

Selenium Attenuates High Glucose-Induced ROS/TLR-4 Involved Apoptosis of Rat Cardiomyocyte

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Received: 30 August 2013 / Accepted: 30 October 2013 / Published online: 10 November 2013
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Abstract The potential mechanism of high glucose-induced cardiomyocyte apoptosis and selenium's protective effects were investigated in this study. Myocytes isolated from neonate rats were cultured in high-glucose medium (25.5 mmol/L glucose) to mimic sustained hyperglycemia. Before high-glucose incubation, myocytes were pretreated by sodium selenite solution. Cell apoptosis was evaluated by annexin V/propidium iodide (PI) staining and caspase activation. Expression of Toll-like receptor 4 (TLR-4) and myeloid differentiation factor 88 (MyD-88) was examined at both mRNA and protein levels. The intracellular reactive oxygen species (ROS) production and glutathione peroxidase (GPx) activity in myocytes were also detected. We found high glucose-induced cell apoptosis and activation of TLR-4/MyD-88/caspase-8/caspase-3 signaling, accompanied by increased production of ROS. Selenium pretreatment attenuated apoptosis in high glucose-incubated myocytes, and mechanically, this protective effect was found to be associated with attenuating oxidative status by increasing activity of GPx, decreasing the generation of ROS, as well as inhibition of the activation of

TLR-4/MyD-88/caspase-8/caspase-3 signaling in myocytes. These results suggest that activation of TLR-4/MyD-88 signaling pathway plays an important role in high glucose-induced cardiomyocyte apoptosis. Additionally, by modulating TLR-4/MyD-88 signaling pathway, which is linked to ROS formation, selenium exerts its antioxidative and antiapoptotic effects in high glucose-incubated myocytes.

Keywords Selenium · High glucose · Apoptosis · Toll-like receptor 4 · Reactive oxygen species

Introduction

There are diverse cardiovascular complications of diabetes, including atherosclerosis, stroke, myocardial infarction, and diabetic cardiomyopathy in which cardiac structure and function are affected by disorders of glucose metabolism. Diabetic cardiomyopathy has been regarded as a major complication and cause of disability and mortality in patients with diabetes [1]. Studies have proposed several mechanisms concerning diabetic cardiomyopathy, including abnormalities in ion homeostasis, lipotoxicity, structural and functional abnormalities, altered substrate metabolism, and so on [2, 3]. Among them, excessive generation of oxidative stress, which is defined as the imbalance between production and elimination of free radicals, plays an important role in the pathogenesis and development of diabetic cardiomyopathy [4]. A previous study suggested that oxidative stress was a critical cause of early-phase cardiac cell apoptosis under the diabetic condition [5], which induced a loss of contractile units and subsequently initiated cardiac remodeling and fibrosis [5, 6].

Toll-like receptor-4 (TLR-4) is a key signaling receptor responsible for triggering innate immune response. The expression of TLR-4 has been observed on cardiac cells, including cardiomyocytes, endothelial cells, and smooth muscle

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cells. Previous studies showed that TLR-4 played a vital role in the development and progression of several types of cardiovascular diseases including atherosclerosis [7], myocardial ischemia reperfusion injury [8], heart failure [9], and pressure overload-induced cardiac hypertrophy [10]. In addition, a previous study suggested that TLR-4 may induce cardiomyocyte apoptosis in cardiomyopathy under the conditions of inflammation and oxidative stress [11]. It is reported that intracellular reactive oxygen species (ROS) could modulate NF- κ B activation through their involvement in TLR-4-mediated cellular response [12], and inducing ROS generation also played an important role in the regulation of TLR4 expression [13].

Selenium (Se) is an important trace element which is now known to protect against oxidative stress-related cell damage. Its antioxidant property is related to glutathione peroxidase (GPx), selenoprotein P (SeLP), and thioredoxin reductase (TrxR), most of the proteins have intracellular ROS scavenging activities [14], and selenium has been regarded as an antioxidant system in cell survival [15]. Although it is reported that sodium selenate could restore cardiac dysfunction induced by hyperglycemia [16], the underlying mechanisms involved in the cytoprotection of selenium against high glucose-induced cardiomyocyte apoptosis remain to be elucidated.

Thus, as a potent inducer of excessive ROS production, high glucose-induced myocyte damage is probably contributed by activation of the innate immune signaling pathway of TLR-4/myeloid differentiation factor 88 (MyD-88). Therefore, we examined the effects of high glucose on ROS generation and myocyte apoptosis, and the involvement of speculated activation of TLR-4 signaling pathway was elucidated in this study. We further investigated whether selenium could alleviate myocyte apoptosis and whether the cytoprotective effect is through the ROS/TLR-4 signaling pathway.

Materials and Methods

Ethics Statement

Neonate male Sprague–Dawley (SD) rats were supplied by the Experimental Animal Center of Xi'an Jiaotong University, China. This study was carried out in accordance with the recommended guidelines for the care and use of laboratory animals issued by the Chinese Council on Animal Research. The protocol was approved by the ethics committee of Xi'an Jiaotong University.

Myocyte Isolation and Cell Culture

Myocytes were isolated from the hearts harvested from the neonate SD rats, according to isolation and cell culture

protocols described in a previous publication [17]. Briefly, after the hearts were harvested from anesthetized rats, standard Krebs–Henseleit perfusion buffer (Sigma-Aldrich) was utilized to perfuse the hearts via cannulated aorta and through coronaries. Then the trimmed and minced heart tissue was digested by Liberase (Roche, 4.5 mg/mL). Isolated myocytes were acquired by filtration using a 100- μ m-diameter sterile cell strainer (BD Falcon), then equilibrated in Tyrode's solution, and then serially titrated by CaCl₂ solution (150 and 300 μ L of 10 mmol/L and 90 and 150 μ L of 100 mmol/L, at a 4-min interval for each step). After centrifugation at 500 rpm for 2 min, the pellet was resuspended in a cell culture medium containing minimum essential medium (MEM) with Hank's buffered salt solution. After being incubated at 37 °C with 5 % CO₂ for 1 h on laminin-coated culture dishes (Invitrogen), the culture medium was replaced by fresh culture media containing MEM, 0.1 mg/mL myocyte bovine serum albumin (Sigma-Aldrich), 2 mM L-glutamine (Invitrogen), and 100 U/mL penicillin–streptomycin (Invitrogen).

Treatments

In this study, isolated cardiomyocytes were equally divided into groups: control group (Ctrl), high-glucose group (HG), high glucose + selenium group (HG + Se), and high glucose + TLR-4 blocker group (HG + TLRB). In Ctrl, the isolated myocytes were cultured in a standard medium with a normal glucose concentration at 5.5 mmol/L. In HG, myocytes were cultured in medium with high glucose concentration at 25.5 mmol/L [18]. This high glucose concentration is comparable to serum glucose levels in severely diabetic rats. In HG + Se, myocytes were cultured in a medium with high glucose concentration for a required period (24, 36, or 48 h) after being pretreated with 20 μ mol/L sodium selenite (Sigma-Aldrich) for 1 h [19]. In HG + TLRB, myocytes were pretreated by 20 μ g/mL of anti-TLR-4 monoclonal antibody (eBioscience) [20, 21] for 1 h and then cultured in medium with high glucose concentration for a required period (24, 36, or 48 h).

Cell Apoptosis Assessment

Myocyte apoptosis was assessed by flow cytometry using Annexin V-FITC and propidium iodide (PI) determination. After treatment as described above, myocytes were suspended in a binding buffer. A 5- μ L PI (Santa Cruz) and a 5- μ L Annexin V-FITC (Santa Cruz) were used to incubate the cell in the dark for 15 min, and then 400 μ L of binding buffer was titrated to each sample. Processed samples were then analyzed by flow cytometer (FACS Calibur, BD).

ROS Generation Detection

The determination of intracellular ROS production was based on the oxidation of 2,7-dichlorofluorescein diacetate (DCFH-DA) to fluorescent 2,7-dichlorofluorescein (DCF). Myocytes were washed by phosphate-buffered saline (PBS) and then incubated with 10 $\mu\text{mol/L}$ of DCFH-DA (Molecular Probes) at 37 °C for 30 min in the dark. Cellular fluorescent was determined by flow cytometry (FACS Calibur, BD). Cells were excited with a laser at 488 nm, and measurements were taken at 530 nm.

Glutathione Peroxidase Activity Assay

Cultured myocytes were washed by PBS, and then cell extracts were prepared by cell lysis buffer (Beyotime). GPx activity in cell extracts was assayed using Glutathione Peroxidase Cellular Activity Assay Kit (Sigma-Aldrich) according to the manufacturer's instructions.

Real-Time Polymerase Chain Reaction

Real-time polymerase chain reaction (RT-PCR) was applied to examine the expression of TLR-4 and Myd-88 at transcription level. Myocytes were lysed, and total RNA was extracted using TRI Reagent RNA Isolation Reagent (Sigma-Aldrich). Two micrograms of total RNA was used in reverse transcription by PrimeScript RT Master Mix (TaKaRa) to serve as template for further amplification of mRNA of TLR-4, Myd-88, and GAPDH. A SYBR Premix Ex Taq™ II (TaKaRa) was used in real-time PCR. Primers of TLR-4 and Myd-88 [21] were designed and synthesized by TaKaRa (Table 1). Results were analyzed by Bio-Rad IQ5 software (Ver2.0, Bio-Rad). Gene expression of TLR-4 and Myd-88 was compared using $\Delta\text{cycle threshold}$ ($\Delta\text{Ct} = \text{Ct}_{\text{Target}} - \text{Ct}_{\text{GAPDH}}$), in which expression of GAPDH was introduced as endogenous reference gene.

Western Blotting

Western blotting was then applied to examine the expression of TLR-4, Myd-88, and indicators of activation of caspases including caspase-8 and caspase-3. Cultured myocytes were washed by PBS and then scraped in RIPA Lysis Buffer (Beyotime) with 1 mmol/L PMSF (Beyotime) on ice. The supernatant was collected after centrifugation at 12,000g at 4 °C for 15 min. Protein concentration of sample was examined by BCA Protein Assay Kit (Santa Cruz). Protein was separated using 10 % SDS-PAGE electrophoretically and then transferred to a PVDF membrane (Millipore). Corresponding antibodies to TLR-4 (Cell Signaling), Myd-88 (Cell Signaling), caspase-8 P18 (Santa Cruz), and caspase-3 P17 (Santa Cruz) were used to detect the immunoblots at 4 °C overnight.

Table 1 Sequences of primers for RT-PCR

Gene	Sequence	Size (bp)
TLR-4	Forward 5'-AGACATCCAAAGGAATACTGCAA-3'	180
	Reverse 5'-GCCTTCATGTCTATAGGTGATGC-3'	
Myd-88	Forward 5'-CTCCATTCTCCTCCAGACACT-3'	196
	Reverse 5'-AAGGAGAGGCAGTTTGGCTTC-3'	
GAPDH	Forward 5'-CATCACTGCCACTCAGAAGA-3'	330
	Reverse 5'-TGAAGTCACAGGAGACAACC-3'	

After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz). Membranes were then developed using Western Blotting Luminal Reagent (Santa Cruz) to perform detection. Intensity of immunoblots was quantified by software Image J2x (Rawak Software).

Statistical Considerations

The data were analyzed by SPSS (ver 17.0, SPSS). One-way ANOVA was utilized to analyze the differences between means. Data were expressed in a mean \pm SD manner. Individual experiments were repeated three times. A *p* value <0.05 was considered statistically significant.

Results

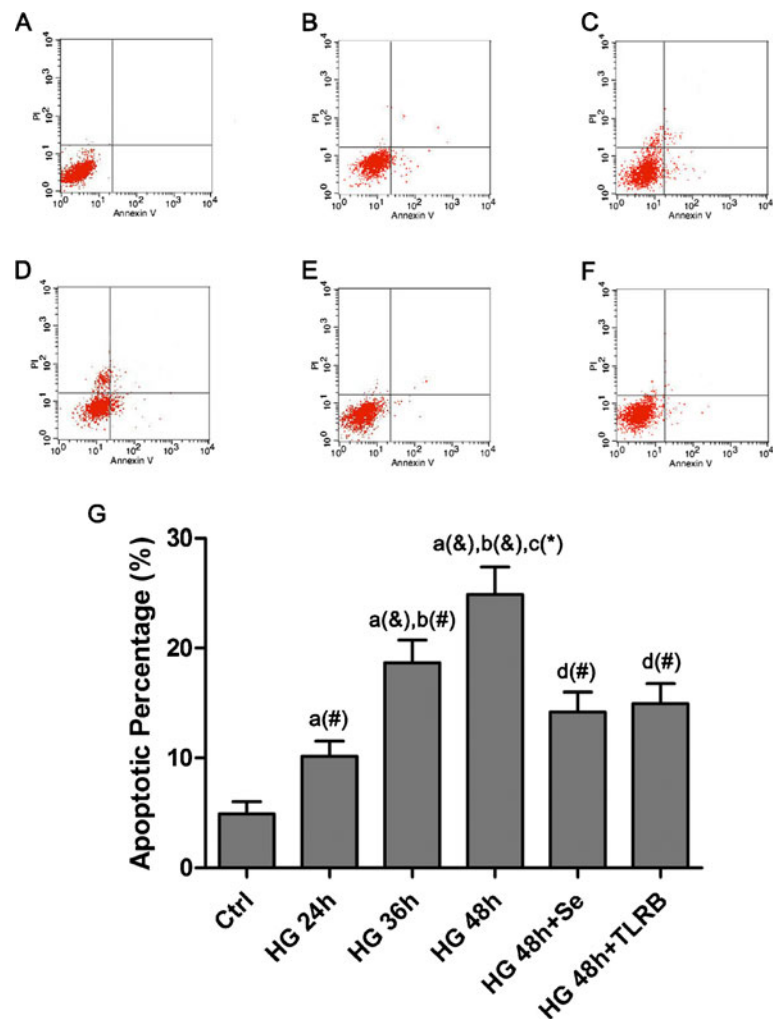
Effects of Selenium on Myocyte Apoptosis Induced by High Glucose Exposure

Apoptosis of annexin V and PI double-stained myocytes which exposed to high glucose was detected and confirmed by flow cytometry. The cell apoptosis was in a time-dependant manner as shown in Fig. 1. The longer the time of exposure to high glucose, the more significantly the apoptosis rate increased at 24, 36, and 48 h in HG, respectively. The apoptosis population of myocytes decreased significantly after being pretreated by 20 $\mu\text{mol/L}$ of sodium selenite for 1 h in HG + Se compared with HG at 48 h. In HG + TLRB, similar phenomenon was observed as 1-h pretreatment of TLR-4 blocker significantly attenuated myocyte apoptosis induced by high-glucose incubation.

Effects of Selenium on Intracellular ROS Generation and GPx Activity in Myocytes Exposed to High Glucose

As shown in Fig. 2a, a significant increase of DCF fluorescence intensity indicating ROS generation was detected by flow cytometer in HG at 48 h after exposure to high glucose.

Fig. 1 Detection of apoptosis after double staining of annexin V and PI by flow cytometry. **a** Myocytes cultured in standard medium (5.5 mM glucose) (Ctrl); **b–d** Myocytes cultured in high-glucose medium (25.5 mM glucose) for 24, 36, and 48 h, respectively (HG 24 h, HG 36 h, and HG 48 h); **e** Myocytes pretreated with 20 $\mu\text{mol/L}$ sodium selenite for 1 h before being cultured in high-glucose medium for 48 h (HG 48 h + Se); **f** Myocytes pretreated with 20 $\mu\text{g/ml}$ TLR-4 blocker peptide for 1 h before cultured in high-glucose medium for 48 h (HG 48 h + TLRB). **g** Columns show the apoptotic percentage in **a–f** charts. Data was presented in a means \pm SD manner. *a* Values are significantly from Ctrl; *b* Values are significantly from HG 24 h; *c* Values are significantly from HG 36 h; *d* Values are significantly from HG 48 h. * $p < 0.05$; # $p < 0.01$; & $p < 0.001$



At the same time, pretreatment of both sodium selenite and TLR-4 blocker in medium resulted in a significant decrease of ROS generation as reflected by DCFH fluorescence intensity. As shown in Fig. 2b, in HG, high-glucose incubation diminished activity of GPx from 86.43 ± 7.49 to 51.88 ± 5.37 $\text{nmol min}^{-1} \text{mg protein}^{-1}$; in HG + TLRB, TLRB pretreatment could not restore the decreased GPx activity induced by high-glucose treatment. However, activity of GPx increased significantly from 51.88 ± 5.37 to 91.15 ± 13.68 $\text{nmol min}^{-1} \text{mg protein}^{-1}$ in HG + Se at 48 h after exposure to high glucose.

Effects of Selenium on mRNA Expressions of TLR-4 and Myd-88 in Myocytes Exposed to High Glucose

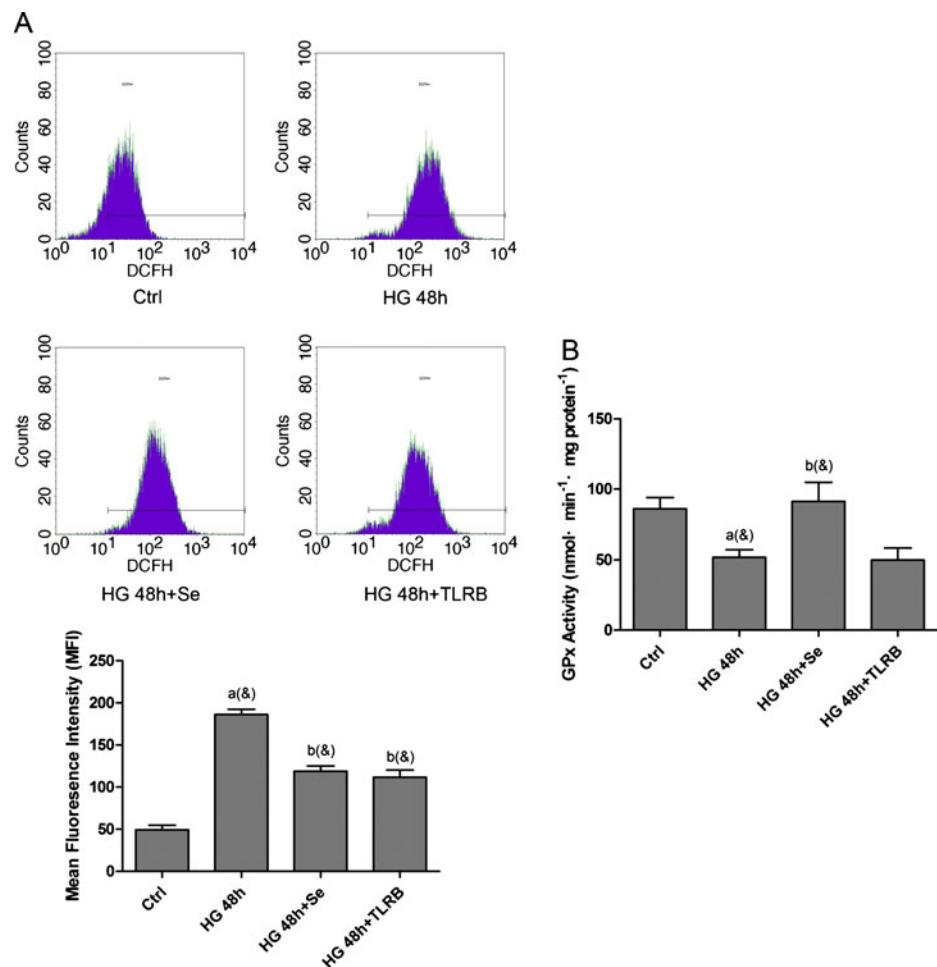
RT-PCR was utilized to analyze the alteration of mRNA expressions of TLR-4 and MyD-88 in myocytes at 48 h after exposure to high glucose. Figure 3 illustrated that compared with untreated myocytes (Ctrl), a dramatic increase of TLR-4 and MyD-88 mRNA expression

was identified in HG 48 h. Meanwhile, in HG 48 h + Se, as shown in Fig. 3, selenium pretreatment reduced upregulation of TLR-4 and MyD-88 mRNA expression compared with (HG 48 h).

Effects of Selenium on Upregulation of TLR-4, MyD-88, Cleaved Caspase-8, and Cleaved Caspase-3 Protein Expressions in Myocytes Exposed to High Glucose

Effects of selenium on regulation of protein expression in TLR-4 signaling pathway in myocytes 48 h after being exposed to high glucose were assessed by western blotting. As shown in Fig. 4, compared with Ctrl, the band intensity of immunoblots of TLR-4 (Fig. 4a), MyD-88, caspase-8 P18, and caspase-3 P17 (Fig. 4b) upshifted significantly after high-glucose incubations. However, in the same time span, selenium pretreatment reversed the upregulation of TLR-4 (Fig. 4a), MyD-88, caspase-8 P18, and caspase-3 P17 (Fig. 4b) protein expression in HG + Se.

Fig. 2 Effects of high-glucose with/without selenium or TLR-4 blocker peptide on ROS production and GPx activity. **a** Charts on the top shows the determination of ROS production in myocytes cultured in standard medium (Ctrl), high-glucose medium for 48 h (HG), high-glucose medium for 48 h after 1 h of pretreatment of Se (HG 48 h + Se) and high-glucose medium for 48 h after 1 h of pretreatment of TLR-4 blocking peptide (HG 48 h + TLRB). Cells were stained by DCFH-DA, followed by a flow cytometry analysis. Columns at the bottom stand for the mean fluorescence intensity (MFI) in each group. Data was presented in a means \pm SD manner. **b** Columns stand for the GPx activity in Ctrl, HG 48 h, HG 48 h + Se, and HG 48 h + TLRB. Data was presented in a means \pm SD manner. *a* Values are significantly different from Ctrl; *b* Values are significantly different from HG 48 h. * p < 0.05; # p < 0.01; & p < 0.001



Discussion

Diabetic cardiomyopathy occurs with a disorder in glucose metabolism, and hyperglycemia showed several negative

effects on the cardiac muscle. Hyperglycemia causes cell damage by increasing ketone body production, which induces the generation of ROS. The increased production of ROS which increases the free radical generation and decreases

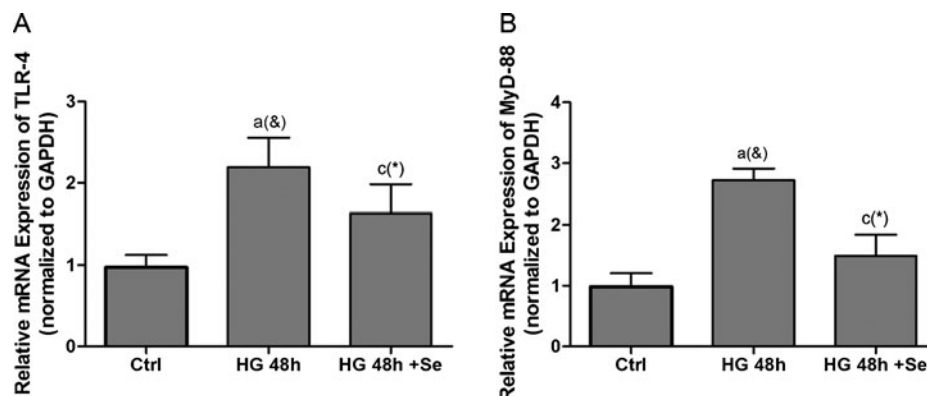


Fig. 3 Real-time PCR assessment of TLR-4 and MyD-88 expression with selenium and/or high glucose in myocytes. Relative mRNA expression levels were normalized to mRNA expression of GAPDH. Columns stand for relative mRNA expression in untreated myocytes (Ctrl), myocytes incubated in high-glucose medium (25.5 mmol/L glucose) for 48 h (HG 48 h), and sodium selenite (20 μ mol/L) for 1 h of pretreatment

of myocytes before incubated by high-glucose medium for 48 h (HG 48 h + Se), respectively, from the left side to the right side. Data was presented in a means \pm SD manner. *a* Values are significantly different from Ctrl; *c* Values are significantly different from HG 48 h. * p < 0.05; # p < 0.01; & p < 0.001

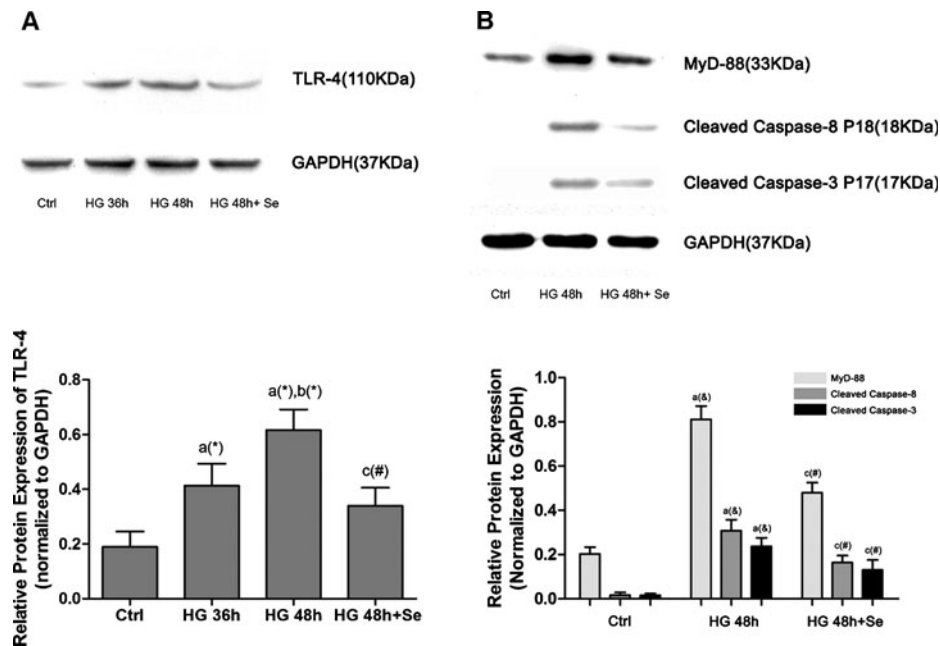


Fig. 4 Effects of selenium and/or high-glucose on TLR-4, MyD-88, cleaved caspase-8, and cleaved caspase-3 expression in myocytes. **a** The *upper part* shows the measurement of TLR-4 protein expression in myocytes by western blotting in myocytes cultured in standard medium (Ctrl), high-glucose medium for 24, 36, and 48 h (HG 24 h, HG 36 h, and HG 48 h), high-glucose medium for 48 h after a 1-h pretreatment of Se (HG 48 h + Se). The *lower part* shows the quantification of immunoblots of TLR-4. *Columns* stand for the relative expression level of TLR-4 (normalized to GAPDH) in described myocyte groups. Data was presented in a means \pm SD manner. **b** Measurement of MyD-88, caspase-8 P18,

and caspase-3 P17 protein level by western blotting. The *upper panel* demonstrates the immunoblots of MyD-88, caspase-8 P18, caspase-3 P17, and GAPDH in Ctrl, HG 48 h, and HG 48 h + Se, respectively. Quantification of immunoblots above is shown in the *lower panel*. *Columns* stand for the relative expression level of MyD-88, caspase-8 P18, and caspase-3 P17 (normalized to GAPDH) in the described myocyte groups. Data was presented in a means \pm SD manner. *a* Values are significantly different from Ctrl; *b* Values are significantly different from HG 36 h; *c* Values are significantly different from HG 48 h. * $p < 0.05$; # $p < 0.01$; & $p < 0.001$

antioxidants is considered as a particularly destructive aspect of oxidative stress. Several studies have shown that oxidative stress could trigger aberrant gene expression, alter signal transduction, and activate many pathways leading to myocyte apoptosis [4, 5, 22, 23]. The resulting myocardial cell loss contributes to the development and progression of diabetic cardiomyopathy. Selenium, as a biological essential trace element, is involved in the production and functioning of several antioxidant enzymes. As an inducer of antioxidants, it is logical to speculate that selenium could attenuate cardiac cell damage and apoptosis under the condition of oxidative stress induced by sustained hyperglycemia. In the present study, cardiomyocytes were incubated in high-glucose medium to mimic sustained hyperglycemia. In agreement with the previous studies, early apoptosis in myocytes were observed in the medium with high glucose concentration. More importantly, our study demonstrated that the TLR-4/MyD-88 signaling pathway was activated, accompanied by the increased level of intracellular ROS in myocytes. Furthermore, the ROS/TLR-4 involved apoptosis was attenuated by supplement of sodium selenite which increased the activity of GPx in myocytes.

TLRs are described to recognize pathogen-associated molecular patterns after infection of bacteria, virus, and fungus,

and also known to recognize damage-associated molecular patterns. At present, ten TLRs have been identified in human, and the most studied TLR in the heart is TLR-4. In addition to the important role of TLRs in mediating cardiac dysfunction under septic conditions, emerging evidence shows that TLRs can also recognize the endogenous ligands and may play a critical role in modulating cardiomyocyte survival and injury [24]. In an *in vivo* study on regional myocardial ischemia, the TLR-4-mutated C3H/HeJ mice suffered smaller myocardial infarct size, lower caspase-3 activity, and reduced cardiac apoptosis compared to the respective control mice [25]. Riad and colleagues demonstrated that after doxorubicin injection, the TLR4-deficient mice showed improved left ventricular function with reduced oxidative and inflammatory stress response including decreased cardiomyocyte apoptosis [11]. MyD-88 is a key adaptor protein in transduction of TLR-4 signaling [26], triggering nuclear translocation and activation of NF- κ B, transcription of inflammatory cytokine genes and activation of mitogen-activated protein kinases (MAPKs) (e.g., JNK and p38), leading to cell apoptosis. Blockade of MyD88-mediated signaling could decrease cardiac apoptosis and eventually attenuate cardiac hypertrophy [27]. In this study, we demonstrated that high glucose triggered cell apoptosis pathways in myocytes. The activation of caspase

cascade including cleavage of caspase-8 and caspase-3 to their cleaved form (activated form) was observed in myocytes in this study. Furthermore, we found that the TLR-4 pathway was significantly activated in the high glucose-treated myocytes, and the apoptosis of myocytes can be attenuated by the TLR-4 blocker. It is considered that by interacting with Fas-associated death domain through MyD-88, one of the TLR-4 adaptor molecules, TLR-4, could activate caspase-8 which is the initiator of caspase cascade reaction [28]. After activation, caspase-8 plays a direct role in downstream activation of caspase-3 which leads to the late events of cell apoptosis as a downstream effector in caspase cascade [29]. Similar to our study, upregulation of TLR-4 and apoptosis were detected in the myocardial tissue of streptozocin (STZ)-treated mice after the appearance of hyperglycemia [30]. Thus, TLR-4 is not only a part of the innate immune system but is also involved in cardiac stress reactions. This signaling pathway may play a critical role in cardiac apoptosis induced by high-glucose incubation.

In addition, it is reported that induction of oxidative stress played an important role in the regulation of TLR-4 expression [13, 31, 32]. Degree of oxidative stress increases after production of oxidizing species or decreases after the activation of antioxidant defense system. As the destructive aspects of oxidative stress, ROS is known to be critical in TLR-4 signaling pathway: intracellular ROS production upregulates TLR2/4 expression [33], but scavenging of ROS prevents the upregulation of TLR-4 mRNA or suppresses TLR-4-mediated cytokine production [13, 34, 35]. Multiple mechanisms have been proposed to account for high glucose- or hyperglycemia-induced toxicity, except for protein kinase C (PKC) activation; increased flux through the hexosamine pathway and increased production of ROS are also considered important ones. Excessive ROS production has been demonstrated to be linked to the activation of caspase-3 and apoptosis in many types of cells [36, 37]. Previous studies also demonstrated that high-glucose treatment increased cell death in cardiomyocytes by stimulating ROS production [38–40], and a principal source of ROS was the one-electron reduction in molecular oxygen to superoxide anion by NADPH oxidase. In monocytes, high glucose could significantly induce TLR-4 expression via PKC and NADPH oxidase activation. The inhibition of oxidative stress-induced formation of ROS significantly suppressed the upregulation of TLR-4 expression, as well as the translocation of TLR-4 to lipid rafts [13, 35]. All these data suggest that the ROS formation may modulate the TLR4 signaling pathway, although the mechanism remains elusive. Moreover, it is likely that ROS production and TLR-4/MyD-88 pathway activation cooperatively mediate the apoptotic procedure, which was observed in diabetic hearts [30]. In this study, we observed that accompanied by upregulation of TLR-4-induced apoptosis, intracellular ROS generation also increased significantly in HG-treated cardiomyocytes. Thus, it

could be proposed that high glucose-induced excessive ROS production was firstly recognized as harmful by TLR-4. Then the activation of TLR-4 and its downstream signaling stimulated caspase signaling and finally resulted in the cardiomyocyte apoptosis.

Selenium deficiency is considered to be associated with increased risk of various diseases, such as cancer, infection, and cardiovascular diseases. The cardioprotective effect of selenium is associated with recovering and modulating activities of antioxidant enzymes including GPx and TrxR [15, 41, 42]. Supplement of selenium in cultured cells has been demonstrated to further increase the GPx activities [43–45], which is the major mechanism against harmful side of ROS production in many cell lines [46, 47]. ROS is regarded as an important contributor in the pathogenesis and development of diabetic cardiomyopathy; the treatment of antioxidant agent protects from hyperglycemia-induced myocyte cell death and compensatory hypertrophy through direct scavenging of ROS [39]. In this present study, we observed the apparent increase of GPx activity and inhibition of ROS formation in selenium-pretreated myocytes after high-glucose incubation. More importantly, the selenium pretreatment also alleviated the expression of TLR-4 and MyD-88 comparably, and the downregulation of caspase-8 and caspase-3 activation was demonstrated as well. These observations suggested that selenium had a protective effect on myocytes under oxidative stress. We made our preliminary conclusion that the inhibition of ROS and inactivation of TLR-4/MyD-88 pathway contributed to the cytoprotection of selenium against high glucose-induced oxidative damage; however, further investigation is needed to determine more specific mechanisms.

Conclusions

These results suggest that activation of ROS/TLR-4/MyD-88 signaling pathway plays an important role in high glucose-induced cardiomyocyte apoptosis. Additionally, by modulating TLR-4/MyD88 signaling pathway which is linked with ROS generation, selenium exerts its antioxidative and antiapoptotic effects in high glucose-incubated cardiomyocytes.

Acknowledgments We thank Ms. Ning Jing for expert technical assistance.

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