Journal of Trace Elements in Medicine and Biology 27 (2013) 52–57

Contents lists available at SciVerse ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Nutrition

Alterations in kidney tissue following zinc supplementation to STZ-induced diabetic rats

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ARTICLE INFO

Article history:
Received 23 February 2012
Accepted 2 July 2012

Keywords:
Diabetes
Zinc
Oxidative stress
Renal damage
Rat

ABSTRACT

Diabetes mellitus is a chronic disease characterized by anomalies forming in carbohydrate, lipid, protein metabolisms and the incidence of this disease varies widely throughout the world. Zinc is an important element which is essential for life and is present in nature. In this study, the animals were divided into four groups. These groups were named as untreated; zinc sulfate; streptozotocin (STZ); STZ and zinc sulfate. STZ (65 mg/kg) was dissolved in a freshly prepared 0.01 M pH 4.5 citrate buffer and given with intraperitoneal injection in a single dose. Zinc sulfate (100 mg/kg) was dissolved in distilled water and given to the animals by gavage at a daily dose for 60 days. The rats were sacrificed under ether anesthesia. This study was aimed to investigate histological and biochemical changes of zinc supplementation on the kidney tissue in STZ-induced diabetic rats. In the current study, histological and histochemical observations showed that the occurred degenerative changes decreased after giving zinc in the kidney tissue of diabetic group. Kidney glutathione (GSH) levels decreased and lipid peroxidation (LPO), nonenzymatic glycosylation (NEG), urea and creatinine levels increased in diabetic rats. GSH levels increased, while LPO, NEG, urea and creatinine levels decreased in the kidney with administration of zinc to diabetic rats. As a result, we observed curative effects of zinc given to diabetic rats. We can say that zinc may be an important antioxidant for the treatment of secondary complications of diabetes in kidney tissue.

Introduction

Diabetes mellitus which accompanies many secondary complications is a heterogeneous metabolic disease and its toxic effects on various organs have been attributed to increase of oxidative stress [1]. Zinc as a trace element is an important cofactor for many proteins related with cell proliferation, differentiation and apoptosis [2,3], and gene expression [4]. There is a relationship between zinc and diabetes because zinc is a component of insulin crystals [2]. It is known that zinc has an important role against the immunomediated free radical attack in the beta cells of endocrine pancreas, but the action mechanism of zinc has yet to be clarified [1,5].

Lectins are glycoproteins or proteins which have binding activity to specific sugar residues of glycoconjugates. They have been used as probes for identification of the carbohydrate moieties on the cell surface or in cytoplasmic organelle [6]. Wheat germ agglutinin (WGA) is a kind of lectin and is generally used for histochemical and cytochemical detection of β1,4 linked N-acetylglucosamine (β1,4 GlcNAc) [7]. The demonstration of complex carbohydrates is important in order to detect functional conditions of kidney tissue [8,9]. Additionally, Wong et al. [10] said that periodic acid-Schiff method is used for identification of glycoconjugates, but using lectins are more specific for identification of different carbohydrate moieties.

Zinc acts by two different mechanisms as an antioxidant. It protects sulfhydryl groups against oxidation in the first mechanism. In the second mechanism, however, it inhibits production of reactive oxygen species [11]. Given exogenous zinc is significant for decrease of oxidative stress in chronic diseases [12]. Oxidative stress may play an important role in chronic diseases caused by zinc deficiency [13]. The aim of this study was to investigate effects of zinc on occurred histopathological changes in kidney tissue of diabetic rats by morphological and biochemical means.

Materials and methods

Animals

In the experiments, 6–6.5-month-old and 150–200 g weight female Swiss albino rats were used. The experiments were reviewed and provided by Institute's Animal Care and Use Committee of Istanbul University. All rats were fed with standard chow and tap water *ad libitum*. In this study the animals were divided into four groups: Group I: control (untreated) animals; Group II:

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http://dx.doi.org/10.1016/j.jtemb.2012.07.006

control animals given zinc sulfate; Group III: diabetic animals; and Group IV: diabetic animals given zinc sulfate. Zinc sulfate was given to the animals by gavage at a daily dose of 100 mg/kg body weight for 60 days. Diabetes was induced by intraperitoneal injection of STZ in a single dose of 65 mg/kg. STZ was dissolved in a freshly prepared 0.01 M pH 4.5 citrate buffer. At the end of the experiment, all rats were fasted overnight. The animals were sacrificed under ether anesthesia.

Histological assays

Kidney tissues were fixed in Bouin's solution for histochemical analyses. After dehydration in ethanol series, kidney tissues were cleared in xylene and embedded in paraffin. Paraffin-embedded tissues were cut as 5 μm sections and stuck on microscope slide. Sections were stained with Masson's trichrome and Periodic-Acid-Schiff (PAS) for histological determinations.

WGA lectin histochemistry

Kidney tissues were fixed in Bouin's solution for lectin histochemistry. Kidney tissues were dehydrated in ethanol series, cleared in xylene and then embedded in paraffin. Paraffin-embedded tissues were cut as 4 μm sections and stuck on microscope slide coated with poly-L-lysine. In this study we used horseradish peroxidase (HRP) conjugated WGA lectin, supplied by EY Laboratories Inc. (USA). After deparaffinization and rehydration process, the slides were incubated for 10 minutes in 3% hydrogen peroxide in phosphate buffered saline (PBS: 10 mM, pH 7.4) in order to reduce endogenous hydrogen peroxidase activity. The sections were then washed twice in PBS and then incubated with 1% BSA for 10 minutes to block non-specific lectin staining. Subsequently, sections were incubated with WGA lectin (20 μg/ml) for 1 hour at room temperature in a humidified chamber. Then sections were rinsed twice in PBS and freshly prepared AEC-H_2O_2 substrate solution was applied for 10 minutes, to visualize the lectin binding reaction in different segments of the kidney. Then they are counterstained with Mayer's hematoxylin. Finally, sections were washed in distilled water and mounted with GVA. To determine lectin reaction specification, sections were incubated with PBS for 1 hour at room temperature without HRP-conjugated lectin.

Biochemical assays

In this study, biochemical investigations were made in serum and kidney tissue from all groups. The blood samples of rats were collected from the tail vein on days 0 and 60. At the end of the experimental period, the animals were fasted overnight and then sacrificed. For biochemical analyses, the tissue samples of kidney were washed with saline and kept frozen until analyzed and used for measurement. These tissue samples were homogenized in cold 0.9% NaCl with a glass homogenizer to make up 10% homogenate (w/v). The homogenates were centrifuged. The supernatant fraction was removed for the determination of GSH, LPO, NEG and protein levels.

In all samples, the 18 hour fasting blood glucose levels were determined by o-toluidine method [14]. Serum urea levels were determined by the method of acetylmonoxime [15]. In the oxime method, diacetyl monoxime is hydrolyzed to the unstable compound diacetyl in acidic medium. Diacetyl reacts with urea to produce a yellow diazine derivative. The color of this product is intensified by addition of thiosemicarbazide. The red color thus formed is measured at 520 nm in a spectrophotometer. Serum creatinine levels were determined by the Jaffe Reaction [16]. The serum is first deproteinized by the addition of sodium tungstate. After alkalinization, picric acid reacts with creatinine with formation of an orange-red dyestuff which is measured spectrophotometrically at 520 nm.

Reduced glutathione (GSH) was determined according to Beutler's method by using Ellman's reagent [17]. The procedure is based on the reduction of Ellman's reagent by SH groups to form 5,5′-dithiobis (2-nitrobenzoic acid) with an intense yellow color, measured spectrophotometrically at 412 nm using a Shimadzu Spectrophotometer. Results were expressed as nmol GSH/mg protein.

Lipid peroxidation in kidney homogenates was assayed by the method of Ledwozyw et al. [18]. In brief, adducts formed following boiling tissue homogenate with thiobarbituric acid is extracted with n-butanol. The difference in optical density at 532 nm is measured in terms of the kidney malondialdehyde (MDA) content, also of TBARS, which is undertaken as an index of lipid peroxidation. Results were expressed as nmol MDA/mg protein. Kidney NEG levels were determined by thiobarbituric acid method [19]. The glucose moiety of glycosylated hemoglobin is converted to 5-hydroxymethylfurfural by heating with oxalic acid. The adduct formed by reacting 2-thiobarbituric acid with hydroxymethylfurfural is measured spectrophotometrically and results are expressed as nmol fructose/mg protein. The protein content in the supernatants was estimated by the method of Lowry using Bovine serum albumin as standard [20].

Statistical analysis

Microscopic analysis for histological assays was made by using 40× objective and 10× ocular system of Olympus CX-45 microscope. Results were scored from zero, through one to three as negative (0), weakly positive (1), moderately positive (2), strongly positive (3) for histological evaluation. Histological score was analyzed by two-way ANOVA and Mann–Whitney *U* tests by using GraphPad Prism version 4.0 computer package. Results were reported as mean ± SE. *P* values less than 0.05 were considered to be significant. Lectin immunoreactivity was analyzed as semiquantative according to labeling density. Biochemical results were evaluated by using an unpaired *t*-test and ANOVA variance analysis using the NCSS statistical computer package. The values were expressed as mean ± SD. Analysis between control and experimental groups was performed using the Mann–Whitney test. $P < 0.05$ was considered as significant.

Histological results

Very important degenerative changes in the kidney tissue of the diabetic animals were observed by light microscope. The increase in hyperemia and mononuclear cell infiltration, the decrease in PAS (+) reaction intensity both of in basal membrane and brush border of proximal tubule and in basal membrane of distal tubule, decreased height and defected continuity in brush border, increased wideness of lumen in proximal tubules, an increase in nucleus and cytoplasmic debris thrown to lumen of proximal tubules, the decrease in glomerular mass, the widening between parietal and visceral leaves of glomerules were observed in diabetic group according to control group. Zinc sulfate reversed the changes in the diabetic animals. The decrease in hyperemia and mononuclear cell infiltration, increasing in PAS (+) reaction intensity in brush border of proximal tubule, increased height and normal continuity in brush border, a decrease in nucleus and cytoplasmic debris thrown to lumen of proximal tubules and the increase in glomerular mass were reported in experimental group given zinc sulfate (Fig. 1A). Histological score in diabetic mice are significantly increased according to control groups ($P < 0.05$). In experimental group given zinc sulfate were not significantly decreased according to diabetic group ($P > 0.05$) (Fig. 1B).