.00 ± 0.79	5.27 ± 1.17*
Diabetes + vitamin E (neonates)	5.87 ± 1.07	6.59 ± 1.21**

*$P < 0.05$ between control group and diabetic animals; ** $P < 0.05$ between animals supplemented and not supplemented with vitamin E

Table 2 Malondialdehyde concentration (nmol/mg protein)

	Serum (mothers)	Liver (mothers)	Uterus (mothers)	Liver (neonates)	Lungs (neonates)
Control	0.07 ± 0.075	0.113 ± 0.009	0.229 ± 0.036	0.204 ± 0.041	0.230 ± 0.024
Diabetes	0.141 ± 0.071*	0.229 ± 0.091**	0.258 ± 0.690	0.421 ± 0.139***	0.270 ± 0.088*
Diabetes + vitamin E	0.027 ± 0.009#	0.073 ± 0.345#	0.123 ± 0.044#	0.125 ± 0.033##	0.076 ± 0.049##

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$ - between control group and diabetic animals
#$P < 0.0001$; ##$P < 0.00001$ - between animals supplemented and not supplemented with vitamin E

Table 3 CuZn dismutase activity (IU/mg protein)

	Liver (mothers)	Uterus (mothers)	Liver (neonates)	Lungs (neonates)
Control	2.175 ± 1.114	0.977 ± 0.535	1.252 ± 0.608	0.610 ± 0.353
Diabetes	1.114 ± 0.303*	0.466 ± 0.330*	0.791 ± 0.456	0.504 ± 0.254
Diabetes + vitamin E	1.904 ± 0.884#	0.930 ± 0.211##	1.422 ± 0.611#	0.796 ± 0.195#

*$P < 0.05$ between control group and diabetic animals
#$P < 0.05$; ##$P < 0.005$ - between animals supplemented and not supplemented with vitamin E

parison with the control group (Table 2). In animals supplemented with vitamin E, MDA levels significantly declined in all tissues studied ($P < 0.0001$).

Analysis of CuZn-dismutase activity revealed the highest values in liver homogenate of control rats (Table 3). SOD activity was supressed in diabetic animals, particularly adults ($P < 0.05$). In the group supplemented with tocopherol CuZn-dismutase activity was significantly higher, particularly in uterine homogenates ($P < 0.005$).

Glutathione peroxidase activity was also diminished in diabetic rats (Table 4), particularly neonates ($P < 0.05$), and remained suppressed in animals fed with vitamin E. Glutathione content was highest in liver homogenate of control rats, and above all markedly diminished in the tissues obtained from diabetic animals (Table 5). The lowest values were found in lung homogenate of neonates ($P < 0.05$). Rats supplemented with vitamin E had remarkably higher glutathione concentrations, and the difference reached statistical significance in neonatal liver and lung homogenates ($P < 0.05$ and < 0.005, respectively).

Table 4 Glutathione peroxidase activity (ng/mg protein)

	Liver (mothers)	Uterus (mothers)	Liver (neonates)	Lungs (neonates)
Control	10.648 ± 6.255	12.730 ± 4.606	9.777 ± 2.037	8.872 ± 2.258
Diabetes	7.271 ± 0.963	10.023 ± 2.054	7.683 ± 1.261*	8.347 ± 1.354
Diabetes + vitamin E	6.779 ± 1.072	8.759 ± 2.544	6.937 ± 3.217	7.049 ± 0.792

*$P < 0.05$ between control group and diabetic animals

Table 5 Glutathione concentration (μmol/L/mg protein)

	Liver (mothers)	Uterus (mothers)	Liver (neonates)	Lungs (neonates)
Control	4.409 ± 1.710	2.277 ± 1.373	3.936 ± 1.098	1.597 ± 0.455
Diabetes	4.037 ± 1.311	1.496 ± 0.666	2.823 ± 1.194	1.184 ± 0.395*
Diabetes + vitamin E	5.059 ± 1.395	1.805 ± 0.439	3.842 ± 1.252#	1.830 ± 0.674##

*$P < 0.05$ between control group and diabetic animals
#$P < 0.05$; ##$P < 0.005$ - between animals supplemented and not supplemented with vitamin E

Discussion

Maternal diabetes increases the risk of embryonic maldevelopment and malformations, but very little is known about the pathophysiological mechanism(s) involved. However, it has been demonstrated that enzymatic scavengers of oxygen free radicals suppress the teratogenic effect of a high concentration of glucose and other metabolic intermediates [4]. This suggests a common mechanism for the generation of oxygen free radicals as one of the key mediators in diabetic pregnancy complications [5]. However, measurement of oxidative stress status (OSS) has not been used in clinical diagnosis and monitoring of diabetic complications, and – more importantly – widely accepted methods for measuring OSS are not yet established. The problem is that active oxygen species and free radicals are so reactive and short living that they are difficult to estimate directly, and most methodologies rather measure lipid peroxide products, such as MDA, which reflect at least partly the oxidative activity of oxygen radicals in the tissues [14]. Highly reactive oxygen free radicals attack membrane phospholipids and cause the conversion of unsaturated fatty acids to lipid peroxides. Peroxidation of fatty acids containing three or more double bonds generates MDA [15]. Lipid peroxides are highly toxic products that damage a number of enzymes, proteins and cell membranes. Moreover, they increase the contractility of small blood vessels and augment their sensitivity to angiotensin II [8, 16-19]. Excessive lipid peroxides also inhibit the synthesis of prostaglandins – one of the crucial factors in normal fetal development [7, 8].

In our study serum and tissue MDA was used as an index of OSS in experimental animals. MDA levels were found to be increased in diabetic female rats and their infants. Our findings are consistent with other reports suggesting that lipid peroxide levels are elevated in plasma, serum, kidney, lens and erythrocyte membranes of diabetic animals [20-22]. A possible explanation for the enhancement of MDA concentration may be a decreased formation of antioxidants in diabetic tissues, which in view of augmented activity of oxygen free radicals allows a consequent increase in MDA production. Our results also revealed a decreased content of glutathione in the tissues obtained from diabetic rats. Those may reflect increased GSH consumption in diabetic animals as compared to healthy controls. The activities of peroxide scavenging system enzymes, e.g. SOD and GPx (a selenium-containing enzyme requiring glutathione) were also significantly reduced in diabetic rats. Glutathione is the substrate for GPx, an enzyme that keeps the cellular concentration of H_2O_2 and organic hydroperoxides low [23]. Interestingly, glutathione also reacts directly with free radicals and can protect cells from singlet oxygen, hydroxyl radicals and superoxide anion radicals. Our findings strongly suggest that OSS is markedly increased and the protection against oxidative stress apparently decreased in some tissues of diabetic animals.

Vitamin E supplementation seemed to at least partially reverse these effects by a suppression of MDA synthesis and its accumulation, restoring GSH content and SOD tissue activity, while GPx activity remained decreased. These findings are somewhat consistent with previous reports of other authors, who have suggested that tocopherol administration might increase intracellular GSH content in erythrocytes either by stimulating glutathione synthetase activity or by reducing GSH utilisation [24, 25]. Masugi and Nakamura [26] found that vitamin E prevents the accumulation of lipid peroxide, but does not control the levels of peroxide scavenging system enzymes such as SOD, catalase and GPx. Recently, Sivan et al. [6] reported that SOD levels were significantly reduced in diabetic animals compared with controls and were not affected by vitamin E supplementation. On the contrary, Yang et al. [27] indicated that the GPx activities in the liver and plasma were significantly lower in vitamin E deficient rats than in the animals fed with tocopherol. In our study SOD activity was found to be markedly higher in rats supplemented with vitamin E, while GPx activity remained suppressed. We believe the latter might reflect alterations in selenium metabolism occurring in pregnancy, especially complicated by diabetes.

In conclusion, our results suggest that diabetic pregnant rats and their neonates are exposed to an increased oxidative stress, but vitamin E supplementation could in part reduce the imbalance between uncontrolled ROS generation and scavenging enzyme activity, and thereby potentially serve as a simple and useful prophylactic factor.

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