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Effect of Sodium Selenite on Lipid Peroxidation and Glutathione in Alloxan Induced Diabetic Rats

Hassan Ahmadvand [1,*]; Ali Khosrowbeygi [1]

[1]Department of Biochemistry, Lorestan University of Medical Sciences, Khorramabad, IR Iran

*Corresponding author: Hassan Ahmadvand, E-mail: hassan_a46@yahoo.com

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Background: Diabetes-related dysfunctions are the major causes of mortality and morbidity for diabetic patients.
Objectives: Selenium is a potent antioxidant. In the present study, we examined antioxidative activities of sodium selenite and possible protective effect of it on serum, liver and kidney lipid peroxidation and glutathione in alloxan-induced type 1 diabetic rats.
Materials and Methods: In this experimental study 40 Sprage Dawley male rats were divided into 4 groups randomly; group I as control, group II as sham treated with sodium selenite (1 mg/kg i.p. daily), group III as diabetic untreated, and group IV as diabetic treated with sodium selenite (1 mg/kg i.p. daily) after induce diabetes, respectively. Diabetes was induced in the 3rd and 4th groups by alloxan injection (s.c.). After 8 weeks, animals were anaesthetized, liver and kidney were then removed immediately and used fresh or kept frozen until analysis. Blood samples were also collected before killing of the rats to measure the lipid peroxidation and glutathione level.
Results: Liver and kidney content of lipid peroxidation decreased in diabetic treated group compared with untreated group. Kidney content of glutathione significantly increased in diabetic treated group compared with untreated group. Serum level of glutathione and liver content of it slightly increased in diabetic treated group compared with untreated group.
Conclusions: This study showed that sodium selenite might be a potent antioxidant and exert beneficial effects on the lipid peroxidation and glutathione in alloxan-induced type 1 diabetic rats.

Keywords: Diabetes Mellitus; Lipid Peroxidation; Rats; Sodium Selenite; Glutathione

1. Background

Diabetes-related dysfunctions are the major causes of mortality and morbidity for diabetic patients (1). Although the precise mechanism by which hyperglycemia induces organ dysfunction is not fully understood, one of the hypothesis to explain this phenomenon is mainly focused on the role of free radicals in these disease states (2). Under physiological conditions, hydrogen peroxide, superoxide and hydroxyl radicals, collectively called reactive oxygen species, are continuously produced and kept under strict control by many enzymes and antioxidants within the cells (3). However, when the finely balanced equilibrium between free radical production and cellular antioxidant defenses is shifted in favor of more free radical production collectively called oxidative stress; this can promote cellular injury (4, 5). Clinical and experimental studies have shown that disturbing of oxidant-antioxidant balance system is involved in the pathogenesis of chronic diseases such as cancer (6), coronary heart disease (7, 8), diabetes and many diabetic complications (9).

A number of natural antioxidant such as vitamin E, coenzyme Q10 and phenolic compounds are known to have hypoglycemic, hypolipidemic and protection of altered antioxidant enzymes and lipid peroxidation in vivo (10). Chemical drugs have many side effects; therefore, looking for new antidiabetic drugs from natural antioxidants sources is still attractive because they are safe and good alternative for treatment of diabetes mellitus. A growing body of research indicates that nutritional deficiencies of antioxidants contribute to the development of diabetes (11, 12). Among antioxidant micronutrients, selenium (Se) is an essential dietary trace element, which plays an important role in a number of biological processes in humans and other species (13). Deficiency of this element induces some pathological conditions, such as cancer, coronary heart disease, and liver necrosis (14-16). Researchers have shown selenium and zinc efficacy on immune system and increase response to influenza and HBV vaccine (17). Also researchers have shown sodium selenite decrease levels of lipid peroxidation (LPO) and NOPs (nitric oxide products) and increase activities of superoxide dismutase, GR (glutathione reductase), and GPX (glutathione peroxidase) in heart diabetes-induced rats (18). Selenium is an essential component of several enzymes such as GPX, TR (thioredoxin reductase) and SeP (selenoprotein P), which contains Seas selenocysteine (19). Various organic and inorganic Se compounds, generally considered to be antioxidants, produced mixed

results when tested in animal models and human subjects (20). Among them, sodium selenite has been shown to be most effective in both in vitro and in vivo (20). The possible protective effects of sodium selenite on serum, kidney and liver lipid peroxidation status and glutathione in alloxan-induced type 1 diabetic rats have not been reported yet.

2. Objectives

Therefore, a study was designed to investigate amelioration of altered serum, kidney and liver lipid peroxidation status and glutathione by sodium selenite in alloxan-induced type 1 diabetic rats.

3. Materials and Methods

3.1. Animals

In this experimental study 40 male mature Sprague-Dawley rats (180-200 g) were obtained from Pasteur Institute of Tehran and were allowed to adapt themselves with the new location for one week. They were kept under standard conditions and were fed a standard rat chow and drinking water ad libitum throughout the study period. This study was approved by the Animal Ethics Committee of the Medical University of Lorestan with accordance to the national health and medical research council guidelines. The rats were divided into four groups (10 per each). The studied groups were as follows: group I as control, group II as sham treated with sodium selenite by 1 mg/kg i.p daily, group III as diabetic without treatment and the group IV as diabetic treatment with sodium selenite.

3.2. Chemicals

Sodium selenite, sodium chloride (NaCl), disodium hydrogen phosphate (Na2HPO4) and trichloroacetic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents used were of analytical grade. 2-thiobarbituric acid (TBA) was obtained from Fluka Chemie (Buchs SG, Switzerland).

3.3. Diabetes Induction

Diabetes was induced after overnight fasting in the second and third groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously (21). Beta cell degradation by alloxan leads to release of more insulin. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 h instead of drinking water. Five days after induction of diabetes, blood samples were gathered from the end part of tails. Blood glucose was measured by glucometer and the rats with blood glucose level of ≥300 mg/dL (16.7 mM/L) were considered as diabetic (22, 23). During the first five days after diabetes induction, 1-3 rats per group died because of alloxan toxicity. The rats were kept at 12:12 h dark-light period in 21±3°C temperature. All animals were allowed free access to food and water ad libitum during the experiment. The third group was treated with sodium selenite by 1 mg/kg i.p. daily (24). The treatment was begun at the first day of diabetes induction. After 8 weeks treatment, animals were anesthetized (Nesdonal 50 mg/kg, i.p.), blood samples were obtained from hearts and allowed to clot for 20 min in laboratory temperature and then centrifuged at 3000 rpm for 10 min for serum separation (21). Liver and kidneys of the rats were also removed immediately and used fresh or kept frozen until the analysis.

3.4. Levels of Malonedialdehyde (MDA)

Serum, liver, and kidney contents of MDA, as a product of lipid peroxidation, were measured by the thiobarbituric acid (TBA) assay. Liver and kidney contents of MDA were also analyzed, using a Shimadzu spectrophotometer (Tokyo, Japan) (25).

3.5. Levels of Glutathione (GSH)

Serum liver and kidney contents of GSH were assayed spectrophotometrically at 412 nm, according to the method of Ellman, using a Shimadzu spectrophotometer (Tokyo, Japan). The contents of GSH were expressed as mM/mg-pr (26). All values are expressed as mean±SD. The data were compared between groups by Mann-whitney U test. Statistical analyses were performed using the SPSS-13 for windows software. A p-value <0.05 was considered statistically significant.

4. Results

Effect of sodium selenite on serum, kidney and liver MDA level of diabetic rats: The levels of MDA in serum, kidney and liver are shown in Table 1. The level of serum MDA in the untreated diabetic rats was significantly (1.6-fold) higher than that of control animals. The level of MDA in the serum of diabetic rats treated with sodium selenite was very low, similar to the level found in the control animals. The treatment of diabetic animal with sodium selenite could significantly (31.24%) inhibit the elevation of MDA in comparison with the untreated diabetic animals.

The level of kidney MDA in the untreated diabetic rats was significantly (1.65-fold) higher than that of control animals. The treatment of diabetic animal with sodium selenite could significantly (27.25%) inhibit the increase of MDA in comparison with the untreated diabetic animals. The level of liver MDA in the untreated diabetic rats was significantly (1.61-fold) higher than that of control animals. The treatment of diabetic animal with sodium selenite could (31.25%) inhibit the increasing of MDA in comparison with the untreated diabetic animals, but it was not statistically significant. The level of MDA in the serum, renal and liver of sham rats treated with sodium selenite were low, similar to the level found in the control animals.

Table 1. The Effect of Sodium Selenite on Serum, Liver and Kidney MDA Content in Alloxan Induced Diabetic rats [a,b]

MDA, nM/mg protein	Serum	Kidney	Liver
Control	89.36 ± 23.48	91.93 ± 18.88 [c]	95.19 ± 25.01 [c]
Sham	106.69 ± 33.39	97.77 ± 15.29 [c]	113.65 ± 35.57 [c]
Diabetic	144.20 ± 32.68 [c]	151.54 ± 30.12	153.61 ± 34.81
Diabetic treated	99.15 ± 17.66 [d]	110.24 ± 30.84 [c]	105.61 ± 18.82 [c]

[a] Abbreviation: MDA, malonedialdehyde.
[b] Values represented as mean ± SD.
[c] $P < 0.05$ as compared with control group.
[d] $P < 0.05$ as compared with diabetic without treatment group.

Table 2. The Effect of Sodium Selenite on Serum, Liver and Kidney GSH Content in Alloxan Induced Diabetic rats[a,b]

GSH, nM/mg Protein	Serum	Kidney	Liver
Control	12.63 ± 1.64 [c]	10.58 ± 3.62 [c]	12.63 ± 1.64 [c]
Sham	10.00 ± 1.42 [c,d]	8.25 ± 1.01 [c,d]	10.00 ± 1.42 [d]
Diabetic	6.87 ± 1.86	5.21 ± 0.74	9.03 ± 1.71
Diabetic treated	7.97 ± 1.62 [d]	6.74 ± 1.48 [c,d]	7.97 ± 1.62 [d]

[a] Abbreviation: GSH, Glutathione.
[b] Values represented as mean ± SD.
[c] $P < 0.05$ as compared with control group.
[d] $P < 0.05$ as compared with diabetic without treatment group.

Effect of sodium selenite on serum, kidney and liver GSH level of diabetic rat: The levels of GSH in serum, kidney and liver are shown in Table 2. The level of serum GSH in the untreated diabetic rats was significantly (1.84-fold) lower than that of control animals. The treatment of diabetic animal with sodium selenite could slightly increase of GSH in comparison with the untreated diabetic animals. The level of renal GSH in the untreated diabetic rats was significantly (2.03-fold) lower than that of control animals. The sodium selenite treated diabetic animals showed significantly elevation (29.36%) in GSH level compared with the untreated animals. The level of liver GSH in the untreated diabetic rats was significantly (1.39-fold) lower than that of control animals.

The treatment of diabetic animal with sodium selenite could not increase (5.43%) in GSH level compared with the untreated samples. The level of GSH in the serum, renal and liver of sham rats treated with sodium selenite was high, similar to the level found in the control animals.

5. Discussion

This study showed that sodium selenite increased serum, renal and liver glutathione and decreased lipid peroxidation in alloxan induced diabetic rats. There is much evidence that oxidative stress play a key role in the most pathogenic pathway of diabetic injuries. Free radicals such as superoxide can induce cell and tissue injuries lipid peroxidation and increase carcinogenesis, inflammation, early aging, cardiovascular diseases and tissue damage in diabetes (27, 28). Also, studies showed that using organic selenium have protective effects on lipid peroxidation in chicken blood during fattening and after fasting. Other study showed that using selenium have protective effects on lipid peroxidation in alloxan induced toxicity rats (29, 30). Antioxidants such as selenium, vitamin E, coenzyme Q10 protect the cells against oxidative stress mediated cellular injuries by converting the toxic free radicals to non-toxic products (17, 29, 31). Therefore use of antioxidant as complementary therapy is useful for diseases that related to oxidative stress. Diabetic animals showed significantly increasing in serum, liver and kidney lipid peroxidation compared with the control group. Treatment of diabetic animals with sodium selenite significantly inhibited increasing of serum and kidney lipid peroxidation in comparison with the untreated diabetic animals. Previous studies showed that using natural antioxidants supplements such as vitamin E (32), α-lipoic acid (33), flavonoids (34), oleanolic acid (35), selenium (36), vanadium, α-carotene, zinc, vitamin C (37, 38), aminoguanidine (39), lycopene (40) and natural phenolic compounds have protective effects on lipid peroxidation in diabetics and chronic disease (41). Several reports showed that selenium has neuroprotective effect and in cerebral ischemia and improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease (42, 43). The protective role of selenium administration against oxidized low density lipoprotein and antioxidant defense in diabetic rats was reported (44). Also, selenium decrease lipid peroxidation and ameliorate oxidative stress in liver and kidneys from Cd-induced oxidative damage (45). Therefore, selenium might be useful as a natural antioxidant for reducing or preventing the complications that related to oxidative stress in diabetes patients. Results of the present study are in accordance with

others showing that sodium selenite can increase GSH level and decrease lipid peroxidation. Therefore, sodium selenite as a natural antioxidant with beneficial effects on GSH level and lipid peroxidation might be helpful in reducing the complications of different tissue damages seen in diabetic patients. Antioxidant therapy is one of the most important treatment strategies in diabetic patients for prevention and slowing of diabetic complications progression such as hyperglycemia, hyperlipemia, hepatic damage, and nephropathy (11). Although the detailed mechanisms of sodium selenite antioxidant function cannot be fully explained by our results, several studies have explained some mechanisms of sodium selenite antioxidant function. Sodium selenite may directly eliminate free radicals in vitro. Therefore, sodium selenite as a good antioxidant with multi-beneficial properties could be proposed as a supplement in diabetic patients without diabetic nephropathy for prevention of its.

This study showed that sodium selenite possess a good antioxidant activity and has beneficial effects, in increasing the reduced serum, renal and liver antioxidant enzymes and GSH levels in alloxan-induced-diabetic rats. Hence, attenuation of lipid peroxidation level and elevation of antioxidant enzymes activities can decrease diabetic complication such as nephropathy in diabetic patients.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis, and manuscript writing.

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