Interrelation between Compensation of Carbohydrate Metabolism and Severity of Manifestations of Oxidative Stress in Type II Diabetes Mellitus

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Glycosylation end-products formed during diabetes mellitus promoted atherogenic oxidative modification of low-density lipoproteins. We evaluated the effects of compensation of carbohydrate metabolism and therapy with antioxidant probucol on parameters of free radical oxidation in patients with type II diabetes mellitus. Compensation of carbohydrate metabolism reduced manifestations of oxidative stress, which was manifested in accelerated enzymatic utilization of reactive oxygen species and lipid peroxides and decreased content of free radical oxidation products in low-density lipoproteins. In patients with type II diabetes mellitus combination therapy with antioxidant probucol decreased the severity of oxidative stress and stabilized carbohydrate metabolism without increasing the dose of hypoglycemic preparations.

Key Words: *type II diabetes mellitus; low-density lipoproteins; free radical oxidation; antioxidants; probucol*

Oxidative stress plays an important role in the development and progression of serious diseases, including cardiovascular and bronchopulmonary pathologies, neurodegenerative disorders, malignant neoplasms, and pathological states induced by adverse environmental factors [1,4]. Recent studies suggest that free radical processes are involved in the pathogenesis of diabetes mellitus and its complications [9,11]. A relationship was revealed between the development of diabetes mellitus and cardiovascular diseases. The risk of death from acute cardiovascular insufficiency (infraction and stroke) increases by 4 times in patients with type II diabetes mellitus [13], which is related to rapid progression of atherosclerosis. A direct correlation was found between the degree of glycemia and severity of vascular damage in patients with diabetes mellitus [13,14].

Activation of free radical oxidation and accumulation of secondary products of lipid peroxidation (*e.g.*, 4-hydroxynonenal and malonyl dialdehyde) contribute to progression of atherosclerosis in patients with diabetes mellitus [1,4]. The interaction of these aldehydes with lysine residues in apoprotein molecule induces modification of low-density lipoproteins (LDL). This modification promotes LDL binding and internalization by monocytes-macrophages and their transformation into foam cells. These changes contribute to preatherogenic lipid infiltration of the vascular wall [1,4]. A correlation was revealed between the formation of oxidized (modified) LDL and accumulation of glycosylation end-products [7]. For example, the intensity of oxidation and modification of LDL sharply increases in the presence of reactive oxygen species (ROS) generated during autooxidation of glucose yielding various aldehydes, including glyoxal, methylglyoxal, 3-deoxyglucosone, and glucosone [7]. It should

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be emphasized that intensive peroxidation of LDL promotes the formation of glycosylation end-products [8], which violates regulatory processes and aggravates pathological changes. Macrophages in the vascular wall internalize not only oxidized LDL, but also glycosylation end-products [10]. Oxidative stress accompanying atherosclerosis causes oxidative modification of LDL and formation of glycosylation end-products, which promotes atherogenic modification of these particles. This vicious cycle can be broken by compensation of carbohydrate metabolism or combination treatment with antioxidants. Here we studied the effects of compensation of carbohydrate metabolism and therapy with antioxidant probucol on free radical oxidation of LDL in patients with type II diabetes mellitus.

MATERIALS AND METHODS

We examined 30 patients (15 men and 15 women aged 57±9.9 years) with a 5.5-6-year-history of type II diabetes mellitus. The body weight index was 30±3.4 kg/m^2. Before examination the patients were characterized by decompensation of carbohydrate metabolism and received peroral hypoglycemic preparations (sulfonylurea alone or in combination with metformin). Two months after compensation of carbohydrate metabolism the patients were divided into 2 groups. Patients of the main group (n=20) received hypoglycemic preparations and probucol in a daily dose of 1 g (0.5 g twice a day for 2 months). Control patients (n=10) received only adequate hypoglycemic therapy.

The concentration of glycated hemoglobin (HbA_{1c}), contents of primary (lipid hydroperoxides) and secondary products (malonic dialdehyde, MDA) of free radical lipid oxidation in LDL, and activities of key antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase in erythrocytes were measured before examination, 2 months after compensation of carbohydrate metabolism (criteria of the European Diabetes Police Group, 1998), and 2 months after the start of probucol therapy. HbA_{1c} level was measured by the method of latex inhibition of immune agglutination on a Bayer DCA-2000 analyzer using Hemoglobin A_{1c} Reagent Kit (Bayer). Fasting venous blood for isolation of LDL was stabilized with 1 mg/ml ethylenediaminetetraacetic acid (anticoagulant and antioxidant). The plasma was centrifuged twice in a NaBr density gradient at 42,000 rpm and 4°C for 2 h (Beckman L-8 refrigerated centrifuge equipped with a fixed-angle rotor) and dialyzed at 4°C for 16 h [2]. Protein content in LDL was measured by the method of Lowry. LDL were dissolved with a solution containing 0.154 M NaCl and 50 mM phosphate buffer (pH 7.4) to a concentration of 50 μg protein/ml. Oxidation of LDL was induced by adding 30 μM $CuSO_4$ at 37°C. Accumulation of lipid hydroperoxides was estimated on a Hitachi 220A spectrophotometer at 233 nm and fixed time intervals. Kinetic curves of LDL oxidation were constructed. The lag-phase reflected induction of oxidation and was proportional to the amount of antioxidants in LDL [2]. The amount of lipid hydroperoxides in LDL was estimated before and after reduction of organic hydroperoxides with triphenylphosphine by a modified method with Fe^{2+} xylenol orange [12]. The concentration of secondary products of free radical lipid oxidation (MDA) was evaluated in the reaction with thiobarbituric acid [2]. SOD activity in erythrocytes was determined by inhibition of nitroblue tetrazolium reduction by superoxide radical $O_2^{\bullet}$ generated in the xanthine-xanthine oxidase system. The formation of formazan was recorded on a Hitachi-557 spectrophotometer at 560 nm [2]. Activity of selenium-containing glutathione peroxidase in erythrocytes was determined by the rate of NADPH oxidation with the substrate tert-butyl hydroperoxide in a coupled glutathione reductase system at 340 nm. The measurements were performed on a FP-901 Labsystems Oy chemical analyzer under kinetic conditions [2]. All reagents were from Sigma.

RESULTS

Blood HbA_{1c} content significantly decreased in patients with type II diabetes mellitus after compensation of carbohydrate metabolism (Table 1). Published data show that the decrease in HbA_{1c} content by 1% has a considerable clinical importance, since it reduces the mortality rate from cardiovascular events by 16% [14]. The content of lipid peroxides and MDA in plasma LDL from these patients decreased by 31 and 47%, respec-

TABLE 1. Effect of Compensation of Carbohydrate Metabolism on the Contents of Primary and Secondary Products of Free Radical Oxidation in Plasma LDL and Activity of Antioxidant Enzymes in Erythrocytes from Patients with Type II Diabetes Mellitus ($M\pm m$, n=30)

Parameters	Carbohydrate metabolism	
	decompensation	compensation
HbA_{1c}, %	8.10±0.03	7.10±0.18*
LDL lipid hydroperoxides, μmol/mg protein	199.0±19.5	138.0±16.6**
LDL MDA, nmol/mg protein	9.5±0.9	5.00±0.58*
SOD, U/g Hb	4288±162	9665±459*
Glutathione peroxidase, U/g Hb	4.10±0.17	5.40±0.33*

Note. *p<0.001 and **p<0.01 compared to decompensation.

TABLE 2. Effect of Hypoglycemic Preparations Alone (Control) or in Combination with Probucol (Daily Dose 1 g) on the Contents of Free Radical Oxidation Products in Plasma LDL from Patients with Type II Diabetes Mellitus (*M*±*m*)

Parameters	Before therapy (*n*=30)	After 2 month	
		control group (*n*=10)	main group (*n*=20)
HbA_{1c}, %	7.10±0.18	8.1±0.5*	7.00±0.21^{++}
Lag-phase of LDL oxidation, min	21.0±1.4	16.0±6.2	71.0±19.7^{+}
LDL lipid hydroperoxides, μmol/mg protein	138.0±16.6	135.0±27.8	75.0±21.4^{++}
LDL MDA, nmol/mg protein	5.00±0.58	8.8±2.5	2.10±0.24^{+}

Note. *p<0.01 compared to parameters before therapy; ^{+}p<0.001 and ^{++}p<0.05 compared to the control.

tively (Table 1). Previous studies showed that in patients with atherosclerosis and other diseases oxidative stress can be accompanied by inactivation of tissue antioxidant enzymes [4]. These changes can be related to inhibition of enzymes with free radicals and products of free radical oxidation [4]. It cannot be excluded that antioxidant enzyme deficiency is a factor promoting oxidative stress [4]. Activity of key antioxidant enzymes utilizing ROS (SOD) and lipid peroxides (glutathione peroxides) increased in the blood of patients with type II diabetes mellitus after compensation of carbohydrate metabolism by 2 times and 32%, respectively (Table 1). Therefore, compensation of carbohydrate metabolism in patients with type II diabetes mellitus is accompanied by a decrease in the severity of oxidative stress (Table 1), which confirms our hypothesis. Compensation of carbohydrate metabolism in patients with type II diabetes mellitus contributes to a decrease in blood content of not only final products of nonenzymatic glycosylation, but also main products of free radical oxidation characterizing the degree of oxidative stress.

Administration of probucol in a daily dose of 1 g to patients with type II diabetes mellitus for 2 months significantly decreased the content of total cholesterol and LDL cholesterol (by 11 and 8%, respectively, p<0.01). Our results are consistent with published data that probucol in high doses produces a weak hypolipidemic effect [3,4]. Probucol modulates lipid metabolism, but had no effect on carbohydrate metabolism. We revealed no significant changes in HbA_{1c} content reflecting compensation of carbohydrate metabolism in these patients (Table 2). *In vitro* and *in vivo* studies demonstrated high antioxidant activity of probucol [3, 4]. Therefore, the lag-phase of Cu^{2+}-induced free radical oxidation of LDL in the plasma from patients with type II diabetes mellitus increased by 3.5 times (Table 2). The contents of primary and secondary products of free radical LDL oxidation decreased by 2 and 2.5 times, respectively. No significant changes in these parameters were found in control group (Ta-_ble 2).

Our results indicate that the synthetic antioxidant probucol used for combination therapy of patients with type II diabetes mellitus reduces manifestations of oxidative stress and stabilizes compensation of carbohydrate metabolism without increasing the dose of hypoglycemic preparations (Table 2). The positive result of treatment with natural antioxidants [5] suggests that the increase in the duration of antioxidant therapy would produce greater success.

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