EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Naftidrofuryl on Lipid Peroxidation in Serum and Erythrocyte Plasma Membrane of Patients with Diabetes Mellitus

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Effect of naftidrofuryl, a blocker of serotonin $5S_2$-receptors (Dusodril-retard), on the malonic dialdehyde content in the serum and erythrocyte membranes is studied in diabetics with and without angiopathies. A 40-day treatment with Dusodril-retard normalizes the serum content of malonic dialdehyde, an intermediate product of lipid peroxidation, and has no effect on the malonic dialdehyde content of the erythrocyte plasma membrane. A negative correlation is established between blood levels of total cholesterol, β lipoproteins, and malonic dialdehyde levels in patients with diabetes mellitus.

Key Words: *naftidrofuryl, lipid peroxidation; diabetes mellitus*

Vascular complications determine the severity of primary disease, premature aging, and death of patients with diabetes mellitus. Oxidative stress, when the excess of free oxygen radicals damages vascular endothelium and peripheral blood cells, is considered to be one of the causes of vascular complications [2,4,6,10]. Angioprotectors with various mechanisms of action — adenosine receptor blockers, phosphodiesterase inhibitors facilitating cATP accumulation (Trental, theophylline), agents normalizing platelet adhesion and aggregation (Dicinon and Doxium), and nicotinic acid — have been successfully used in complex therapy of diabetes mellitus [3]. Blockers of serotonin $5S_2$-receptors act as vasodilators and improve blood supply and tissue metabolism. These agents were used in the treatment of vasculopathies, but, as we are aware, not in the therapy of vascular complications occurring in diabetes mellitus. Therefore, the use of serotonin $5S_2$-receptor blockers as angioprotective agents to treat microangiopathies in patients with diabetes mellitus seems quite interesting.

The objective of the present study was to examine the effect of the serotonin $5S_2$-receptor blocker naftidrofuryl (Dusodril-retard, Byk Gulden), on the content of malonic dialdehyde (MDA) in serum and erythrocyte plasma membrane of patients with insulin-dependent diabetes mellitus (IDDM). This parameter is an obligatory metabolic marker of oxidative stress.

MATERIALS AND METHODS

Sixteen patients (11 women and 5 men, mean age 26.2 years) and 15 healthy donors (mean age 35.2 years) were enrolled in the study. The patients had a 13.3-year (1-21.9 years) history of diabetes mellitus. Ten patients developed diabetes-related com-

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plications: nephropathy (transitory proteinuria, 3 patients), retinopathy (angiopathy of the retina, 3 patients), and both (4 patients). Blood was drawn from the ulnar vein after an overnight fast before and 40 days after treatment with Dusodril-retard (100 mg, twice daily). For isolation of the erythrocyte plasma membranes blood was collected in vials with 3.8% sodium citrate (9:1, v/v). Erythrocytes were pelleted in a Beckman centrifuge (1500g, 15 min, 0-4°C), washed 3 times with cold physiological saline (150 mM NaCl, 5 mM sodium phosphate buffer, pH 7.4), and stored at -20°C. Blood serum was prepared routinely.

The MDA content in erythrocytes and serum was determined by spectrofluorimetry, Hb by the hemoglobincyanide method, protein content by the method of Lowry, serum glucose by the glucose oxidase method, and glycosylated hemoglobin (HbA1c) by affinity chromatography using a BioRad kit; total serum cholesterol was determined spectrophotometrically with Cormay diagnostic kits, and the content of β lipoproteins was measured turbidimetrically by the method of Burstein and Samay.

The data were processed by analysis of variances using Statistica for Windows software. The significance of differences was evaluated by the Student's *t* test for paired and unpaired variables. The relationship between the studied parameters was assessed by the Spearman's range test.

RESULTS

At the beginning of the study, 3 patients were at the state of decompensation (HbA1c>10%), 5 patients were at the state of compensation (HbA1c 6-8%), and 8 patients were at the state of subcompensation (HbA1c 8-10%). The mean level of HbA1c was 8.51±0.58. The degree of glycemia was approximately the same in patients with IDDM with and without angiopathies and declined during the treatment (HbA1c 7.49±0.56). The body weight index was significantly higher in patients without angiopathies than in patients with angiopathies ($p<0.01$). Serum content of anti-insulin antibodies in patients with IDDM was considerably higher than in normal subjects. In patients with angiopathies, the level of C peptide was lower, while that of immunoreactive insulin was higher than in patients without angiopathies. These differences were statistically insignificant ($p<0.1$) due to a wide-range variation of these parameters in patients without angiopathies. Serum content of lipid peroxidation (LPO) products in patients with IDDM was 35% higher than in the control ($p<0.001$), being independent of vascular complications. A 40-day treatment with naftidrofuryl (Dusodril-retard, 100 mg twice daily after meal) led to a reduction in the serum content of MDA, an intermediate LPO product, to the normal level (paired *T* test, $p<0.001$). The content of MDA in the erythrocyte membranes was the same in patients with and without angiopathies (26% higher than in healthy donors, $p<0.001$), remaining increased throughout the Dusodril-retard therapy (Table 1). There was no significant correlation between glycemia, duration of the disease, age, body weight index, and LPO indexes for serum and erythrocyte membranes in patients with IDDM. A negative correlation was established between total blood cholesterol and serum MDA ($r=-0.75$, $p<0.005$) and between serum β lipoproteins and MDA contents in patients with IDDM ($r=-0.62$, $p<0.05$).

There is considerable evidence indicating that free radicals are involved both in the pathogenesis of diabetes mellitus and the development of vascular complications [2,4,7,8,10,14]. However, the reason for increased production of free radicals in diabetes mellitus remains unclear. Hyperglycemia was shown to induce LPO in erythrocyte membranes [9]. It was suggested that activation of LPO and conformational changes in the erythrocyte plasma membrane occurring in diabetes mellitus are caused by generation of hydroxyl anion-radical during autoxidation of glucose [8]. Free radical reactions are probably stimulated by formation of free oxygen radicals in the sorbitol pathway of glucose metabolism [11]. We believe that tissue hypoxia which develops in diabetes mellitus [3,15] and activated LPO [4,6] are responsible for increased formation of free radicals. This may account for the sustained increase in the blood content of LPO products in patients with diabetes mellitus despite compensation of carbohydrate metabolism [2]. Presumably, normalization of serum MDA level in patients treated with Dusodril-retard results from alleviation of tissue hypoxia due to vasodilation, and normalization of blood filterability [1] and metabolism in the zone with disturbed circulation. Although Dusodril-retard lowers the plasma content of prooxidants, the MDA content in erythrocyte membranes remains high, indicating that peroxidation processes in these cells depend not only on plasma prooxidants, but also on structural and functional changes in their plasma membrane occurring in diabetes mellitus [12,13]. This can be indirectly confirmed by significant correlation between serum cholesterol and lipoprotein contents, on the one hand, and serum MDA but not erythrocyte membrane MDA content, on the other.

Thus, naftidrofuryl applied as an angioprotector in patients with IDDM only partially prevents the development of angiopathies. By reducing hypoxia

TABLE 1. Characteristics of Patients with IDDM

Characteristic	IDDM (n=16)	IDDM with angiopathies (n=10)	IDDM without angiopathies (n=6)	Donors (n=15)
Age, years	26.18±1.79	26.30±2.12	26.00±3.46	35.23±5.21
Body weight index, kg/m²	20.81±0.69	19.78±0.87	22.52±0.75**	
History of diabetes, years	13.32±2.56	16.33±3.50	8.30±2.75	
Immunoreactive insulin, μU/ml	13.96±2.10	17.02±2.22	8.88±3.49	3-25
Anti-insulin antibodies, %	18.7±4.06*	20.2±5.21*	16.2±6.99	7.0±3.0
C-peptide, nmol/liter	0.24±0.11	0.09±0.03	0.48±0.28	0.12-1.25
HbA1c before treatment, %	8.51±0.58	8.53±0.80	8.46±0.79	4.0-6.0
HbA1c after treatment, %	7.49±0.56	7.66±0.84	7.25±0.67	
Total cholesterol, mmol/liter	6.05±0.53	6.47±0.84	5.38±0.33	3.1-5.2
Blood β lipoproteins, g/liter	5.97±0.61	6.17±1.00	5.68±0.41	3.0-4.5
Serum MDA before treatment, rel. units/mg protein	0.92±0.05**	0.94±0.06***	0.88±0.09*	0.65±0.06
Serum MDA after treatment, rel. units/mg protein	0.68±0.05+	0.68±0.08+	0.68±0.07	
Erythrocyte MDA before treatment, rel. units/mg Hb	1.17±0.06***	1.18±0.08**	1.16±0.08***	0.93±0.05
Erythrocyte MDA after treatment, rel. units/mg Hb	1.28±0.06***	1.28±0.06***	1.27±0.15***	

Note. *p<0.05, **p<0.01, ***p<0.001 compared with the control; +p<0.001 compared with the corresponding parameter before treatment, **p<0.01 compared with the corresponding parameter in patients with angiopathies.

and normalizing lipid peroxidation in the plasma, naftidrofuryl probably abolishes the inhibiting effect of the increased concentration of LPO products on the synthesis of prostacyclin in the endothelium, thus acting not only as a vasodilator but also as a true angioprotector. However, the risk of angiopathies still remains due to disturbed peroxidation status of erythrocytes and rheological properties of the blood [5,10].

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