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

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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,

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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

expressed as the content of malondialdehyde (MDA) in nanomoles per milligram of protein [29].

2.6. Estimation of pancreatic iNOS activity

The iNOS activity assay is based on the catalysis of L-arginine and dioxygen reaction to generate NO by iNOS, which is essentially Ca^{2+}-independent [30]. One unit of iNOS was defined as a production in the NO of 1 nmol/min and was expressed in units per milligram of protein.

2.7. Determination of pancreatic nitrite and nitrate

NO level was determined indirectly by quantification of their oxidized products of degradation (NO_2^- and NO_3^-), using nitrate reductase [31] and Griess reagent according to Moshage et al. [32].

2.8. Statistical analysis

All results are expressed as mean±S.D. Significance was assessed by using the one-way analysis of variance (ANOVA), and a P value less than .05 was considered to be statistically significant.

3. Results

3.1. Changes in blood glucose levels of diabetic animals

The mean blood glucose levels of normal control, untreated diabetes mellitus and selenite-treated diabetes mellitus groups were given in Fig. 1. At the end of the experimental period (14 days after selenite administration to diabetic mice), blood glucose level in untreated diabetes mellitus group was much greater than that of normal control group. However, blood glucose level in selenite-treated diabetes mellitus group on day 15 was significantly decreased by 25% (P<.05) compared with the initial level found in the same diabetes mellitus group before selenite treatment on day 1, but still higher than that in normal control group.

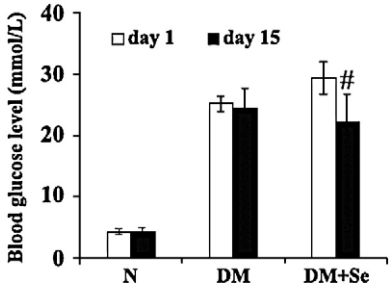

Fig. 1. Blood glucose levels in normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated with diabetes mellitus group (DM+Se). Values are means±S.D. (n=7). $^{\#}P$<.05 represents the comparison between blood glucose level in selenite-treated diabetes mellitus group on Day 15 and the initial level found in the same diabetes mellitus group before selenite treatment on Day 1.

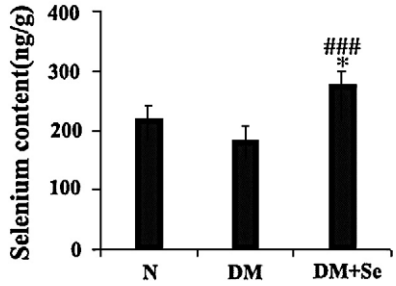

Fig. 2. Pancreatic selenium contents in normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Values are expressed as means±S.D. (n=7). *P<.05 compared with normal control group; $^{\#\#\#}P$<.001 compared with untreated diabetes mellitus group.

3.2. Effect of selenium supplementation on pancreatic selenium contents, GPx mRNA expression and activity

In the present study, although the mouse feed contained 0.112 μg Se per gram, selenium content of tissue pancreas could increase significantly due to selenium supplementation. The contents of selenium in pancreas from normal control, untreated diabetes mellitus and selenite-treated diabetes mellitus groups were shown in Fig. 2. Pancreatic selenium content in untreated diabetes mellitus group was 84.0% of that observed in normal control group, but there was no significant difference between those two groups. However, the pancreatic selenium content in selenite-treated diabetes mellitus group was 51.0% (P<.001) and 26.7% (P<.05) greater than that in untreated diabetes mellitus group and normal control group, respectively.

The mRNA expression and activity of GPx are regulated by the selenium status of tissues. As shown in Fig. 3, mRNA expression and activity of GPx in untreated diabetes mellitus group were 63.9% (P<.01) and 31.2 % (P<.01) less than those in normal control group (Fig. 3), respectively. However, compared with untreated diabetes mellitus group, mRNA expression and activity of GPx in selenite-treated diabetes mellitus group were significantly increased by 79.7% (P<.05) and 21.0% (P<.05), respectively, but did not

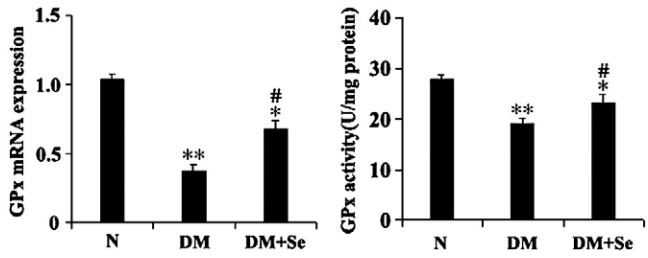

Fig. 3. GPx mRNA expression and activity in the pancreas of normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Results of real-time PCR are shown as GPx expression relative to housekeeping gene GAPDH. Values are expressed as means±S.D. (n=7). *P<.05, **P<.01, compared with normal control group; $^{\#}P$<.05, compared with untreated diabetes mellitus group.

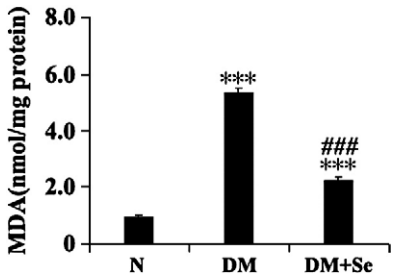

Fig. 4. Lipid peroxidation level measured as MDA content in the pancreas of normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Values are expressed as means±SD (n=7). ***P<.001, compared with normal control group; ###P<.001, compared with untreated diabetes mellitus group.

return to normal level yet, and they were 35.1% (P<.05) and 16.7% (P<.05) lower than those in normal control group, respectively.

3.3. Influence of selenite on pancreatic lipid peroxidation levels

As shown in Fig. 4, in the pancreas, the MDA content in untreated diabetes mellitus group was as high as 5.74 times (P<.001) of that in normal control group. Whereas, selenite played an important role in reducing MDA content enhanced by diabetes mellitus. The MDA content in selenite-treated diabetes mellitus group significantly reduced by 58.4% (P<.001) compared with untreated diabetes mellitus group, and was still 138.6% (P<.001) greater than that in normal control group.

3.4. Inhibitory role of selenite on pancreatic mRNA expressions of IL-1β, TNF-α and IFN-γ

To investigate the influences of selenium on pancreatic proinflammatory cytokines in diabetic animals, we measured the proinflammatory cytokines IL-1β, TNF-α and IFN-γ mRNA expressions by real-time PCR method. As shown in Fig. 5, proinflammatory cytokines IL-1β, TNF-α and IFN-γ mRNA expressions were significantly elevated in untreated diabetes mellitus group, and they were as high as 2.33- (P<.01), 2.64- (P<.001) and 2.11-fold (P<.01) of those in normal control group, respectively. However, in selenite-treated diabetes mellitus group, they were 43.2% (P<.01), 37.5% (P<.01) and 33.9 % (P<.05) lower than those in untreated diabetes mellitus group, respectively, and were 132.6% (P<.05), 164.8% (P<.01) and 140.0% (P<.05) of those observed in normal control group, respectively.

3.5. Suppressive effect of selenite on pancreatic iNOS mRNA expression and activity

Next, we assayed the pancreatic iNOS mRNA expression and activity of different groups. The results showed that iNOS mRNA expression and activity were both significantly augmented in the pancreas of untreated diabetes mellitus group (Fig. 6), and they were 101.0% (P<.001) and 73.2% (P<.001) higher than those in normal control group, respectively. However, compared with untreated diabetes mellitus group, iNOS mRNA expression and activity in selenite-treated diabetes mellitus group were significantly decreased by 35.5% (P<.01) and 34.9% (P<.01), respectively, and were 29.5% and 12.8% greater than those in normal control group, respectively, but there was no significant difference between selenite-treated diabetes mellitus group and normal control group.

3.6. Depressive effect of selenite on pancreatic nitrite and nitrate levels

NO level was determined indirectly by quantification of their oxidized products of degradation (NO_2^- and NO_3^-). As shown in Fig. 7, pancreatic nitrite and nitrate levels in untreated diabetes mellitus group were 137.6% (P<.01) of normal control group levels. However, their levels in selenite-treated diabetes mellitus group were decreased by 18.1% (P<.05) compared with untreated diabetes mellitus group and increased by 12.6% compared with normal control group, respectively, and they were not significantly different

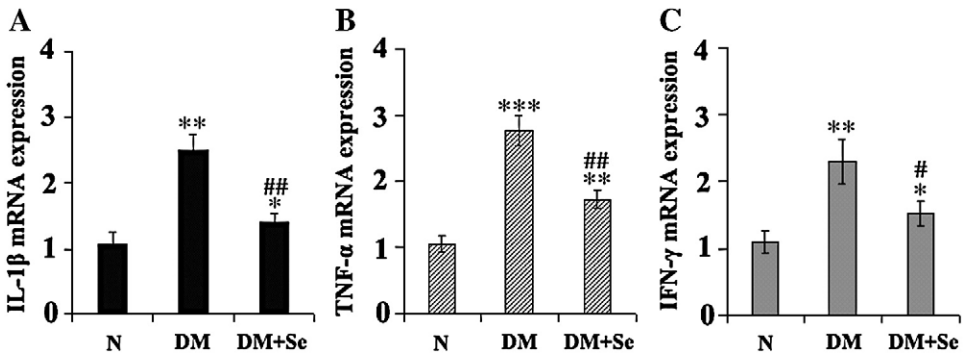

Fig. 5. Proinflammatory cytokines IL-1β (a), TNF-α (b) and IFN-γ (c) mRNA expressions in the pancreas of normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Results of real-time PCR are shown as IL-1β, TNF-α and IFN-γ expression relative to housekeeping gene GAPDH, respectively. Values are expressed as means±S.D. (n=7). *P<.05; **P<.01; ***P<.001, compared with normal control group; #P<.05; ##P<.01, compared with untreated diabetes mellitus group.

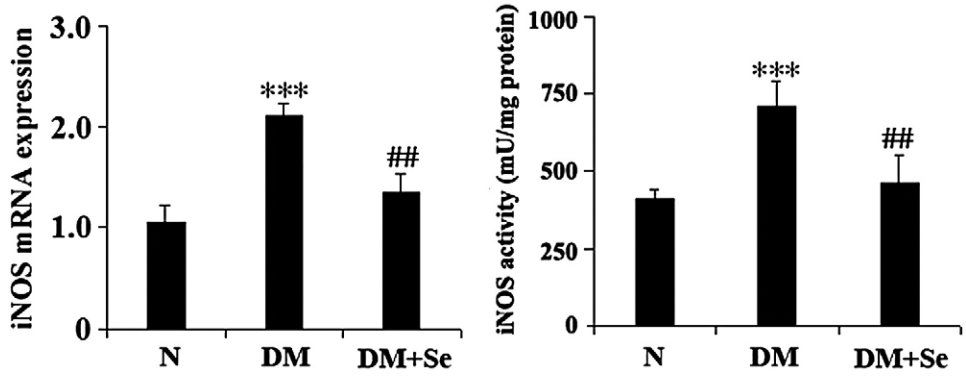

Fig. 6. Pancreas iNOS mRNA expression and activity in normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Results of real-time PCR are shown as iNOS expression relative to housekeeping gene GAPDH. Values are expressed as means±SD (n=7). ***P<.001 compared with normal control group; $^{\#\#}P$<.01, compared with untreated diabetes mellitus group. No significant differences were found between DM +Se and control groups.

between selenite-treated diabetes mellitus group and normal control group.

4. Discussion

Proinflammatory cytokines as IL-1β, TNF-α and IFN-γ and free radical NO have been implicated as inflammatory mediators that lead to inflammation and lesion of pancreatic islets and make β-cells impaired, dysfunctional and associated with Type 1 diabetes mellitus [1–4,33]. Recent studies suggest that ROS and NO are generated in both cytokine-stimulated [5,34,35] and STZ-treated β-cells [9,36], and they mediate the deleterious effects of proinflammatory cytokines or STZ on β-cells dysfunction and destruction [2,35]. As a toxic inflammatory effector molecule on the impairment of islet β-cells, NO is mainly produced by iNOS in inflammatory macrophages [4] and the inflamed pancreatic β-cells themselves [37]. Moreover, it has been suggested by Tanaka et al. [36] that NO produced spontaneously from STZ may take part in pancreatic β-cell damage because STZ has a nitrosourea group in its molecule.

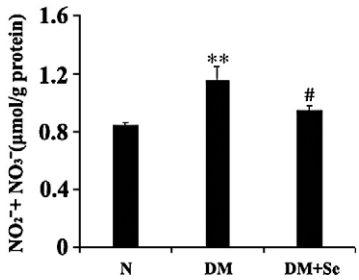

Fig. 7. NO content (measured as nitrite and nitrate level) in the pancreas from normal control group (N), untreated diabetes mellitus group (DM) and selenite-treated diabetes mellitus group (DM+Se). Values are expressed as means±SD (n=7). **P<.01, compared with normal control group; $^{\#}P$<.05, compared with untreated diabetes mellitus group. No significant differences were found between DM+Se and control groups.

NO mediates cytotoxicity through several routes: NO rapidly reacts with the superoxide anion, which results in the formation of peroxynitrite and hydroxyl radicals, both powerful oxidants [34,38–40], and excess NO generated in cells may inhibit mitochondrial metabolism and contribute to protein modification and DNA cleavage, any one of which could lead to β-cells death [37–41].

It has been reported that overexpression of protective enzymes could efficiently prevent the synergistic toxicity of ROS and NO [42], and selenium supplementation has some beneficial effects against the development of diabetes by exhibiting antioxidant properties in experimental models of diabetes mellitus [13]. In addition, selenium exerts insulin-mimetic effects in streptozotocin-induced diabetic rodents [14–16]. In this study, blood glucose level was significantly decreased by the pharmacological dose of selenite treatment in selenite-treated diabetes mellitus group on Day 15 compared with the initial level found in the same diabetes mellitus group before selenite treatment on Day 1 (Fig. 1). Regarding hypoglycemic effect of selenium on diabetic animals, the dose of selenite chosen in the current study was 2 mg/kg body weight per day (11.6 mmol/kg body weight per day), which was in accordance with the studies of McNeill et al. [14] and Sheng et al. [23] (10–15 mmol /kg body weight per day).

However, selenite administration to diabetes mellitus group mice had no effect on elevating plasma insulin level (data not shown). That was in agreement with previous studies by Sheng et al. [23] and McNeill et al. [14]. However, the mechanism remains virtually unknown. We presume it may be related with the inhibiting effect of selenium on proinflammatory cytokines and iNOS. Therefore, the main purpose of this study was to investigate the effect of selenium on mRNA expressions of proinflammatory cytokines and iNOS in the pancreas of diabetic model mice. The results showed that mRNA levels of IL-1β, TNF-α, IFN-γ and iNOS; activity of iNOS and content of NO (measured as nitrite and nitrate levels) were significantly increased in

pancreas of untreated diabetes mellitus group compared to normal control group. Furthermore, selenite administration to diabetic mice could significantly reverse those increases. This is the first evidence of the effect of selenium on mRNA expressions of proinflammatory cytokines in the diabetic model mice.

GPx system is thought to be largely responsible for oxidative defense; GPx-1 is the most abundant biochemical form of body selenium, and its activity could indicate the level of selenium and selenoproteins in organism [43,44]. As shown in Figs. 2 and 3, selenite administration to diabetic mice significantly augmented pancreatic selenium content and reversed decreased mRNA expression and activity of GPx-1. Meanwhile, pancreatic ROS level (measured as MDA) was significantly higher in untreated diabetic group than that in normal control group. However, selenite treatment could significantly reduce the elevated MDA in diabetes mellitus (Fig. 4). That was consistent with the outcomes of previous investigations that antioxidants administration decreased pancreatic augmented lipid peroxides in diabetes mellitus [45–47].

Besides its well-known antioxidant property essential for mammals, selenium is important for mammals' immune function too [17–19]. It has been suggested that selenium exerts its effects on inflammation and immune responses by the mechanism involving the stimulation of immunological cells such as T lymphocytes in the immune system [17,18], the increased GPx activity [48], attenuation of the mutation and virulence of viruses [49] or the inhibitory effect on iNOS [50–52]. Experiments in vitro have shown that selenium supplementation resulted in the diminished ROS production [51,52], the decreased mRNA and protein levels of iNOS and subsequent reduction in NO production [50–52], and there is a higher GPx activity in selenium supplemented cells than that in selenium deficient cells [51]. Supplementation of micronutrient complex (selenium, vitamin E, vanadium and chromium) significantly decreased the elevated expression of TNF-α in the blood lymphocytes of STZ-induced diabetic C57BL mice [53]. In the present study, selenite treatment to diabetic mice significantly increased pancreatic selenium content, GPx mRNA expression and activity, which were helpful to scavenge ROS; reduce the mRNA expressions of proinflammatory cytokines IL-1β, TNF-α and IFN-γ and attenuate iNOS-mediated NO production. Meanwhile, we cannot exclude that other selenoproteins may also play the same role with GPx.

In summary, administration of the pharmacological dose of selenium could improve blood glucose level of the streptozotocin-induced diabetic mice to a certain extent; furthermore, it may suppress the mRNA expressions of proinflammatory cytokines IL-1β, TNF-α and IFN-γ; inhibit the mRNA expression and activity of iNOS and reduce the levels of NO and ROS. The results suggested that hypoglycemic role of selenium may relate with its inhibiting effect on augmentation of proinflammatory cytokines and ROS/reactive nitrogen species by streptozotocin inducing in the pancreas of diabetic mice.

Acknowledgments

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