Catalase (antioxidant enzyme) activity in streptozotocin-induced diabetic rats

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Abstract

Background: High concentration and/or inadequate removal of reactive oxygen species may result in oxidative stress that may cause severe metabolic malfunction. An imbalance in antioxidant enzymes has been related to specific pathologies such as diabetic complications. Catalase catalyzes the reduction of hydroperoxides, thereby protecting mammalian cells against oxidative damage. In addition, catalase is active in neutralizing reactive oxygen species and so removes cellular superoxide and peroxides before they react with metal catalysts to form more reactive species. We investigated the status of catalase activity in erythrocytes of streptozotocin (STZ)-induced diabetic rats. Method: Catalase activity was measured by using spectrophotometric techniques. Result: Catalase activity increased in diabetic rats compared to control group [25.7 ± 2.8 vs. 16.3 ± 2.1 mmol H₂O₂ per min/ mg of protein, mean ± SD, p < 0.05]. Our results show that catalase activity increased significantly in the erythrocytes of STZ-induced diabetic rats. (Int J Diabetes Metab 15: 22-24, 2007)

Key words: Antioxidant enzyme, Catalase, diabetic, oxygen free radicals.

Introduction

There are significant differences in the activities of antioxidant enzymes between diabetic and non-diabetic patients.¹ The relationship between serum lipids, lipoproteins, and the erythrocyte antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) has been investigated in non-insulin-dependent diabetic patients.² Erythrocyte antioxidant enzyme activities were measured in 105 non-insulin dependent diabetic patients, among whom 38 had microvascular complications of diabetes.³ Free radicals and lipid peroxide are easily formed in the diabetic state and may play an important role in the development of diabetic complications.⁴ A positive correlation between thiobarbituric acid reactive products and glucose, glycated hemoglobin and fructosamine in the serum of diabetic patients was observed.⁵ Impairment by streptozotocin (STZ) of antioxidant enzymes may contribute to STZ-induced experimental diabetes.⁶ SOD, GPx and catalase were assayed in the erythrocytes of a diabetic population on various treatment regimens in order to investigate any relationships between their activities and diabetes markers.⁷ Reactive oxygen species (ROS) are constantly formed in the human body and are removed by an antioxidant defense system. In healthy individuals the generation of ROS appears to be in approximate balance with antioxidant defense. An imbalance between ROS and antioxidant defenses in favor of the former has been described as oxidative stress. In some human diseases, increased oxidative stress may make an important contribution to disease pathology.⁸,⁹ ROS are generally cytotoxic because of the oxidative damage they can cause to cellular components. However, at low concentrations, ROS may function as physiological mediators of cellular responses.¹⁰ Oxidative stress, which is associated with the formation of lipid peroxides, is suggested to contribute to pathological processes in aging and many disease such as diabetes, atherosclerosis and cataract.¹¹ Increased oxidative stress as a result of increased free radical formation has also been suggested as a contributor to vascular damage in diabetes.¹²,¹³ Low levels of ROS are indispensable in many biochemical processes, including intracellular messaging in cell differentiation and cell progression or the arrest of growth, apoptosis,¹⁴ immunity¹⁵ and defense against microorganisms.¹⁶,¹⁷ In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunction and damage to biological macromolecules.¹⁸,¹⁹ Oxidative stress derived from excessive superoxide production and an imbalance in antioxidant enzymes has been related to many other pathologies such as chronic granulomatous diseases, diabetic complications, hepatitis, rheumatoid arthritis, influenza virus, ulcer, pneumonia, HIV infection, cataract and glaucoma.¹⁹,²⁰ The aim of this study was to investigate catalase activity in the erythrocytes of STZ-induced diabetic rats.

Materials And Methods

Materials

2,4-dinitrophenylhydrazine, H₂O₂, NaCl, EDTA, trichloroacetic acid, HCl, ethanol-ethyl acetate, guanidine hydrochloride and streptozotocin (STZ) were purchased from Sigma (St. Louis, Mo, USA). Distilled water was used in all experiments.
Catalase activity in diabetes

Figure 1: Catalase activity in the erythrocytes of normal and diabetic rats. Each column represents the mean value ± SD of 6 separate experiments, p < 0.05. (column 1: diabetic group; column 2: control group).

Animals
Male rats (10 weeks old and between 220 and 260 g in body weight) were randomly assigned to two groups. One group of rats (diabetic group) received an intraperitoneal injection of STZ (50 mg/kg) after anesthesia with diethyl ether. STZ was dissolved in a citrate solution (0.15 M citric acid and 0.25 M sodium phosphate, pH 4.65). Another group (control group) received an equivalent volume of citrate buffer alone. Control and diabetic rats were caged separately but housed under similar conditions. Both groups of animals were fed with the same diet and water ad libitum. All experimental manipulations were carried out with the animals under diethyl ether anesthesia. On the day of the experiments, blood samples were collected and catalase activities were determined.

Preparation of blood samples and lysates
Blood was collected into heparinized syringes through puncture of the left heart ventricle or tail. Erythrocytes were obtained after centrifugation at 600-1400 × g for 10 min. Erythrocytes were washed twice with 0.9% sodium chloride and were centrifuged under the same conditions. A 5% erythrocyte suspension in 0.15 M NaCl – 10 mM sodium phosphate buffer, pH 7.4 was lyzed by freezing (-20°C) for 24 h and was used for catalase measurement.

Assay of catalase activity
Catalase (EC.1.11.1.6) catalyses the decomposition of hydrogen peroxide to give water and molecular oxygen. Catalase activity was determined according to a previously reported method.21 The decomposition of H₂O₂ can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a measure of catalase activity. The results obtained for the sample containing 500 μL haemolysate dilution and 500μL substrate solution (10 mM H₂O₂ prepared in 50 mM phosphate buffer, pH 7.0) were compared with those of a blank containing 1 ml phosphate buffer, instead of substrate solution and 500μL of hemolysate dilution. The reaction was initiated by the addition of the substrate solution and incubated at 20 °C for about 1 min. Catalase activity was expressed as mmol H₂O₂/min/mg protein. An enzyme unit was defined as the amount of enzyme that catalyzes the release of one μmol of H₂O₂ per min at 20 °C. Specific activity was calculated in terms of units per mg of protein.

Results
All rats injected with STZ developed severe diabetes as indicated by increasing serum glucose concentrations. Serum glucose levels in diabetic rats were elevated (approximately 2-fold) as compared with controls. Catalase activity in the diabetic rat group was increased compared to control group [25.7 ± 2.8 vs. 16.3 ± 2.1 mmol H₂O₂/ min/mg of protein; mean ± SD. P<0.05] Catalase activities in diabetic and control rats are reported in Figure 1.

Discussion
Higher amounts of ROS have been shown to play a role in the development of diabetic complications as well as in a number of other disease states. As a safeguard against the accumulation of ROS, intracellular enzymatic antioxidant activities exist. ROS generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations.3,6,8 Oxidative stress induced by excessive production of superoxide and an imbalance in antioxidant enzymes has been linked to the development of diabetic complications. ROS are key participants in the damage caused by diabetic complications. Diabetes is one of the pathological processes known to be related to an unbalanced production of ROS, such as hydroxyl radicals (HO), superoxide anions (O2) and H₂O₂. Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. We found a significantly higher catalase activity in diabetic rats compared with controls. Our results confirm previous data indicating enhanced ROS levels in diabetes mellitus.3,2,14 An overproduction of ROS especially in diabetes can not be properly balanced by antioxidant enzymes. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of this enzyme. Our results indicate the presence of some alteration in oxidant–antioxidant balance in the erythrocytes of diabetic rats. The increase in the erythrocyte antioxidant enzymes such as catalase is related to the oxidative damage of membrane protein and lipid by increased oxygen free radicals in the body.

References
1. Durdi Qujeq & Timur Rezvani
