EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Combination Therapy with Coenzyme Q10 on Functional and Metabolic Parameters in Patients with Type 1 Diabetes Mellitus

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Functional state of the kidneys, severity of metabolic disturbances, intensity of LPO, and activity of the antioxidant system in 30 patients (18-36 years old) with type 1 diabetes mellitus and diabetic nephropathy of different compensation were studied before and after standard therapy or combination treatment with coenzyme Q10. Similar parameters were evaluated in 20 healthy subjects of the same age group. The development of metabolic disturbances in patients with type 1 diabetes mellitus (decompensated form) was accompanied by activation of LPO and inhibition of the antioxidant system. These patients were characterized by oxidative stress, diabetic nephropathy with associated proteinuria, and impairment of water excretion, electrolyte excretion, and nitrogen excretion in the kidneys. Combination therapy with coenzyme Q10 had a positive effect on LPO and antioxidant system. This treatment was followed by the relief of hyperglycemia, decrease in the concentrations of glycosylated hemoglobin and LDL cholesterol, and improvement of nitrogen metabolism.

Key Words: *diabetes mellitus; diabetic nephropathy; lipid peroxidation; antioxidant system; coenzyme Q10*

The development of vascular complications of diabetes mellitus (DM) is an urgent problem. According to WHO reports, the annual increase in the number of these patients is 5-7%. The number of patients increases 2-fold every 10-15 years [1,2,7]. Diabetic nephropathy (DNP) is one of the most severe complications of DM, which decreases the quality of life and life span of patients [3,4,10]. DNP ranks first among other angiopathies. Clinical and experimental studies showed that the development of hyperglycemia in DM is followed by the induction of free radical oxidation in the kidneys, which serves as a damaging factor [11,12]. However, only few combination studies have attempted to investigate the nature of metabolic disturbances in patients with type 1 DM of different compensation and associated nephropathy. Little is known about the possible approaches to optimize therapy with drugs that inhibit the formation of free radicals.

Here we studied the intensity of LPO and type of metabolic disturbances in patients with type 1 DM of different compensation. Moreover, we evaluated the effect of combination therapy with coenzyme Q10 in these patients.

MATERIALS AND METHODS

The study was performed at the Republican Endocrinologic Dispensary and Department of Pathobioche-

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mistry (Institute of Biomedical Studies, Vladikavkaz Research Center). We examined 30 patients with type 1 DM (12 men and 18 women, 18-36 years old). The mean duration of the disease was 6-8 years. The patients had DNP of different compensation degree. The control group consisted of 20 healthy subjects without DM. The severity and compensatory stage of type 1 DM were evaluated from standard clinical and laboratory parameters [3].

Depending on the degree of metabolic compensation, these patients were divided into 3 groups. Group 1 patients (n=10) had decompensated form of DM before the start of therapy. Group 2 patients (n=10) with subcompensated DM received standard therapy with insulin products. Group 3 patients (n=10) with compensated DM received combination therapy with insulin and coenzyme Q10 in a daily dose of 1 g (2 capsules of 0.5 g) with meal.

Blood samples were taken from the cubital vein after overnight fast. The serum was obtained by centrifugation at 2500 rpm and 4°C for 15 min. Glucose concentration was measured by the glucose oxidase method. The concentration of glycated hemoglobin in the blood was measured calorimetrically with Lachema kits. The contents of total cholesterol (TC), triglycerides, and HDL CS were estimated with BIOKON kits. The content of LDL CS was calculated by the Friedewald formula. The concentrations of total protein, urea, creatinine, hydroperoxides, and MDA and activities of catalase [5] and SOD [9] were measured in the blood serum.

A change in diuresis, degree of proteinuria, glomerular filtration rate, percentage of tubular water reabsorption, excretion of sodium and potassium, concentration of sodium and potassium in the blood, filtration charge of sodium and potassium, and tubular sodium reabsorption (calculated as described elsewhere [8]) served as the criteria of DNP. The content of nonenzymatic cationic proteins was measured by the cytochemical method [6].

The results were analyzed Microsoft Excel software. The data are presented as $M\pm SEM$. The significance of differences between patients with type 1 DM and control subjects, as well as between pre- and post-treatment parameters, was evaluated by Student's t test at $p<0.05$.

RESULTS

The patients with decompensated or subcompensated DM and DNP were characterized by proteinuria and reduced glomerular filtration rate and tubular water reabsorption, which resulted in an increase in diurnal diuresis (Table 1). We revealed an increase in urine sodium excretion and decrease in urine potassium excretion. No significant changes were found in the filtration charge of sodium. Tubular sodium reabsorption was reduced under these conditions. The degree of proteinuria decreased, while the glomerular filtration rate and tubular sodium reabsorption increased in patients with subcompensated DM after standard therapy. The observed changes were followed by a decrease in water and sodium excretion with urine. However, these parameters did not reach the control level. The concentrations of glucose and glycated hemoglobin in the blood were significantly increased in patients with decompensated and subcompensated forms of type 1 DM (10.40±0.71 and 8.60±0.71%, respectively, *vs*. 5.30±0.81% in the control; $p<0.001$; Table 1). Lipid metabolic disturbances manifested in an increase in the concentrations of total CS and LDL CS and decrease in the content of HDL CS. It illustrates the elevated level of atherogenic lipoproteins in the blood. Type 1 DM patients were characterized by accumulation of hydroperoxides and MDA in the blood (Table 1). LPO products in the blood have a destabilizing effect on lipid matrix of lysosomes. The content of nonenzymatic cationic proteins in neutrophil lysosomes and mean cytochemical index were reduced under these conditions (1.05±0.03). These changes illustrate the impairment of nonspecific body resistance. The mean cytochemical index increased to 1.25±0.07 after combination therapy ($p<0.01$ compared to the control, 1.58±0.03). Oxidative stress was accompanied by a decrease in SOD activity and increase in catalase activity in all patients with decompensated and subcompensated forms of type 1 DM.

Free radical chain reactions are the cause of systemic changes and organ diseases in patients with type 1 DM. Therefore, antioxidant agent coenzyme Q10 was used in combination with insulin treatment. Combination therapy was followed by a greater decrease in the concentrations of hydroperoxides and MDA (final product) in the blood. The content of these compounds remained high in group 2 patients. Analysis of the antioxidant system (enzyme component of LPO) showed that SOD activity increases and practically does not differ from the control level on day 14-20 of combination therapy. Catalase activity decreased under these conditions. A positive correlation was found between MDA concentration and catalase activity in coenzyme Q10-receiving patients (r=+0.57, p=0.05). These patients were characterized by a negative correlation between the decrease in MDA concentration and increase in SOD activity (r=-0.46, p=0.03). Combination therapy was accompanied by a significant decrease in the concentration of glycated hemoglobin and content of urea, creatinine, total CS, and LDL CS in the blood serum. The contents of total protein and HDL CS were elevated after treatment. The severity of nephropathy was significantly reduced under these conditions. We

TABLE 1. Metabolic and Functional Parameters in Patients with Type 1 DM and DNP during Various Stages of Disease Compensation (*M*±*SEM*)

Parameter	Group			
	control	1	2	3
Glucose, mmol/liter	5.06±0.10	14.6±0.5$^{****+++}$	10.6±0.2$^{****+++}$	7.133±0.261^{****ooo}
Glycated hemoglobin, %	5.30±0.81	10.40±0.71****	8.60±0.71^{****+}	5.30±0.81^{ooo}
Total protein, g/liter	74.60±1.24	58.20±1.66****	73.390±0.544^{+++}	80.090±1.532^{***ooo}
Urea, mmol/liter	4.81±0.12	8.54±0.62****	6.44±0.32$^{****+++}$	5.62±0.25^{***o}
Creatinine, μmol/liter	88.14±1.21	105.000±5.235***	92.910±1.139^{***+}	75.250±1.532^{****ooo}
Total CS, mmol/liter	5.04±0.10	6.86±0.43****	5.226±0.112^{+++}	3.797±0.200^{****ooo}
LDL CS, mmol/liter	2.92±0.13	4.61±0.59****	3.48±0.59***	3.08±0.36***
HDL CS, mmol/liter	1.42±0.05	1.15±0.04****	1.24±0.05**	1.39±0.06^{+}
Hydroperoxides, mmol/liter	0.97±0.09	1.30±0.29	1.04±0.20	0.95±0.16
MDA, nmol/ml	2.930±1.177	5.70±0.80**	4.20±0.61	3.80±0.62
Catalase, mcat/liter	251.1±42.8	564.8±43.2****	438.0±34.4^{++}	329.0±23.7^{ooo}
SOD, U/mg protein	3.550±0.156	2.450±0.061***	2.650±0.045^{+++}	3.150±0.035^{oooo}
Proteinuria, g/24 h	0.045±0.010	1.18±0.24	0.98±0.18^{++++}	0.068±0.080^{oooo}
GFR, ml/min	126.40±8.56	76.84±8.66	84.28±9.40^{+++}	122.8±8.6^{oooo}
RH_2O%	99.20±0.16	97.59±0.36	98.25±0.28^{+++}	99.32±0.18^{oo}
Sodium excretion, μmol/100 g/h	63.4±3.6	98.5±2.9^{ooo}	85.49±3.10^{++++}	69.50±2.75^{oooo}
NaFc, μmol/100 g/h	2006.4±118.4	1750.0±111.5	1850.0±98.6	1950.0±101.5
RNa%	98.85±0.16	97.50±0.18^{oo}	98.05±0.15^{++++}	98.79±0.12
Potassium excretion, μmol/100 g/h	38.48±2.50	20.00±1.98^{o}	25.05±2.05^{++++}	32.98±2.24^{oooo}
Diuresis, ml/24 h	1380±115	2230±140^{o}	1870±160^{++++}	1360±80^{oooo}

Note. $^{*}p<0.05$, $^{**}p<0.02$, $^{***}p<0.01$, and $^{****}p<0.001$ compared to the control group; $^{+}p<0.05$, $^{++}p<0.02$, $^{+++}p<0.01$, and $^{++++}p<0.001$ compared to group 1; $^{o}p<0.05$, $^{oo}p<0.02$, $^{ooo}p<0.01$, and $^{oooo}p<0.001$ compared to group 2. GFR, glomerular filtration rate; NaFc, sodium filtration charge; RNa%, tubular sodium reabsorption; RH_2O%, percentage of tubular water reabsorption.

revealed an increase in the glomerular filtration rate and tubular water reabsorption, reduction of diurnal diuresis (to the control level), and significant decrease in the degree of proteinuria and electrolyte excretion (sodium and potassium). It resulted from increased filtration charges of sodium and potassium and intensification of tubular sodium reabsorption (Table 1). Our results indicate that metabolic disturbances have an important role in the development of DNP in patients with type 1 DM. Therefore, these disorders can be corrected by metabolic drugs.

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