Analysis of Serum Activities of Matrix Metalloproteinases and α1-Proteinase Inhibitor in Patients with Type 2 Diabetes Mellitus

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Activities of matrix metalloproteinases 2 and 7 and α1-proteinase inhibitor were measured in patients with type 2 diabetes mellitus. Correlations between enzyme activity and the main parameters of carbohydrate metabolism were studied. In patients, significant reduction of matrix metalloproteinase activity in the serum was noted, which correlated with the decrease in blood concentration of C-peptide (r=0.8). At the same time, serum activity of α1-proteinase inhibitor increased. No significant relationships between patient's age, glucose concentration, fructosamine concentration, and matrix metalloproteinase activity were observed.

Key Words: *matrix metalloproteinases; type 2 diabetes mellitus*

Type 2 diabetes mellitus (DM) is associated with hyperglycemia, appearance of modified lipoproteins, oxidative stress, and increase in serum TNF-α concentration; these processes, in turn, modulate activity of matrix metalloproteinases (MMP) [9]. Disordes in matrix degradation in type 2 DM may result in endothelial dysfunction and related complications: diabetic nephropathy, retinopathy, *etc*. [5]. MMP account for 70% of matrix degradation [1,3].

Here we analyzed activities of MMP-2,7 and α1-proteinase inhibitor (α1-PI) in patients with type 2 DM. Correlations between enzyme activity and main parameters of carbohydrate metabolism were studied.

MATERIALS AND METHODS

Fifty-six patients with type 2 DM and vascular complications (arterial hypertension, CHD, diabetic nephropathy) were examined. Mean age of the patients was 52.5±5.0 years and duration of the disease was ~5.5 years. The control group comprised clinically healthy one-time donors (N=39). Diagnosis was established according to criteria of WHO Expert Committee for DM. The study was carried out in accordance to ethic principles of Declaration of Helsinki (2000).

MMP-2,7 activity in blood samples was measured using fluorescent substrate MCA-Pro-Ley-Gly-Leu-DPA-Ala-Arg-NH_2 (ICN Biomedicals Inc.) [6]. Measurements were carried out on spectrofluorometer (Shimadzu RF-5301 PC) at excitation and emission wavelengths of 325 and 393 nm, respectively. Activity was expressed in μmol MCA/liter/h.

α1-PI activity was determined using the method based on trypsin cleavage of a synthetic substrate benzoyl-arginine-ethyl ether hydrochloride with benzoyl-arginine formation. Activity was expressed in inhibitory units (IU) per ml serum.

C-peptide concentration was measured by competitive ELISA (DRG). Biochemical parameters of blood serum (glucose, fructosamin) were measured on an automatic analyzer KONELAB (ThermoLabSystems).

The results were treated using Statistica software. Intergroup differences were assessed using Student's t test and were considered significant at p<0.05.

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TABLE 1. Basic Parameters of Carbohydrate Metabolism in Serum from type 2 DM Patients with Different MMP Activity

Group	Age, years	MMP activity, μmol MCA/liter/h	Glucose, mmol/liter	Fructosamine, μmol/liter	C-peptide, pmol/liter
Group 1 (reduced MMP activity)	60.7±14.5	132.7±48.5*	10.4±3.4	311.4 ±37.5	232±81*
Group 2 (normal MMP activity)	46.8±13.6	241.7±31.9	9.3±2.3	337.5±40.2	1708±930

Note. *$p<0.001$ in comparison to group 2.

RESULTS

Serum MMP activity in healthy subjects was 281.8± 25.9 μmol MCA/liter/h; while in patients with type 2 DM it was 180.4±21.6 μmol MCA/liter/h ($p<0.05$). α1-PI activity in control group was 25.2±2.5 IU/ml, in patients with type 2 DM it was 39.9±7.9 IU/ml of blood serum ($p<0.05$). The patients were divided into two groups: with MMP activity substantially below the normal (group 1, N=31, Table 1) and with enzyme activities within normal ranges (group 2, N=25).

Serum glucose and fructosamine concentrations were elevated in both groups. C-peptide concentration in group 1 was lower than in group 2 ($p<0.001$).

Thus, patients with type 2 DM had significantly reduced serum activity of MMP-2,7 (~50%). Low proteolytic MMP activity was also noted in the arterial vascular wall [7], skin fibroblasts [8], and cultured keratinocytes from wound extracts of patients with DM [4]. Reduced MMP activity results in enhanced deposition of collagen and other components of extracellular matrix, which leads to diabetic vascular complications [2].

Simultaneously, α1-PI serum activity increased by 1.5 times. High inhibitor concentration is probably responsible for reduced MMP activity.

Serum C-peptide concentration was reduced in patients with significantly decreased MMP activity (r=0.8). No significant correlations between patient age, glucose and fructosamine concentrations, and MMP activity were observed.

We assume that reduced MMP-2,7 activity may result in decreased proteolytic process, splitting of C-peptide from proinsulin in blood serum. Recent studies showed that MMP promotes insulin degradation [5], which may result in disturbed C-peptide splitting from insulin molecule. It is also possible that reduced concentration of C-peptide is associated with altered insulin secretory capacity of insular apparatus of the pancreas. Irrespective of its causes this decrease in C-peptide concentration attests to transformation of type 2 DM into type 1 DM.

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