Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 530–536
Journal of Nutritional Biochemistry

Effect of selenium on pancreatic proinflammatory cytokines in streptozotocin-induced diabetic mice

Jinhong Zeng, Jun Zhou, Kaixun Huang*

Department of Chemistry, Huazhong University of Science and Technology, Wuhan, Hubei 430074, People's Republic of China

Received 10 February 2008; received in revised form 16 May 2008; accepted 27 May 2008

Abstract

To investigate the effects of selenium on mRNA expressions of proinflammatory cytokines and inducible nitric oxide synthase (iNOS) in the pancreas of streptozotocin-induced diabetic mice, the animals were divided into three groups in this study: a normal control group, an untreated diabetes mellitus group and a selenite-treated diabetes mellitus group. Selenite was administered to the diabetic mice in selenite-treated diabetes mellitus group for 2 weeks with an oral dose of 2 mg/kg body weight per day by gavage. The results showed that pancreatic selenium content and glutathione peroxidase mRNA expression and activity were decreased by 16.0%, 63.9% ($P<.01$) and 31.2 % ($P<.01$), respectively, in untreated diabetes mellitus group compared with normal control group, and they were significantly increased by 51.0% ($P<.001$), 79.7% ($P<.05$) and 21.0% ($P<.05$), respectively, in selenite-treated diabetes mellitus group compared with untreated diabetes mellitus group. Meanwhile, pancreatic mRNA expressions of proinflammatory cytokines interleukin-1β, tumor necrosis factor-α and interferon-gamma; mRNA expression and activity of iNOS and content of nitric oxide were significantly increased by 133.0% ($P<.01$), 164.0% ($P<.001$), 111.0% ($P<.01$), 101.0% ($P<.001$), 73.2% ($P<.001$) and 37.6% ($P<.01$), respectively, in untreated diabetes mellitus group compared with normal control group, and they were decreased by 43.2% ($P<.01$), 37.5% ($P<.01$), 33.9 % ($P<.05$), 35.5% ($P<.01$), 34.9% ($P<.01$) and 18.1% ($P<.05$), respectively, in selenite-treated diabetes mellitus group compared with untreated diabetes mellitus group. In conclusion, the chosen pharmacological dose of selenium provides partial correction of these effects towards control values. Moreover, the results suggested that the hypoglycemic role of selenium may relate with its inhibiting effect on augmentation of proinflammatory cytokines and reactive oxygen species/reactive nitrogen species by streptozotocin inducing in the pancreas of diabetic mice.

Keywords: Selenite; Proinflammatory cytokines; iNOS; Diabetic mellitus; Streptozotocin

1. Introduction

Type 1 diabetes mellitus is a chronic lifelong disease that occurs when the pancreas does not produce enough insulin to properly control blood sugar levels. Proinflammataory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ) that are released by the infiltrating immune cells around pancreatic islets are involved in the pathogenesis of Type 1 diabetes mellitus. These cytokines exert cytotoxic and inhibitory effects on pancreatic β-cells and have been shown to represent major effector molecules involved in β-cell destruction and the induction of β-cell-specific autoimmunity [1–4]. The deleterious effects of proinflammatory cytokines on rodent islets are mediated in large part by reactive oxygen species (ROS) and nitric oxide (NO) [2,4–6]. It has been documented that proinflammataory cytokines IL-1β, TNF-α and IFN-γ and ROS are overproduced in the pancreas of streptozotocin-diabetic animals [7–9]. Anti-inflammatory agents or free radicals scavengers have been shown to prevent pancreas against damage and ameliorate the development of diabetes mellitus [7,8,10].

Selenium, as an essential nutritional trace element for humans and many other forms of life, plays an important role in many physiological processes and exerts its biological effect through selenoproteins [11,12]. Recently, selenium has been considered as a potential agent for the prevention of diabetes mellitus. It has been reported that selenium supplementation has some beneficial effects against the development of diabetes by exhibiting antioxidant properties in experimental models of diabetes mellitus [13]. In addition,

* Corresponding author. Tel.: +86 27 8754 3532; fax: +86 27 8754 3632.
E-mail address: hxxzrf@mail.hust.edu.cn (K. Huang).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2008.05.012

it exerts insulin-mimetic actions in streptozotocin-induced diabetic rodents [14–16].

Selenium is needed for the proper functioning of immune system, especially the functions of macrophages, T lymphocytes and some other immunological cells [17–19]. It may be considered to play an anti-inflammatory role in patients with systemic inflammatory response syndrome [20,21]. However, little information is available about the influences of selenium on pancreatic proinflammatory cytokines and inducible nitric oxide synthase (iNOS) in diabetic animals. Thus, the effects of selenium treatment on selenium content, mRNA expressions and activities of glutathione peroxidase (GPx) as well as iNOS; mRNA expressions of proinflammatory cytokines IL-1β, TNF-α and IFN-γ and NO content in the pancreas of streptozotocin-induced experimental diabetic mice are investigated.

2. Materials and methods

2.1. Materials

Streptozotocin (STZ) was purchased from Sigma. TRIzol reagent was obtained from Invitrogen. Molony murine leukemia virus (M-MLV) reverse transcriptase and oligo (dT) were purchased from Promega. SYBR Green PCR Master Mix was obtained from Toyobo (Japan) and dNTP was from Roche. All other chemicals were of the highest commercial grade available.

2.2. Animals and diabetic animals

Male Kun-Ming mice (body weight: 18–20 g), bought from Hubei Research Centre for Laboratory Animal, were kept in an air-conditioned animal house with a normal day/night cycle and fed with mouse chow containing 0.112 μg selenium/g diet (purchased from Hubei Research Centre for Laboratory Animal) and tap water ad libitum. These animals were randomly assigned to two major groups: the diabetic and the normal control. Mice fasted for 24 h were intraperitoneally injected with a single dose of STZ at 150 mg/kg body weight (dissolved in 0.1 M citrate buffer, pH 4.5) [22], and normal control group was injected with citrate vehicle alone. At 96 h after STZ injection, mice with blood glucose levels of over 15 mmol/L in the whole blood samples obtained from the tail vein of the overnight fasted animals and measured by a glucose test strip (Roche Diagnostic, Indianapolis, IN, USA) were considered to be diabetic. Thus, diabetic animals were randomly divided into two groups having seven animals per group: untreated diabetic group and selenite-treated diabetic group (orally administered a dose of 2 mg /kg body weight per day sodium selenite dissolved in redistilled water by gavage) [23]. This dose of sodium selenite administered to the diabetic mice would lead to a daily selenium uptake of 23.3 μg per mouse at a mean body weight of 25.5 g. The consumption of about 3.0 g feed per day would result in a mean daily selenium uptake of about 0.34 μg per mouse. Thus, this dose of selenium would be considered pharmacological. The recorded time began on the day when selenite was administrated to diabetic mice.

After 2 weeks of selenite treatment, the mice were deprived of food overnight and killed by means of exsanguination after anesthesia with diethyl ether. The pancreas tissue was rapidly removed and then kept it at −80°C until use.

2.3. Real-time polymerase chain reaction analysis

Total RNAs were extracted from pancreatic tissue in 1 ml of TRIzol reagent according to the manufacturer's instructions. Complementary DNA (cDNA) was prepared by incubation of the RNA with M-MLV reverse transcriptase, dNTP, and oligo (dT) at 37°C for 60 min in Tris (hydroxymethyl) aminomethane hydrochloride buffer (50 mM, pH 8.3). Then the enzyme was inactivated by incubation at 95°C for 5 min.

Real-time polymerase chain reaction (PCR) amplification was carried out with a reaction mixture composed of 5 μl of the template cDNA, 25 μl of 2×SYBR Green PCR Master Mix and 400-nM forward and reverse primers, respectively. Reactions were run on MJR Opticon (MJ Research, Watertown, MA, USA). Primer sets for GPx were 5′-gcggccctggcattg-3′ (forward) and 5′-ggaccagcgcccatctg-3′ (reverse) [24]; for IL-1β, 5′-caaccaacaagtgatattctccatg-3′ (forward) and 5′-gatccacactctccagctgca-3′ (reverse); for TNF-α, 5′-catcttctcaaaattcgagtgacaa-3′ (forward) and 5′-tgggagtagacaaggtacaaccc-3′ (reverse); for IFN-γ, 5′-tcaagtggcatagatgtggaagaa-3′(forward) and 5′-tggctctgcaggattttcatg-3′ (reverse); for iNOS, 5′-cagctgggctgtacaaacctt-3′ (forward) and 5′-cattggaagtgaagcgtttcg-3′ (reverse) and for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as an internal control were 5′-ttcaccaccatggagaaggc-3′ (forward) and 5′-ggcatggactgtggtcatga-3′ (reverse) [25]. Real-time PCR conditions for them were 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Expression levels of target genes were related to the expression level of the housekeeping gene GAPDH, and relative expression levels were calculated with the $2^{-\Delta\Delta Ct}$ rule [26].

2.4. Measurement of content of selenium and activity of GPx

The contents of selenium in pancreas tissue and mouse chow were determined by the fluorimetric method according to Watkinson [27]. GPx activity was measured according to the method of Hafeman et al. [28]. One unit of GPx was defined as a decrease of 1 μmol/L in the concentration of glutathione per minute per milligram of protein after the nonenzymatic reaction was subtracted and was expressed in unit per milligram of protein. The protein content was determined using the method of Bradford with bovine serum albumin as standard.

2.5. Lipid peroxidation detection

Lipid peroxidation products, thiobarbituric acid-reactive substances, were measured by a standard method and were