Malondialdehyde and carbonyl contents in the erythrocytes of streptozotocin-induced diabetic rats

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Abstract

Oxidative stress is involved in degenerative disease, including atherosclerosis and diabetes mellitus. Impaired antioxidant defense mechanism may be an important factor in the pathogenesis of diabetes. Oxidative stress can be measured by monitoring the changes in blood malondialdehyde and carbonyl content. Determination of carbonyl level is used as an index of the extent of the oxidative damage of protein. Moreover, malondialdehyde level is a marker of lipid oxidation. We investigated the status of lipid peroxidation and protein oxidative damage in erythrocytes of streptozotocin (STZ) induced diabetic rats by measuring erythrocyte malondialdehyde and carbonyl levels using a spectrophotometer. We found that carbonyl and malondialdehyde levels were significantly increased in the erythrocytes of STZ-induced diabetic rats. This observation indirectly suggests an increase in free radical-mediated damage of the cell membrane.

*Key words***:** *Malondialdehyde, carbonyl, diabetes, antioxidant.*

Introduction

Antioxidants work together in human blood cells against toxic reactive oxygen species.[1,2] Reactive oxygen species (ROS) cause lipid peroxidation and oxidation of some specific proteins, thus affecting many intra- and intercellular systems.[3,4] Total superoxide dismutase, catalase, and glutathione peroxidase activities are higher in the liver of STZ-treated rats.[5] The levels of malondialdehyde and protein carbonyl, as markers of lipid and protein oxidation do not increase in aminoguanidine-treated rats.[6] The antioxidant enzyme superoxide dismutase activity is significantly higher in the erythrocytes of diabetic patients independently of the presence of microvascular complications.[7] The levels of malondialdehyde and carbonyls in the liver, kidney and pancreas mitochondria are significantly increased in STZ-treated rats.[8]. Significant increases in lipid peroxidation, as measured by thiobarbituric acid, and protein oxidation, as measured by protein carbonyl content, were observed 6 weeks after the occurrence of diabetes.[9] A broad derangement in nonenzymatic biochemistry involving both lipids and carbohydrates exists in diabetic glomerular lesions.[10] Protein oxidation may, therefore, represent an important factor in the development of symptoms in diabetic patients.[11] Higher concentrations of malondialdehyde and carbonyl proteins were found in ocular tissues of diabetic compared to nondiabetic patients.[12] The aim of the present study was to investigate the status of lipid peroxidation

and protein oxidative damage in the erythrocytes of STZ-induced diabetic rats by measuring erythrocyte malondialdehyde and carbonyl levels.

Materials and method

Experimental Animals

Male Wistar rats, 10 weeks old weighing between 220g and 260g, were randomly assigned to two groups. One group of rats (diabetic group) received a single tail-vein injection of STZ (50 mg/kg) under light anaesthesia with diethyl ether. STZ was dissolved in a citrate buffered 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 solution 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 anaesthesia. On the day of the experiments, blood sample was collected and malondialdehyde and carbonyl contents were determined. All animals survived the study without any sign of illness during the course of the study.

Preparation of blood samples and lysates

Blood samples were collected into heparinized syringes through a puncture in the left ventricle of the heart. Erythrocytes were obtained after centrifugation at 600g for 10 min. Erythrocytes were washed twice with 0.9% sodium chloride and were centrifuged under the same condition. The 5% erythrocyte suspension in 0.15 M NaCl –10 mM sodium phosphate buffer, pH 7.4 - was lysed through freezing (-20°C) for 24 h and was used for the measurement of malondialdehyde and carbonyl contents.

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Determination of carbonyl level

Determination of carbonyl level in proteins is used as an index of the extent of protein oxidative damage. Determination of carbonyl level in proteins was carried out as previously described.[13] Briefly, one ml of haemolysate was placed in one glass tube and 3 ml of 8 mM 2, 4-dinitrophenylhydrazine in 3 M HCL was added. The tubes were incubated for 50 min at 37 °C in the dark and vortexed every 10 min. Then, 3 ml of 25% (w/v) trichloroacetic acid was added and the tube was left on ice for 8 min and then centrifuged for 3 min in a tabletop centrifuge to collect the protein precipitates. This pellet was washed using 3ml 15% trichloroacetic acid. Next, the pellet was washed 2 times with 4 ml of ethanol-ethyl acetate (1:1), (v/v). The final precipitate was dissolved in 2 ml 5M guanidine hydrochloride solution and was incubated for 15 min at 37°C with mixing. Any insoluble materials were removed by repeated centrifugation. Carbonyl level was calculated from the peak absorbance of the spectra at 355-390nm, using an absorption coefficient of 22000 M^{-1} cm^{-1}. Results were expressed as mmol/mg of protein.

Determination of malondialdehyde

Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substance method. This method evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress. Determination of malondialdehyde level in proteins is used as an index of the extent of lipid peroxidation. Concentration of malondialdehyde was measured by the thiobarbituric acid test.[14] Briefly 1 mM EDTA (Sigma, St. Louis, MO, USA) was added to a 0.5 ml haemolysate and was mixed with 1 ml cold 15 % (w/v) trichloroacetic acid to precipitate proteins. The supernatant was treated with 1 ml 0.5 % (w/v) thiobarbituric acid in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 535 nm and the concentration of thiobarbituric acid reactive substance was calculated by using malondialdehyde as a standard. Results were expressed as mmol thiobarbituric acid reactive substances/mg of protein. The protein contents of various enzyme extracts relative to standard solutions of bovine serum albumin were determined by the method of Lowry et al. [15]

Results

All rats injected with streptozotocin developed diabetes as indicated by an increasing serum glucose level (range 228 - 283 mg/dl). Serum glucose levels in diabetic rats were elevated approximately 2-fold as compared to controls. Figure 1 shows the levels of carbonyl in erythrocytes of STZ-induced diabetes rats. The result showed that the level of carbonyl increased significantly (p<0.05) in the erythrocytes of STZ-diabetic rats compared to controls. Malondialdehyde levels in diabetic and control rats are shown in Figure 2. Malondialdehyde content in erythrocytes of streptozotocin induced diabetes rats group was significantly (p<0.05) elevated compared to that of the control group.
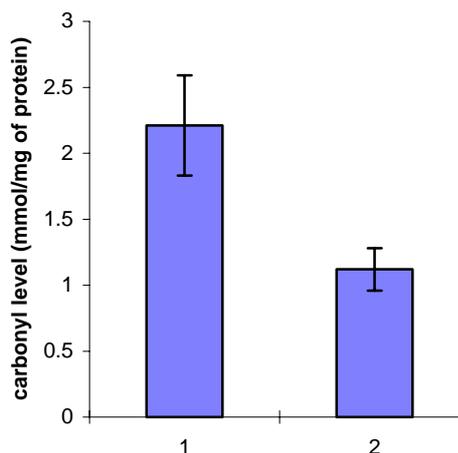

Figure1: Carbonyl levels in diabetic and control rats. Each column represents the mean value ± SD of 6 separate experiments, P<0.05. (Column 1: diabetic group; Column 2: control group).

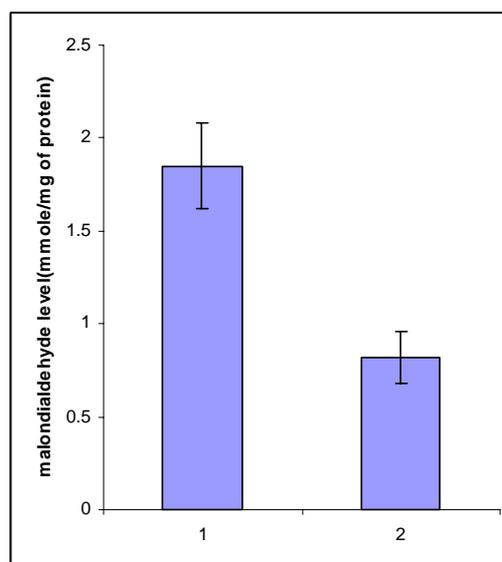**Figure 2:** Malondialdehyde levels in diabetic and control rats. Each column represents the mean value ± SD of 6 separate experiments, P<0.05. (Column 1: diabetic group; Column 2: control group).

Discussion

Diabetes is one of the many pathological processes known to be related to an unbalanced production of reactive oxygen species, such as hydroxyl radicals (HO), superoxide anions (O_2) and hydrogen peroxide (H_2O_2). Reactive oxygen species generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations.[3,4] Determination of malondialdehyde by thiobarbituric acid is used as an index of the extent of lipid peroxidation. Malondialdehyde, used as the best available measure of global reactive oxygen species is substantially elevated in diabetes.[8, 9]

In our study, we observed increased malondialdehyde and carbonyl content in the erythrocytes of streptozotocin induced diabetic rats. The increase in the blood carbonyl level is related to the oxidative damage of protein by elevated oxygen free radicals in the diabetic group. Increased blood malondialdehyde levels also suggest oxygen free radical-mediated damage of the membrane lipid.

These findings may indicate that the mechanism of lipid and protein metabolism is impaired in erythrocytes of STZ-induced diabetic rats. The balance between oxidative stress and antioxidant defence mechanism may be impaired by depletion of enzymatic antioxidants and increased blood levels of malondialdehyde and carbonyl in subjects with diabetes. In the present study, we find some interesting similarities between our findings and those reported earlier in the literature.[3,6,9] Our study showed that malondialdehyde and carbonyl levels were significantly increased in erythrocytes of streptozotocin induced diabetes rats compared with the levels in control subjects. These indirectly suggest an increased production of oxygen free radicals in diabetic rats. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. Also, the increase in the blood carbonyl level is related to the oxidative damage of the protein by increased oxygen free radicals in the diabetic group. Our results confirm previous data of enhanced reactive oxygen species levels in diabetes mellitus.[8,9,10] In conclusion, these results indicate the presence of some variations in oxidant-antioxidant balance in the erythrocytes of diabetic rats.

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