Original paper

Selected antioxidative enzyme activities in patients with diabetes mellitus type 2

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Abstract

Background: Chronic hyperglycaemia is considered to be the main reason of diabetes complications. In the prolonged increased glucose concentration the increased generation of reactive oxygen species (ROS) occurs, as well as the disturbances of pro- and antioxidative balance i.e. the oxidative stress. In respect of the great role that the oxidative stress may play in the pathogenesis of type 2 diabetes and its chronic complications the aim of this paper was to evaluate the activity of two selected antioxidative enzymes: superoxide dysmutase (SOD) and catalase (CAT) in erythrocytes of type 2 diabetes patients at different stages of the metabolic balance.

Material and methods: 61 type 2 diabetes patients took part in the study. Among them 31 patients met the criteria of the metabolic balance, while 30 patients suffered from metabolically unbalanced diabetes. The control group included 40 healthy people. The activity of SOD-1 in red blood cells was determined using the Misra and Fridovich method. The activity of CAT was determined using the Beers and Sizer method.

Results: While comparing the activity of SOD-1 and CAT in each studied group, in the group of type 2 diabetes patients at the stage of the metabolic balance it was found to be the highest

Conclusions: Taking into account early development of chronic complications of type 2 diabetes it seems that the differences observed in the antioxidative enzymes activity in type 2 diabetes patients with different degrees of the metabolic balance may be used to monitor the clinical process of the disease.

Key words: enzyme activities, chronic hyperglycaemia, diabetes, reactive oxygen species.

Background

The frequent occurrence of diabetes mellitus, particularly type 2, as well as clinical disturbances of this disease have been considerably growing all over the world. It is worth noting that in type 2 diabetes the processes whose consequence is the development of chronic disturbances i.e. macro- and/or microangiopathy begin many years before diagnosing the full symptoms disease [1]. That is the reason why intense research is being done which would

enable identification of diabetes early markers, particularly type 2, and possible organ complications. Despite the research into the elements of diabetes pathogenesis, their molecular or cellular basis still has not been fully explained. Chronic hyperglycaemia is considered to be the main reason of diabetes complications. In the prolonged increased glucose concentration the increased generation of reactive oxygen species (ROS) occurs, as well as the disturbances of pro- and antioxidative balance i.e. the oxidative stress. It is caused by the increased activity of some independent metabolic processes such as autooxidation of monosaccharides, intensification of non-enzymatic glycation, activation of protein kinase C (PKC) and phospholipase A_2 as well as the increased activation of the intracellular polyolic process [2]. In diabetes with an increased glucose concentration in blood the intensification of non-enzymatic protein glycation occurs.

The initial product of this reaction is the so-called Shiff base spontaneously transforming into the Amadori product which is the case of the glycated haemoglobin (Hb A_{1C}). These initial reactions are reversible and depend on the reagent concentration. The series of consecutive reactions leads to forming, among others, advanced glycation end products (AGE) which take part in tissue reconstruction. They stimulate the cellular response through the receptor specific for AGE (RAGE) which is situated in the cellular membrane of macrophages and endothelium. Due to joining the receptors AGE become another source of ROS and they increase the oxidative stress [3].

The intensified production of ROS is accompanied by some disturbances in the antioxidative systems. Although the former examinations of diabetes patients have not brought any unmistakable results, the reduction of Cu, Zn-superoxide dismutase (SOD-1) activity or the glutathion system activity in prolonged hyperglycaemia has been suggested. Moreover, in diabetes the reduced tissue concentration of natural antioxidants such as ascorbic acid and α-tocopherol have been observed [4, 5]. The reduced activity of antioxidants in type 2 diabetes is not only the result of hyperglycaemia but it is also caused by hyperinsulinism and the reduced sensitiveness of distal tissues to insulin. Hyperinsulinism is accompanied by the rise of catecholamine concentration, which also influences the intensification of ROS production [3, 6].

In respect of the great role that the oxidative stress may play in the pathogenesis of type 2 diabetes and its chronic complications the aim of this paper was to evaluate the activity of two selected antioxidative enzymes: superoxide dysmutase (SOD) and catalase (CAT) in erythrocytes of type 2 diabetes patients at different stages of the metabolic balance.

Material and methods

61 type 2 diabetes patients took part in the study. Among them 31 patients (the average age 64.3±9.8 years met the criteria of the metabolic balance (DM_W) while 30 patients (the average age 62.7±10.2 years) suffered from metabolically unbalanced diabetes (DM_N). The control group included 40 healthy people (the average age 37.1±8.2 years), it was typical of the given region and it was possible to relate the obtained results to it. Before the tested people were included in the study, they consciously agreed to participate in it. The Ethical Commission of the Military Academy of Lodz gave their permission (number 139/01) to carry out the investigation.

The qualification of research subjects took place on the basis of: anamnesis and physical examination, body mass index (BMI), routine lab tests and glycated haemoglobin concentration (HbA_{1c}). They did not take any medicines of proved antioxidative properties, which was the precondition for being included in the study. The blood for the tests was taken each time from the unfed patients (from the diabetes patients before administering the morning dose of hypoglycaemic drugs from the cubital vein on heparin (250 units of heparin/1 ml of blood).

The haemoglobin concentration was determined using the cyanmethaemoglobin method and standard reagents (Biomed, Poland).

The activity of superoxide dismutase (SOD-1; EC 1.15.1.1) in red blood cells was determined using the Misra and Fridovich method [7]. The enzyme activity was determined on the basis of the dismutase delaying the adrenalin oxygenation reaction in which superoxide anion takes part.

The activity of catalase (CAT; EC 1.11.16) was determined using the Beers and Sizer method [8] based on measuring the decomposition rate of hydrogen peroxide by the enzyme.

The U-Mann-Whitney Test and the ANOVA variance analysis were used in the statistical analysis.

Results

No statistically essential differences were found between BMI (<25 kg/m^2), arterial blood pressure (<140/90 mmHg), creatinine concentration, urea and urine albumin excretion (<30 mg/24 h during 24 h urine collection) between the tested groups and the obtained values were within the standard norms of the laboratory where the tests were carried out.

Glycaemia in the unfed control group was statistically essentially lower (x=4.8±1.9 mmol/l) compared with the type 2 diabetes patients metabolically balanced (x=6.8±1.7 mmol) and metabolically unbalanced (x=8.1±1.4 mmol/l) ($p<0.05$). The differences in the glycaemia values between the group with type 2 metabolically balanced diabetes and the one with type 2 metabolically unbalanced diabetes were statistically significant ($p<0.05$). The glycated haemoglobin concentration (HbA_{1c}) in the group of metabolically unbalanced type 2 diabetes patients was statistically significantly higher (x=8.2±1.7%) compared with the group with metabolically balanced diabetes

(x=6.9±1.3%) and the control group (x=5.6±1.1%). The concentration of total cholesterol and triglycerides in the blood plasma in the group of metabolically unbalanced type 2 diabetes patients were significantly higher (x=6.2±1.4 and x=2.9±1.3 mmol/l, respectively) compared with metabolically balanced type 2 diabetes patients (x=5.8±1.4 mmol/l and x=1.9±0.7 mmol/l, respectively) and the control group (x=3.8±1.1 mmol/l and x=1.1±0.3 mmol/l, respectively).

The activity of SOD-1 in red blood cells

While comparing the activity of SOD-1 in each studied group, in the group of type 2 diabetes patients at the stage of the metabolic balance it was found to be the highest (x=2156.0±158.8 U/gHb). The differences in the activity between this group of patients and the one with diabetes at the stage of being metabolically unbalanced (x=1979.8±123.1 U/gHb) were statistically significant ($p<0.05$). While comparing the SOD-1 activity values in each group of type 2 diabetes patients with the control group (x=2394.2±203 U/gHb), a statistically significant reduction of the enzyme activity was observed ($p<0.05$) (Figure 1).

The CAT activity in red blood cells

While comparing the CAT activity in each group of patients at the stage of being metabolically balanced (x=15.5±1.7 Bergmeyer U/gHb) and unbalanced (x=12.2±1.0 Bergmeyer U/gHb), the statistically significantly higher CAT activity in the group with metabolically balanced diabetes was observed ($p<0.05$).

However, while comparing the mean CAT activities in each group of patients with the enzyme activity in the control group (x=18.4±1.7 Bergmeyer U/gHb), the statistically significantly reduced CAT activity was observed in each group of type 2 diabetes patients ($p<0.05$) (Figure 2).

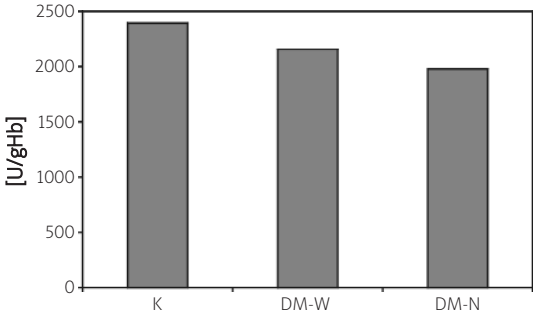
Discussion

The factor initiating the intensification of oxidative stress in diabetes might be prolonged hyperglycaemia. It has been confirmed with the proposition submitted by Ceriello regarding "glucose toxicity" [9]. Oxidative stress in diabetes manifests itself through, among others, the increased concentration of one of the end products of lipid peroxidation i.e. malondialdehyde (MDA) in the blood, directly proportionally to the glycated haemoglobin concentration, the increased concentration of hydroxy-lipids and hydrogen peroxide as well as the decrease of reduced glutathion in erythrocytes [2].

Similarly to other diseases, the oxidative stress in diabetes is the result of the increased production of reactive oxygen species on the one hand, on the other hand, however, of the reduced activity of the antioxidant system.

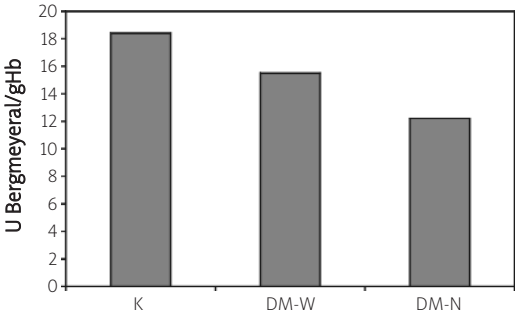
In many studies, both clinical and experimental, some changes in the activity of the enzymatic antioxidative systems in diabetes have been found. There is a lot of divergence, however, in the evaluation of the enzyme activity in diabetic patients. Both the increase and the reduction of the antioxidative enzyme activity in animal experimental models have been observed [10]. In our earlier studies the reduction of the key cellular antioxidative enzyme activity in erythrocytes in patients with diabetic nephropathy was found [11]. In our current studies the statistically significantly higher SOD-1 activity in the group of metabolically balanced type 2 diabetes patients, compared with the patients with poor metabolic control, was found.

While comparing the obtained values of SOD-1 activity in each group of type 2 diabetes patients at different stages of the metabolic balance with the control group, the considerable reduction of the enzyme activity, particularly in patients with poor metabolic control of diabetes, was observed. The reduced SOD-1 activity was reported in some earlier publications. Matkowics et al. [12] were the first to suggest the connection between the reduced activity of superoxide dismutase and diabetes. Crouch et al. [13] pointed out that in experimental diabetes in rats the activity of superoxide dismutase both in red blood cells and retina was reduced by 50% compared with healthy animals. Kawamura et al. [14] found the



K – control group, DM-W – metabolically balanced diabetes, DM-N – metabolically unbalanced diabetes

Figure 1. The activity of SOD-1 in red blood cells



K – control group, DM-W – metabolically balanced diabetes, DM-N – metabolically unbalanced diabetes

Figure 2. The CAT activity in red blood cells

increased amount of glycated form of SOD-1 in erythrocytes in type 1 diabetes patients which was connected with the reduced activity of the enzyme.

As far as the other antioxidative enzyme, i.e. CAT is concerned, its lowest activity was also found in the group of patients with metabolically unbalanced diabetes. While comparing mean CAT activities in each group of patients with the enzyme activity in the control group, the reduced CAT activity in groups of metabolically unbalanced type 2 diabetes patients was observed. It is consistent with the results of our earlier experimental studies where the reduced catalase activity, both in erythrocytes and in the kidney homogenate of the rats with streptozocine diabetes, was found [15].

The reduced catalase activity may be the result of the increased production of superoxide anion and an excess of hydrogen peroxide, which is the case in the increased glucose concentration in the plasma. Superoxide anion is CAT inhibitor, whereas hydrogen peroxide might be a precursor of other reactive oxygen species which in turn might initiate lipid peroxidation processes, which was found by, among others, Salahudeen et al. in his studies [16, 17].

The reduced catalase activity observed in our studies, as well as those by other authors, confirms that the enzyme may be easily inactivated due to the influence of ROS, particularly in poor metabolic control and prolonged hyperglycaemia.

The disturbances of the antioxidative protective system in erythrocytes in type 2 diabetes patients observed in our studies may indicate higher susceptibility of "diabetic" erythrocytes to the oxidative stress. It has been confirmed in other authors' studies [4, 18, 19]. These disturbances are bigger in poor metabolic balance of diabetes which has been proved in our studies. The increased glycated haemoglobin concentration in diabetes, particularly the poorly controlled one, might not only be instrumental in the intensification of the oxidative stress but influence erythrocyte oxidative damage and rheologic changes connected with it as well, which may add to the development of vascular diabetic complications.

In conclusion, in the red blood cells of type 2 diabetes patients, compared with the control group, the disturbances within the enzymatic antioxidative barrier were found, particularly in-patients with poor metabolic control of diabetes. The obtained results confirm the thesis of glucose toxicity. Prolonged hyperglycaemia leads to the intensification of oxidative stress and weakening antioxidative mechanisms by activating various metabolic processes.

Taking into account early development of chronic complications of type 2 diabetes it seems that the differences observed in the antioxidative enzymes activity in type 2 diabetes patients with different degrees of the metabolic balance may be used to monitor the clinical process of the disease and may be prognostically significant in case of the development of chronic complications of the disease and thus indicate new therapeutic directions.

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