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Influence of alloxan-induced diabetes and selenite treatment on blood glucose and glutathione levels in mice

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Abstract

Many clinical studies reported that diabetic patients had lower glutathione contents in erythrocytes or plasma. Recently, selenium, an essential trace element with well-known antioxidant characteristics, has been found to have insulin-mimetic properties. But seldom information is available about the influence of selenium on glutathione changes induced by diabetes mellitus in animals. Therefore, this study was designed to compare the impacts of selenite treatment on glutathione (GSH) levels of blood and tissues such as brain, kidney, liver, spleen and testis in mice. Four groups were used in this study: a control group, a diabetic group, a selenite-treated normal group and a selenite-treated diabetic group. Selenite was administered to the mice for 4 weeks with an oral dose of 2 mg kg^{-1} day^{-1} by gavage. The blood glucose level, and GSH level in blood and tissues were determined. The results show that the selenite-treated diabetic group had significantly lower blood glucose levels than the diabetic group. Moreover, alloxan-induced diabetes significantly decreased GSH levels in blood, kidney, liver and testis compared to the controls. Selenite treatment of the diabetic mice only improved the GSH levels in liver and brain. On the other hand, selenite administered to the normal mice reduced GSH levels in the liver compared to the controls. In conclusion, this study suggests that selenite treatment of diabetic mice with an effective dose would be beneficial for the antioxidant system of liver and brain although it exerts a toxic effect on the liver of normal mice.
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Keywords: Selenite; Alloxan; Diabetes mellitus; Glutathione; Glucose

Introduction

Reduced glutathione (GSH) is the prevalent non-protein thiol in mammalian cells. GSH has many biological functions, such as maintaining membrane protein sulfydryl groups in the reduced form, acting as a substrate for GSH peroxidase and detoxification of xenobiotics. Therefore, the maintenance of the GSH level is crucial for cellular defense against oxidative injury and cellular integrity [1,2].

It has been reported that diabetic patients had lower GSH concentrations in erythrocytes and plasma [3–5]. In diabetic animals, a reduction of the GSH level was also observed both in erythrocytes [6,7] and in aortic tissue [8].

Selenium has been proved to have insulin-mimetic properties in vitro [9] and in vivo [10] and exert antioxidant characteristics in diabetic animal models [11]. However, seldom information is available about the influence of selenium treatment on GSH levels of tissues in diabetic animals. Therefore, this study was

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designed to systematically compare the influence of selenite treatment on GSH levels in blood and tissues such as brain, kidney, liver, spleen and testis in Kun-Ming mice with diabetes induced by alloxan. We found that mice with alloxan-induced diabetes had lower GSH levels in blood, kidney, liver and testis compared to the controls, and that selenite treatment of the diabetic mice with an oral dose of $2\,mg\,kg^{-1}\,day^{-1}$ only improved GSH levels in liver and brain.

Materials and methods

Materials

Alloxan, *o*-phthalaldehyde (OPA), diethylenetriaminepentaacetic acid (DTPA) and *N*-ethylmaleimide (NEM) were purchased from Sigma Co. GSH was obtained from Amresco Co. All other chemicals were of the highest commercial grade available in the domestic market. The water used was freshly prepared redistilled water.

Animals, induction of diabetes and sodium selenite treatment

Fifty male Kun-Ming mice obtained from Hubei Research Animal Center, weighing 20 ± 2 g, were kept in an air-conditioned animal house with a normal day/night cycle. The mice were fed with mouse chow (also purchased from Hubei Research Animal Center) and tap water ad libitum. Twenty mice were used as controls; they were again randomly divided into two subgroups: the control group and the selenite-treated normal group. Thirty mice, after 48 h of fasting, were intraperitoneally injected with $200\,mg\,kg^{-1}$ alloxan dissolved in $50\,mmol\,l^{-1}$ citrate buffer (pH 3.0). Seventy-two hours after alloxan injection, whole blood samples were obtained from the tail vein of the overnight fasted mice and their glucose levels were tested by glucose test strips (Roche Diagnostic Corp., Indiannapolis, IN, USA). Twenty mice with glucose levels over $15\,mmol\,l^{-1}$ were included in the diabetic group and then randomly assigned to two subgroups: the selenite-treated diabetic group and the diabetic group without any treatment.

The mice in the selenite-treated diabetic and normal group were treated with a dose of $2\,mg\,kg^{-1}$ selenite dissolved in redistilled water by gavage. The body weight and blood glucose level were measured once a week when the mice were fasted overnight. After 4 weeks of selenite treatment, the mice were anesthetized with ether, and blood samples were drawn from postcava into heparinized tubes. An aliquot of whole blood was removed and hemolyzed with a ninefold volume of redistilled water for the GSH assay. The remaining blood was centrifuged 15 min at 1500*g* for isolation of plasma. After the blood was collected, tissues of brain, liver, kidney, spleen and testis were removed, weighed and rinsed three times with cold physiological saline, and stored at $-75\,^{\circ}C$ for the GSH assay.

GSH assay

The content of reduced GSH in whole blood samples was measured with an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB).

Levels of reduced GSH in brain, liver, spleen, kidney and testis where determined using a fluorescence method [12]. A 10% homogenate of the tissue samples was prepared in an ice-cold homogenization solution ($20\,mmol\,l^{-1}$ HCl, $5\,mmol\,l^{-1}$ DTPA, $10\,mmol\,l^{-1}$ ascorbic acid, and 5% trichloroacetic acid (TCA)) using a glass piston homogenizer for 1 min. The suspension was centrifuged at 14,000*g* and the resulting supernatant solution was centrifuged through a 0.45-μm microcentrifuge filter (Millipore Corp., USA), yielding a 5% deproteinized homogenate that was used for the OPA assay procedure. The following solutions were required to perform the OPA assay: redox quenching buffer (RQB) ($20\,mmol\,l^{-1}$ HCl, $5\,mmol\,l^{-1}$ DTPA, $10\,mmol\,l^{-1}$ ascorbic acid); 5% TCA in RQB (TCA-RQB); $7.5\,mmol\,l^{-1}$ NEM in RQB; $1.0\,mol\,l^{-1}$ potassium phosphate (KPi) buffer (pH 7.0); $0.1\,mol\,l^{-1}$ KPi buffer (pH 6.9); $5.0\,mg\,ml^{-1}$ OPA in methanol. The OPA solution was prepared just before use. $0.1\,mmol\,l^{-1}$ GSH was prepared as a standard. In brief, a mixture of sample (or standard), TCA-RQB, NEM and $1\,mol\,l^{-1}$ KPi was incubated for 5 min at room temperature (20–21 °C), $0.1\,mol\,l^{-1}$ KPi and OPA were added and incubated for 30 min at room temperature before measuring the OPA-derived fluorescence at 365 nm excitation and 430 nm emission [12].

Blood glucose assay

Blood glucose was determined by the glucose oxidase method (blood glucose assay kit, Shanghai Rongsheng Biotech Co., Shanghai, China).

Plasma insulin determination

The insulin level in plasma was estimated using an insulin radioimmunoassy kit (Huaxi Institute of diabetic technology, Chengdu, Sichuan Province, China).

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