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

Streptozotosinle Diyabetik Sıçanların Eritrositlerinde Katalaz Aktivitesi

Durdi Qujeq, Timur Rezvani*

Department of Biochemistry and Biophysics, Faculyt of Medicine, *Faculty of Medicine, Babol University of Medical Sciences, Babil, Iran

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 spectrophtometric 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 türleri metal katalizi ile daha reaktif türlere dönüşmek için reaksiyona girmeden reaktif oksijen türlerini nötralize eder ve hücre içinden superoksit ve peroksitleri uzaklaştırır. Biz streptozotosin ile diyabetik hale getirilmiş sıçanların eritrositlerinde katalaz aktivitesi durumunu araştırdık.

Katalaz aktivitesi spektrofotometrik teknikle ölçüldü. Katalaz aktivitesi diyabetik sıçanlarda kontrol grubuna gore artmış olarak bulundu [25.7 ±2.8 (mean ± SD) vs. 16.3 ± 2.1], mmol H2O2 per min/ mg of protein, P<0.05.

Bulgularımıza gore streptozotosinle oluşturulmuş diyabetik sıçanlarda katalaz aktivitesi anlamlı olarak artmıştır.*Turk Jem 2007; 11: 79-80*

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

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 peroxide, 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.

Address for Correspondence: Dr. Durdi Qujeq, Department of Biochemistry and Biophysics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
Phone: :+98-111-2229591-5(363) Fax: 0098-111-2290181 E-mail: dqujeq@hotmail.com

Materials and Methods

Materials

2,4 -dinitrophenylhydrazine, H2O2, NaCL, EDTA, trichloroacetic acid, HcL, ethanol, ethyl acetate and streptozotocine were purchased from Sigma, St. Louis, Mo, USA.

Animals

Male rats ,were randomly assigned two groups. One group of rats (diabetic group) received a 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 diabetics 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 increased compared to control group [25.7 ±2.8 (mean ± SD) vs. 16.3 ± 2.1], mmol H2O2 per min/ mg of protein, $P<0.05$.

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 diabetic complications. Reactive oxygen species are key participants in damage caused by diabetic complications (1, 6).

We found significantly increased catalase activity in diabetic rats as compared with control subjects. Our results confirm previous data of an enhanced reactive oxygen species 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 oxygen free radicals in the body.

References

1. Halliwell B. The role of oxygen radicals in human disease, with particular reference to the vascular system. Haemostasis. 1993; 23: 118-26.
2. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41: 1819-28.
3. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB. et al. Epidermal growth factor induced generation of hydrogen peroxide. The Journal of Biological Chemistry 1997; 272: 217-21.
4. Mano T, Shinohara R, Nagasaka A, Nakagawa H, Uchimura K, Hayashi R, Nakano I, Tsugawa T, Watanabe F, Kobayashi T, Fujiwara K, Nakai A, Itoh M. Scavenging effect of nicorandil on free radicals and lipid peroxide in streptozotocin-induced diabetic rats. Metabolism. 2000 Diabet Med 1999; 16: 74-8.
5. Jandric-Balen M, Bozikov V, Bistrovic D, Jandric I, Bozikov J, Romic Z, Balen I. Antioxidant enzymes activity in patients with peripheral vascular disease, with and without presence of diabetes mellitus. Coll Antropol 2003; 27: 735-43.
6. Winkler R, Moser M. Alterations of antioxidant tissue defense enzymes and related metabolic parameters in streptozotocin-diabetic rats--effects of iodine treatment. Wien Klin Wochenschr 1992; 104: 409-13.
7. Gallou G, Ruelland A, Legras B, Maugendre D, Allannic H, Cloarec L. Plasma malondialdehyde in type 1 and type 2 diabetic patients. Clin Chim Acta 1993; 214: 227-34.
8. Jennings PE, Jones AF, Florkowski CM, Lunec J, Barnett AH. Increased diene conjugates in diabetic subjects with microangiopathy . Diabetic Med 1987; 4: 452-6.
9. Lyons TJ. Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabetic Med 1991; 8: 411-9.
10. Ghosh J, Myers CE. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. Proc Natl Acad Sci 1998; 95: 13182-7.
11. Yin GY, Yin YF, He XF. Effects of zhuchun pill on immunity and endocrine function of elderly with kidney -yang deficiency. Chung Kuo Chung His Chieh Ho Tsa Chih 1995; 15: 601-3.
12. Bae YS, Kang SW, Seo MS. Epidermal growth factor-induced generation of hydrogen peroxide. J Biol Chem 1997; 272: 217-21.
13. Lee YJ, Galoforo SS, Berns CM. Glucose deprivation -induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. J Biol Chem 1998; 273: 5294-9.
14. Chopra S, Wallace HM. Induction of spermidine/spermine N1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen species. Biochem Pharmacol 1998; 55: 1119-23.
15. Czene S, Tiback M, Harm S, Ringdahl M. PH-dependent DNA cleavage in permeabilized human fibroblasts. Biochem J 1997; 323: 337-41.
16. Wojtaszek P. Oxidative burst: an early plant response to pathogen infection. Biochem J 1997; 322: 681-92.
17. Abei H. Catalse Methods Enzymatic analysis (Bergmeyer HU, Ed), Verlag Chemie, Weinheim 1974; 673-78.