The Status of Catalase Activity in Erythrocytes of Steptozotocin (STZ) Induced Diabetic Rats

Introduction

Reactive oxygen species (ROS) are constantly formed in the human body and removed by an antioxidant defense system. In healthy individuals, the generation of ROS appears to be approximately in 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 disease, increased oxidative stress may make an important contribution to disease pathology (1,2). 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 response (3).

Free radicals and lipid peroxides, easily formed in the diabetic state, play an important role in the development of diabetic complications (4). There is significant difference in activity of antioxidant enzymes between diabetic and non-diabetic patients (5). Impairment by streptozotocin of antioxidant enzymes may contribute to streptozotocin-dependent experimental diabetes (6). Increased oxidative stress as a result of increased free radical formation has also been suggested as a contributor to vascular damage in diabetes (7-9).

Low levels of ROS are indispensable in many biochemical processes, including intracellular messaging in the cell differentiation and cell progression or the arrest of growth, apoptosis (10), immunity (11), and defense against micro-organism (12,13). In contrast, high doses and /or inadequate removal of ROS result in oxidative stress, which may cause server metabolic malfunction and damage to biological macromolecules (14-16). The aim of this work was to investigate, an antioxidant enzyme, catalase activity in the erythrocyte of steptozotocin –induced diabetic rats.

Abstract

Catalase, catalyses the reduction of hydroperoxides, thereby protecting mammalian cells against oxidative damage. Also, catalase active in reactive oxygen species neutralization and within cells removes superoxide and peroxides before they react with metal catalysis to form more reactive species. We investigated the status of catalase activity, in erythrocytes of streptozotocin (STZ) induced diabetic rats. Catalase activity was measured by using spectrophotometric techniques. Catalase activity in the diabetic rats group was increased compared to control group [25.7 ±2.8 (mean ± SD) vs. 16.3 ± 2.1], mmol H2O2 per min/ mg of protein, P<0.05. Our results showed that catalase activity was significantly increased in the erythrocytes of STZ-induced diabetic rats. Turk Jem 2007; 11: 79-80

Key words: Antioxidant enzyme, catalase, streptozotocin , oxygen free radicals

Özet

Katalaz hidroksiperoksitleri katalize edip redükte ederek memeli hücrelerini oksidatif hasara karşı korur. Aynı zamanda katalaz reaktif oksijen türeli metal katalizi ile daha reaktif türlerle dönüşüm için reaksiyona girdiğinde, reaktif oksijen türelerini nötralize eder ve hücre içinde super- oksit ve peroksitleri uzaklaştırır. Biz streptozotosin ile diyabetik hale getirilmiş çıkanların eritrositlerinde katalaz aktivitesi durumunu araştırdık. Katalaz aktivitesi specrofotometr teknikle ölçüldü. Katalaz aktivitesi diyabetik çıkanlarda kontrol grubuna göre artış olarak bulundu [25.7 ±2.8 (mean ± SD) vs. 16.3 ± 2.1], mmol H2O2 per min/ mg of protein, P<0.05.


Anahtar kelimeler: Antioxidan enzim, katalaz, streptozotosin, serbest oksijen radikalleri

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**Materials and Methods**

**Materials**

2,4-dinitrophenylhydrazine, H2O2, NaCl, EDTA, trichloroacetic acid, HCl, ethanol, ethyl acetate and streptozotocin were purchased from Sigma, St. Louis, Mo, USA.

**Animals**

Male rats were randomly assigned two groups. One group of rats (diabetic group) received an intraperitoneal injection of streptozotocin (50 mg/kg) anesthesia with diethyl ether. All rats injected with streptozotocin developed diabetes as indicated by an increasing serum glucose level. The rats which had developed diabetes Serum glucose levels in diabetic rats were elevated approximately 2-fold as compared to controls. Also, the rats which had developed diabetes as indicated by glucosuria, indicated by glucose test for urine. Duration of diabetes was 3 month. 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 experiments manipulation were carried out with the animals under diethyl ether anesthesia. On the day of the experiments, a blood sample was collected and catalase activity was determined.

**Preparation of blood samples and lysates**

Blood was collected by a heparinized syringe through puncture of the left heart ventricle or tail. Erythrocytes were obtained after centrifugation at 600 g for 10 min. Erythrocytes were washed twice with 0.9% sodium chloride and were centrifuged under the same conditions. The 5% erythrocyte suspension in 0.15 M NaCl–10 mM sodium phosphate buffer, pH 7.4 was lazed through freezing (-20°C) for 24 hr and was used for enzyme measurement. Blood glucose was determined by the kit method (Pars Azmon, IRAN).

**Assay of catalase activity**

Catalase activity was determined according to the previously reported method (17) 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 for the sample, containing 500 mL hemolysate dilution an 500mL substrate solution (10 mM H2O2 prepared in 50 mM phosphate buffer PH 7.0), were compared with a blank. The reaction starts by addition of the substrate solution and follows at 20°C for about 1 min. Catalase activity was expressed as mM H2O2 /min/ mg protein. An enzyme unit was defined as the amount of enzyme that catalyzes the release of one mmol of H2O2 per min at 20°C. Specific activity was in terms of units per mg of protein.

**Results**

Serum glucose levels in diabetic rats were elevated approximately 2-fold as compared with controls. In diabetic rats we observed a decrease in body. Also, the rats which had developed diabetes as indicated by glucosuria, indicated by glucose test for urine. Catalase activity in the diabetic rats group was observed a decrease in body. Also, the rats which had developed diabetes Serum glucose levels in diabetic rats were elevated approximately 2-fold as compared to controls. Also, the rats which had developed diabetes as indicated by glucosuria, indicated by glucose test for urine. Duration of diabetes was 3 month. 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 experiments manipulation were carried out with the animals under diethyl ether anesthesia. On the day of the experiments, a blood sample was collected and catalase activity was determined.

**Discussion**

Reactive oxygen species generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations (1, 6). Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. An imbalance in antioxidant enzymes has been related to diabetic complications. Reactive oxygen species are key participants in damage caused by diabetic complications (1, 6). We found a significantly increased catalase activity in diabetic rats as compared with control subjects. Our results confirm previous data of an enhanced catalase activity levels in diabetes mellitus (7,9). An overproduction of reactive oxygen species especially in diabetes can not be properly balanced by the antioxidant enzymes. Therefore, when oxidative stress arises as consequence of a pathologic event, a defense system promotes the regulation and expression of this enzyme. Our results indicate the presence of some variation in oxidant–antioxidant balance of erythrocyte in diabetic group. The increase in the erythrocyte antioxidant enzymes such as catalase is related to the oxidative damage of membrane protein and lipid by increased free radicals in the body.

**References**