

Amelioration of altered serum, liver, and kidney antioxidant enzymes activities by sodium selenite in alloxan-induced diabetic rats

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Abstract

Background: The aim of this study was to evaluate the possible protective effect of sodium selenite on serum, liver, and kidney antioxidant enzymes activities in alloxan-induced type 1 diabetic rats.

Methods: Forty Sprague-Dawley male rats were randomly divided into four groups; Group one as control, Group two as sham-treated with sodium selenite by 1 mg/kg intraperitoneal (i.p.) injections daily, Group three as diabetic untreated, and Group four as diabetic treated with sodium selenite by 1 mg/kg i.p. injections daily. Diabetes was induced in the third and fourth groups by subcutaneous alloxan injections. After eight weeks the animals were euthanized and livers and kidneys were immediately removed and used fresh or kept frozen until analysis. Before the rats were killed blood samples were also collected to measure glutathione peroxidase (GPX) and catalase (CAT) activities in sera.

Results: Glutathione peroxidase and CAT activities serum, liver, and kidney were all significantly less in the diabetic rats than in the controls. Sodium selenite treatment of the diabetic rats resulted in significant increases in GPX activity in the kidneys and livers, and CAT activity in the sera and livers.

Conclusions: Our results indicate that sodium selenite might be a potent antioxidant that exerts beneficial effects on both GPX and CAT activities in alloxan-induced type 1 diabetic rats.

Keywords: Diabetes, Rat, Sodium selenite, Antioxidant enzymes activity

Introduction

Chronic diseases such as diabetes and cardiovascular disease increase oxidants and decrease antioxidants in patients (1, 2). Conditions that increase oxidants and reduce antioxidants also exacerbate these diseases (1-5). In diabetic patients, hyperglycemia causes glycation of macromolecules such as proteins, lipids, and nucleic acids (6). The glycation of macromolecules can alter their functions, increase lipid peroxidation, and reduce antioxidant enzyme activity, causing damage to the cells (7). Clinical and experimental studies have shown that disturbing the

balance of the oxidant-antioxidant system can contribute to the pathogenesis of chronic diseases such as cancer, cardiovascular disease, diabetes, and many diabetic complications (8).

Selenium is an antioxidant. It can act as an antioxidant directly or as a cofactor in antioxidant enzymes such as glutathione peroxidase (GPX) in a number of biological processes in humans and other species (9). Selenium deficiencies can induce cancer, coronary heart disease, and liver necrosis (10-14). The possible protective effects of sodium selenite on

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serum, kidney, and liver antioxidant enzyme activities in alloxan-induced type 1 diabetic rats have not yet been reported. Therefore, this study was designed to investigate the effect of sodium selenite on the antioxidant enzymes GPX and catalase (CAT) activities in serum, kidney, and liver from alloxan-induced type 1 diabetic rats.

Materials and Methods

Animals

Thirty mature male Sprague–Dawley rats (180-200 g) were obtained from the Pasteur Institute of Tehran and allowed to adapt to the new location for one week. This study was approved by the Animal Ethics Committee of the Medical University of Lorestan according to the National Health and Medical Research Council guidelines. The rats were divided into four groups of 10. The groups were as follows: Group 1 as control, Group 2 as non-diabetic treated with 1 mg/kg intraperitoneal (i.p.) daily sodium selenite, Group 3 as diabetic without sodium selenite treatment, and Group 4 as diabetic treated with 1 mg/kg i.p. daily sodium selenite.

Diabetes induction

Diabetes was induced after overnight fasting in the third and fourth groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously (5). Beta cell degradation by alloxan leads to increased insulin release. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 hr in place of drinking water. Five days after induction of diabetes, blood samples were collected from tails. Blood glucose was measured by glucometer and the rats with blood glucose levels ≥ 300 mg/dl (16.7 mmol/l) were defined as diabetic (15, 16). During the first five days after diabetes induction, 1-3 rats per group died from alloxan toxicity. The rats were kept at 12/12 dark-light periods in 21 ± 3 °C. All animals were allowed free access to food and water during the experiment. The sodium selenite treatment of Group 4 began on the first day of diabetes induction. After eight weeks, animals were euthanized with Nesdonal (50 mg/kg, i.p.). Blood samples were obtained from hearts and allowed to clot for 20 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes in a bench top centrifuge and serum was collected (5). Livers and kidneys were removed

immediately and used fresh or kept frozen for further analyses.

CAT Activity

Catalase activity was estimated using the method of Sinha. The reaction was started by the addition of 20 μ l of sample in 2 ml of 30 mmol/l hydrogen peroxide (H_2O_2) in 50 mmol/l potassium phosphate buffer pH 7.0. Enzyme units are expressed as mmol/l of consumed H_2O_2 per min g or ml (18).

GPX Activity

GPX activity was determined using a GPX assay kit (Randox Lab., Ltd., UK) according to the manufacturer's protocol.

Statistical analysis

All values are expressed as means \pm standard deviations (SD). The data were compared between groups by the Mann-Whitney U test. Statistical analyses were performed using the SPSS 13 for Windows software. *p* values < 0.05 were considered statistically significant.

Results

Effect of sodium selenite on serum, kidney, and liver GPX activity in diabetic rats

The GPX activity in serum, kidney, and liver are shown in Table 1. Serum GPX activity in the untreated diabetic rats was significantly less than that of the controls (44.86 ± 10.89 vs. 69.19 ± 20.11 U/mg protein). Serum GPX activity of the selenium-treated diabetic rats was not significantly different from that of the untreated diabetic rats (52.24 ± 13.76 vs. 44.86 ± 10.89 U/mg protein). Renal GPX activity in the untreated diabetic rats was significantly less (1.81-fold) than that of the controls (37.06 ± 16.49 vs. 66.92 ± 8.25 U/mg protein). Renal GPX activity in the selenium-treated diabetic rats was significantly greater than that of the untreated diabetic rats (65.04 ± 7.73 vs. 37.06 ± 16.49 U/mg protein). Liver GPX activity in the untreated diabetic rats was significantly less (1.48-fold) than that of the controls (42.77 ± 6.78 vs. 63.52 ± 13.30 U/mg protein). Liver GPX activity in the sodium selenite-treated diabetic rats was significantly greater (22.63%) than that of the untreated diabetic rats (52.45 ± 15.99 vs. 42.77 ± 6.78 U/mg protein). The GPX activities in the kidneys and

livers of the sodium selenite-treated non-diabetic rats were relatively high and similar to the levels found in the control animals.

Effect of sodium selenite on serum, kidney, and liver CAT activity in diabetic rats

Serum, kidney, and liver CAT activities are shown in Table 2. Serum CAT activity in the untreated diabetic

rats was significantly less (1.79-fold) than that of the controls (46.00 ± 7.27 vs. 83.49 ± 29.71 U/mg protein). Serum CAT activity of sodium selenite-treated diabetic rats was significantly greater than that of the diabetic untreated rats (71.63 ± 19.68 vs. 46.00 ± 7.27 U/mg protein).

Table 1. The effect of sodium selenite on serum, liver, and kidney GPX activity in alloxan-induced diabetic rats (Values represented as mean \pm S.D).

Experimental groups		Serum	Kidney	Liver
GPX activity (U/mg protein)	Control (non-diabetic untreated)	$69.19 \pm 20.11^*$	$66.92 \pm 8.25^*$	$63.52 \pm 13.30^*$
	Non-diabetic treated	$70.86 \pm 13.76^*$	$70.16 \pm 5.11^*$	$68.67 \pm 13.33^*$
	Diabetic untreated	44.86 ± 10.89	37.06 ± 16.49	42.77 ± 6.78
	Diabetic treated	$52.241 \pm 13.76^\#$	$65.04 \pm 7.73^*$	$52.45 \pm 15.99^* \# \surd$

* $P < .05$ compared with diabetic untreated group.

^\# $P < .05$ compared with controls.

\surd $P < .05$ compared with the non diabetic treated group.

Table 2. The effect of sodium selenite on serum, liver, and kidney CAT activity in alloxan-induced diabetic rats (Values represented as mean \pm S.D).

Experimental groups		Serum	Kidney	Liver
CAT activity (U/mg protein)	Control (non-diabetic untreated)	$83.49 \pm 29.71^*$	$89.88 \pm 19.01^*$	$84.94 \pm 23.55^*$
	Non-diabetic treated	$76.45 \pm 21.43^*$	53.84 ± 20.02	$61.26 \pm 11.77^*$
	Diabetic untreated	46.00 ± 7.27	46.75 ± 10.39	26.13 ± 7.94
	Diabetic treated	$71.63 \pm 19.68^*$	$45.71 \pm 23.75^\#$	$58.84 \pm 23.91^* \#$

* $P < .05$ compared with diabetic untreated group.

^\# $P < .05$ compared with controls.

Renal CAT activity in the untreated diabetic rats was significantly less (1.92-fold) than that of the controls (46.75 ± 10.39 vs. 89.88 ± 19.01 U/mg protein). Renal CAT activity in the sodium selenium-treated diabetic rats was not significantly different from that of the untreated diabetic rats (45.71 ± 23.75 vs 46.75 ± 10.39 U/mg protein).

Liver CAT activity in the untreated diabetic rats was significantly less (3.25-fold) than that of controls (26.13 ± 7.94 vs 84.94 ± 23.55 U/mg protein), while liver CAT activity in the sodium selenite-treated diabetic rats was significantly greater than that of the untreated diabetic rats (58.84 ± 23.91 vs 26.13 ± 7.94 U/mg protein). The CAT activities in the sera, kidneys, and livers of the sodium selenite-treated diabetic rats were all somewhat less than those of the controls, but greater than those of the diabetic untreated rats in sera and livers.

Discussion

This study showed that sodium selenite can increase the reduced serum, renal, and liver GPX and CAT in alloxan-induced diabetic rats. There is much evidence that free radicals play a key role in the most pathogenic pathway of diabetic injuries (8). Free radicals such as superoxide can increase lipid peroxidation, carcinogenesis, inflammation, early aging, cardiovascular diseases, and tissue damage in diabetes (19-24). Antioxidants, such as vitamin E, coenzyme Q10, and rosmarinic acid, and antioxidant enzymes, such as CAT, SOD, and GPX decrease the increased lipid peroxidation that occurs in carcinogenesis, inflammation, early aging, cardiovascular diseases, and diabetes (24). Much evidence indicates that oxidative stress plays a key role in the pathogenesis of diabetes (24).

These antioxidants and antioxidant enzymes protect the cells against oxidative stress-mediated injuries by converting the toxic free radicals to non-toxic products (19, 20). Therefore the use of antioxidants as complementary therapies can be useful for the treatment of diseases related to oxidative stress. Glutathione peroxidase and CAT activities, as markers of antioxidant enzymes status, were significantly less in the untreated diabetic rats than in the controls. Sodium selenite treatment significantly increased serum, renal, and liver GPX and CAT activities compared with the untreated animals. Sodium selenite treatment also resulted in similar serum GPX and CAT activities of the treated animals similar to as those of the control group. The antioxidant enzymes GPX and CAT are considered to be indicators of antioxidant status (25). Previous studies showed that antioxidants such as glibenclamide and metformin (26), vitamins E and C, melatonin (27), zinc (28), vanadium and Trigonella (29, 30), salidroside (31), flavonoids (32), taurine (26), N-acetyl cysteine, L-arginine (33), lycopene (34), succinic acid monoethyl ester (35), rutin (36), aminoguanidine (37), and natural phenolic compounds (38) increased antioxidant enzymes and antioxidant status in diabetics. Research also showed that vitamins E and C together reduced lipid peroxidation and increased the antioxidants growth stimulating hormone (GSH), SOD, and GPX in chlorpyrifos-ethyl-induced lung toxicity in rats (39). Also, one previous study showed that sodium selenite can increase GSH, SOD, GPX, and CAT, and decrease lipid peroxidation in liver and kidneys from Cd-induced oxidative damage (11).

The results of our study agree with others showing

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