Long-term hyperglycaemia decreases vascular fraction of extracellular superoxide dismutase

To the Editor: Diabetes mellitus is accompanied by more active processes of generating free oxygen radicals and simultaneously by a decreased rate of their removal. The intensity of these processes is proportional to the level of glycaemia and its duration. In diabetes, particularly when complicated by microangiopathy, a higher rate of oxidation of ascorbic acid, which is a non-specific scavenger of the superoxide radical was found [1]. The concentration of free radicals in body fluids of patients with diabetes could rise also as a result of changes in the activity of enzymes liberating oxygen radicals or a decrease in the activity of scavenging enzymes. A close inverse relationship between non-enzymatic glycation of proteins and the activity of superoxide dismutase in erythrocytes has been shown [2]. The only enzyme breaking down the superoxide radical in extracellular space is the extracellular superoxide dismutase (EC-SOD). Part of this enzyme is released into the bloodstream by fibroblasts and another is present on the surface of blood vessels. Administration of heparin into the bloodstream leads to the liberation of this fraction from the endothelium and its increased activity in plasma. Another study [3] has shown that glycation of EC-SOD decreases its affinity to heparin-Sepharose. Patients with diabetes have a higher percentage of glycated extracellular superoxide dismutase. Superoxide dismutase protects against an increase in the permeability of vessels caused by the superoxide radical and hypoxia. Reduced activity of the vascular fraction of EC-SOD could lead to higher levels of superoxide radical on the surface of endothelium and could activate the mechanism of diabetic microangiopathy.

From 37 healthy subjects, samples containing 3 to 4 ml of venous blood were drawn into heparinized tubes. Then they were given intravenously appropriate dose of heparin and after 15, 30, 60 and 120 min once again 3 to 4 ml of venous blood was taken from the other forearm vein. After collection EC-SOD activity was assessed in the obtained plasma. Heparin (Heparinum "Polfa") was given in different doses: 50 IU/kg, 75 IU/kg, 100 IU/kg, 125 IU/kg, 250 IU/kg of body weight. Analysis of the results obtained showed that the

optimal dose of Heparinum "Polfa" is a 5000 IU dose, whereas the optimal time for the second blood collection is 30 min.

A total of 38 non-insulin dependent patients with diabetes mellitus (18 men and 20 women) of 22 to 69 years of age (45.2 ± 15.2) participated in the study. The duration of disease was 1 to 32 years (15 ± 8). The control group was comprised of 22 healthy subjects (11 men and 11 women) of 21 to 66 years of age (44.1 ± 13.0). Patients were divided into three subgroups in relation to the level of fructosamine (sFRA). Subgroup 1 (DM 1) included 15 patients, 7 men (M) and 8 women (F), $sFRA < 300 \mu\text{mol/l}$; subgroup 2 (DM 2) included 16 patients, 8 men, 8 women, $300 \leq sFRA \leq 400 \mu\text{mol/l}$; subgroup 3 (DM 3) included 7 patients, 3 men and 4 women, $sFRA > 400 \mu\text{mol/l}$. Samples of 5 ml of blood were collected from each patient twice, first time fasting, in standard conditions, and then 30 min after an intravenous administration of 5000 IU of Heparin "Polfa". Superoxide dismutase activity was determined using spectrophotometric method [4]. Serum fructosamine concentration was determined using Fructosamine Test (La Roche, Basel, Switzerland). The concentration of glucose in plasma was measured by standard laboratory methods. Glycosylated haemoglobin was determined using ion-exchange liquid chromatography.

The study was approved by Bioethics Committee of Pomeranian Medical University and was carried out in accordance with the Declaration of Helsinki as revised in 2000. All patients gave their informed consent.

The results were statistically analysed; arithmetic mean and standard deviation (SD) were calculated. Statistical significance of the difference was calculated using a Student's *t* test for paired and unpaired variables, statistical significance of the difference between groups where the number of patients was lower than 11, was calculated using Mann-Whitney's test. Correlations were obtained using linear regression equation.

Fasting glycaemia values were: $4.5 \pm 0.8 \text{ mmol/l}$ in the control group, $9.3 \pm 2.7 \text{ mmol/l}$ in DM 1, $9.3 \pm 2.9 \text{ mmol/l}$ in DM 2 and 9.4 ± 2.7 in the DM 3 group. HbA_{1c} [%] were: 3.6 ± 0.6 in the control group, 7.67 ± 3.1 in DM 1, 7.74 ± 2.7 in DM 2 and 7.83 ± 2.64 in the DM 3 group. Both fasting glycaemia and HbA_{1c} were lower in the control than in all diabetic groups ($p < 0.001$) (Table 1).

Preheparinic EC-SOD activities in control and DM 1 groups were similar ($9.86 \pm 1.01 \text{ U/ml}$ vs $9.47 \pm 0.61 \text{ U/ml}$, NS). The basal EC-SOD activities in DM 2 ($8.79 \pm 0.73 \text{ U/ml}$) and DM 3 ($7.63 \pm 0.73 \text{ U/ml}$) were lower ($p < 0.001$) compared to the control group. Postheparinic EC-SOD activities in all diabetic groups (DM 1 $17.69 \pm 1.63 \text{ U/ml}$, DM 2 $13.10 \pm 2.11 \text{ U/ml}$ and DM 3 $9.96 \pm 0.87 \text{ U/ml}$) were lower ($p < 0.001$) compared to the

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Table 1. Concentrations of glycaemia, HbA_{1c} , basal and postheparinic EC-SOD values in patients and in the control group

	Control group <i>n</i> =22	DM 1 <i>n</i> =15	DM 2 <i>n</i> =16	DM 3 <i>n</i> =7
Glycaemia (mmol/l)	4.5 ± 0.8	9.3 ± 2.7^a	9.3 ± 2.9^a	9.4 ± 2.7^a
HbA_{1c} (%)	3.6 ± 0.6	7.67 ± 3.1^a	7.74 ± 2.7^a	7.83 ± 2.64^a
Preheparinic activity of EC SOD (U/ml)	9.86 ± 1.01	9.47 ± 0.61	8.79 ± 0.73^a	7.63 ± 0.73^a
Postheparinic activity of EC SOD (U/ml)	20.33 ± 1.68	17.69 ± 1.63^a	$13.1 \pm 2.11^{a,b}$	$9.96 \pm 0.87^{a,b}$
Δ EC SOD (U/ml)	10.47 ± 0.67	8.22 ± 1.02	$4.31 \pm 1.38^{a,b}$	$2.33 \pm 0.14^{a,b}$

Values are expressed as means $\pm$ SD

Δ EC SOD = postheparinic activity of EC SOD – preheparinic activity of EC SOD

^a $p < 0.001$ compared to control group

^b $p < 0.05$ DM 3 and DM 2 compared to DM 1

control group (20.33 ± 1.68 U/mL) (Table 1). In patients with diabetes negative correlation was found between preheparinic EC-SOD activity and fasting glycaemia ($r = -0.48$, $p < 0.005$), fructosamine ($r = -0.72$, $p < 0.001$), HbA_{1c} ($r = -0.56$, $p < 0.001$). Negative correlation was also found between postheparinic EC-SOD activity and fasting glycaemia ($r = -0.67$, $p < 0.001$), fructosamine ($r = -0.87$, $p < 0.001$), HbA_{1c} ($r = -0.75$, $p < 0.001$).

There are no data available concerning postheparinic activity of EC-SOD in patients with diabetes. At present there are only studies concerning basal activity [5], glycation of EC-SOD in vitro [3] and postheparinic activity of this enzyme in healthy subjects [6]. The results presented in our study are similar to the results of the authors cited. Comparison of correlation coefficients between the amount of EC-SOD and indicators of diabetic control can suggest that in vivo the state of EC-SOD glycation balance is achieved during approximately 2 to 3 weeks, which corresponds to the half-life of fructosamine. Similar duration of hyperglycaemia influences glycation of extracellular superoxide dismutase. Non-enzymatic glycation leads to decreased activity of vascular EC-SOD. Such changes in enzymes activity possibly enhance the reactions of free radicals (not changed superoxide radical generation and reduced abilities of its inactivation). The superoxide radical because of its negative charge does not penetrate cell membranes [7], so its action will be limited to the surface of vessels i.e. endothelial cells. Protective action of SOD on vascular permeability was described earlier [8]. Our own results show apparently lower postheparinic SOD activities in patients with diabetes compared to healthy subjects and their close association with long-term hyperglycaemia.

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