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Original Articles

Effect of Vitamins A, E, C and Omega-3 Fatty Acids on Lipid Peroxidation in Streptozotocin Induced Diabetic Rats

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Abstract

Background: To assess the effect of supplementation with vitamins A, E and C and ω-3 fatty acids on lipid peroxidation in streptozotocin (STZ) induced diabetic rats.

Methods: Sixty four male wistar rats weighting 250g were divided into four groups as normal control, diabetic control, diabetic with vitamin A, E as well as C supplementation and diabetic with ω-3 fatty acids supplementation. After four weeks of treatment the rats were anesthetized and malondialdehyde (MDA) levels were investigated in blood samples, liver and heart homogenate.

Results: In diabetic rats MDA level in plasma, liver and heart was significantly more elevated than normal control rats ($P< 0.05$). Vitamin A, E and C supplementation caused significant decrease in plasma, liver and heart MDA ($P< 0.05$). A significant decrease in heart MDA ($P< 0.05$) was observed in diabetic rats with ω-3 fatty acids supplementation.

Conclusion: Supplementation of vitamin A, E and C and ω-3 fatty acids was found to decrease lipid peroxidation to some extent in diabetic rats and they can be valuable candidates in the treatment of the complications of diabetes.

Keywords: *Vitamin C, Vitamin E, Vitamin A, Omega3 fatty acid, Malondialdehyde*

Introduction

Diabetes is widely known to induce metabolic derangement leading to oxidant-antioxidant imbalance. The putative role of reactive oxygen species (ROS) in the development of diabetic complications has been investigated for several decades (1, 2). Propagating lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress, often with cytopathological consequences (3). Eukaryotic cells are equipped with a variety of primary and secondary defenses against the deleterious effects of oxidative stress. As lethal injury can occur when these defenses are overwhelmed, supplementation with antioxidant vitamins such as vitamins A, E and C may be beneficial (4). Vitamin E is a hydrophobic antioxidant found in lipoproteins and membranes and can afford primary as well as secondary stage protection. It is the most efficient scavenger of lipid peroxyl radicals (5). Vitamin C is a hydrophilic molecule that can scavenge several radicals, among them the hydroxyl radical. It is likely that vitamins E and C act in a synergistic manner, by vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vita-

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min C (6). Vitamin A is a hydrophobic antioxidant that its decrease in diabetes causes retinopathy (7). Supplementation with ω-3 fatty acids can be useful to prevent diabetic complications. Long-chain ω-3 fatty acids are incorporated into cell membranes and have anti-inflammatory properties that may be relevant for the prevention of type 1 diabetes, such as decreased expression of HLA class II molecules on activated human monocytes (8) and reduced expression of interleukin 1ß (9). The long-chain ω-3 fatty acids play an important role in eicosanoid metabolism, and there is evidence for aberrant prostaglandin metabolism in children with type 1 diabetes (10, 11). Together, these data suggest that the anti-inflammatory ω-3 fatty acids such as DHA and EPA may reduce the risk of disease development.

Thus the present study was designed to assess the effects of supplementation with vitamin A, E and C and ω-3 fatty acids on lipid peroxidation in the liver, heart and blood of streptozotocin induced diabetic rats.

Materials and Methods

Animals and diets Sixty four male Sprague-Dawley rats (15-wk-old) weighting 250g were used in this study. The rats received standard chow diet and water ad libitum during the experimental period and were maintained at an environmental temperature of 18-23 ºC with a 12-h light/ 12-h dark cycle. The rats were randomly divided into four groups of 16 rats each as normal control, diabetic control, diabetic with vitamin A, E and C supplementation and diabetic with ω-3 fatty acids supplementation. The rats in the normal control group received only standard diet and water. In the other three groups diabetes was induced by intravascular injection of a single dose of STZ (40 mg/ kg body weight). Only those animals with a blood glucose level more than 300 mg/dl a week after injection were regarded as diabetic. In the diabetic group with vitamin A, E and C supplementation each rat received vitamin A 106 mg/ kg B.W., vitamin E 250 mg/ kg B.W. and vitamin C 200mg/ kg B.W. daily by a gavage. Each rat in the diabetic group with ω-3 fatty acids supplementation daily received ω-3 fatty acids 300mg/ kg B.W. by a gavage.

Tissue preparation After four weeks of feeding period the rats were anesthetized and arterial blood samples were withdrawn with EDTA. After centrifugation of blood samples at 2500×g for 5 min, the plasma was separated and stored at -70 °C until biochemical analysis. Liver and heart tissues were promptly excised and washed with physiological saline, then dried by a filter paper and stored at -70 °C.

Preparation of tissue homogenate For homogenate preparation to determine malondialdehyde 1:11 (W/V) tissue homogenate was prepared in 50mM K phosphate buffer, pH 7.4, 150mM KCl, and 200 mM EDTA by a Potter-Elvehjem tissue homogenizer and then centrifuged at 15000×g for 30 min. Protein concentration in the homogenate was determined by the biuret assay using bovine serum albumin as a standard (12).

Lipid peroxide MDA contents in liver and heart homogenates and plasma were measured by the thiobarbituric acid (TBA) method. MDA in tissue homogenates was measured using a modified TBA assay following promotion with Fe/ascorbate (13, 14).

Statistical Analysis All values are expressed as mean±SD. Independent sample t-test was performed to compare the means of each two groups. A value of $P< 0.05$ was considered to be statistically significant. All data were analyzed using SPSS software.

Results

The values and statistical comparisons of MDA in experimental groups are shown in Table 1, 2 and 3. Lipid peroxidation as reflected by MDA level value was significantly higher in plasma ($P< 0.05$), heart ($P< 0.01$) and liver ($P< 0.05$) of diabetic rats vs. control group. Vitamins A, E and C supplementation caused significant decrease

($P< 0.05$) in MDA levels of plasma, heart and liver in diabetic rats with vitamin A, E and C supplementation vs. diabetic control group.

Supplementation with ω-3 fatty acids made no significant change in MDA levels of plasma and liver but significantly decreased heart MDA ($P< 0.01$).

Table 1: Values and statistical comparison of MDA in the samples of normal control and diabetic control groups (mean±SD).

Sample	Normal control group (n=16)	Diabetic control group (n=16)	*t*-test results
Plasma MDA (nmol/ml)	1.33±0.28	1.57±0.25	t= 2.49 Df = 30 P= 0.018
Liver MDA (nmol/mg protein)	0.44±0.12	0.57±0.15	t = 2.59 Df = 30 P=0.014
Heart MDA (nmol/mg protein)	0.82±0.17	1.08±0.32	t = 2.81 Df = 30 P=0.009

Table 2: Values and statistical comparison of MDA in the samples of diabetic control and Diabetic + vitamins A, E, C groups (mean ± SD)

Sample	Diabetic control group (n=16)	Diabetic + vitamins A, E, C group (n=16)	*t*-test results
Plasma MDA (nmol/ml)	1.57±0.25	1.35±0.28	t = 2.29 Df = 30 P=0.029
Liver MDA (nmol/mg protein)	0.57±0.15	0.45±0.18	t = 2.10 Df = 30 P=0.043
Heart MDA (nmol/mg protein)	1.08±0.32	0.87±0.21	t =2.20 Df = 30 P=0.036

Table 3: Values and statistical comparison of MDA in the samples of diabetic control and Diabetic + ω-3 fatty acids groups (mean ± SD)

Sample	Diabetic control group (n=16)	Diabetic + ω-3 fatty acids group (n=16)	*t*-test results
Plasma MDA (nmol/ml)	1.57±0.25	1.51±0.30	t = 0.62 Df = 30 P=0.53
Liver MDA (nmol/mg protein)	0.57±0.15	0.51±0.15	t = 1.13 Df =30 P=0.26
Heart MDA (nmol/mg protein)	1.08±0.32	0.82±0.16	t = 2.81 Df = 30 P=0.009

Discussion

This experimental study of STZ- induced diabetes mellitus reveals that lipid peroxidation, which is one of the deleterious effects of oxidative stress, increased substantially in the heart, liver and plasma. Further vitamins supplementation lowered MDA in diabetic rats. Supplementation with ω-3 fatty acids decreased heart MDA. Hepatic MDA is reported to be increased in most of the current literature (15-17) and unchanged in some (18). Glucose auto-oxidation, protein glycation, and the interaction of advanced glycation end-products with their specific receptors on macrophages are the main mechanisms of increased production of oxygen free radicals in diabetes (19- 21). As MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells, increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status (22). It seems that applied vitamins act synergistically, and hence a combination of them provides a better effect. The ability of ascorbic acid to reduce α-tocopheroxyl radical to generate α-tocopherol and possibly to inhibit oxidation induced by α-tocopheroxyl radical has been demonstrated in many in vitro and *in vivo* studies (23, 24). A synergistic effect of vitamin C, E and A could be expected based on the different environments where they act. Increased oxygen free radical production lowers the intracellular Mg concentration and, in light of such evidence, vitamin E administration might also regulate the intracellular Mg concentration. Vitamin C acts in the hydrophilic milieu scavenging reactive oxygen spices (24). α-tocopherol and vitamin A, in the hydrophobic domains of the bilayer, will inhibit the lipid oxidation free radical chain reaction and Zn, located in the interphase of the bilayer, will prevent iron or copper binding to the membrane (25). In 1997 Mete et al. reported an increase in the MDA level of plasma and liver of diabetic rats (13). In 2004 Seven et al. reported a significant increase in the MDA level of the livers of diabetic rats (26). Farvid et al. have found that supplementation with vitamins C, E and Mg decreases MDA level in type II diabetic patients (27).

In the present study, these interactions have been confirmed by significant decrease in MDA in diabetic rats supplemented with vitamins A, E and C. Supplementation with ω-3 fatty acids decreased heart MDA. The specific biologic mechanism for the beneficial effects of ω-3 fatty acids on lipid peroxidation has not been fully established. Several data indicate that ω-3 fatty acids may play a key role in decreasing the cardiovascular complications of diabetes (28). According to the results of this study decreased cardiovascular complications may be due to the decreased lipid peroxidation in heart tissue.

In conclusion, the results of the present study indicated that in diabetic, a combination of vitamins or ω-3 fatty acids decreased lipid peroxidation and MDA level by decreasing free radicals production or inhibiting their oxidative damage. Further studies are needed to clarify the cellular mechanism(s) of this effect.

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