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The Protective Effect of Selenium on Bovine Mammary Epithelial Cell Injury Caused by Depression of Thioredoxin Reductase

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Abstract To elucidate the effect of selenium (Se) on antioxidant function of mammary glands in dairy cows and the underlying mechanism, an experiment was conducted using a single-factor completely randomized design study. Bovine mammary epithelial cells (BMECs) were randomly divided into four groups: control, Se treatment, 2,4-dinitrochlorobenzene (DNCB) inhibition, and Se prevention. Treatment of BMECs with Se was found to significantly reverse decreased cell proliferation and the expression of thioredoxin reductase (TrxR) after DNCB exposure. DNCB-induced activation of apoptosis signaling kinase-1 (ASK-1), which activates the mitogen-activated protein kinase (MAPK) pathway, was reduced in BMECs treated with Se. Additionally, our results indicated that Se treatment resulted in lower intracellular accumulation of arachidonic acid (ARA) and 15-hydroperoxyeicosatetraenoic acid (15-HPETE) due to suppressed expression of cytosolic phospholipase A$_2$ (cPLA$_2$) regulated by p38MAPK and c-Jun N-terminal kinase (JNK) in DNCB-stimulated BMECs. Taken together, these findings suggest that Se treatment improved the antioxidant function of dairy cow mammary glands and protected cells from oxidative damage primarily by increasing the activity of TrxR, inhibiting the activation of the MAPK signaling pathway, and thus decreasing the content of ARA and its related metabolites.

Introduction

Dairy cattle experience oxidative stress due to the high metabolic rate of bovine mammary epithelial cells during lactation. This stress leads to decreased immune function, milk production, and milk quality, ultimately resulting in economic losses [1]. The essential micronutrient selenium (Se) occurs in the amino acid selenocysteine in selenoproteins, such as thioredoxin reductase (TrxR), which exert various effects while maintaining the cell reduction-oxidation balance [2]. However, the exact mechanism by which Se maintains the redox state of a cell is still unclear.

Arachidonic acid (ARA) is a type of polyunsaturated fatty acid (PUFA) that can be released from esterified lipid pools by the action of specific lipases, such as cytosolic phospholipase A2 (cPLA$_2$) [3]. ARA is the common precursor of eicosanoids and exists in phospholipids of the cell membrane. ARA has a positive physiological function at some concentrations. However, stimulation of the cell membrane by reactive oxygen species (ROS), lipopolysaccharides (LPS), cytokines, and NO increases ARA secretion; excess ARA production can lead to the blocking of the antioxidant signaling pathway and oxidative stress [4]. Studies of heart mitochondria and COS-7 cells indicate that the massive release and metabolism of ARA induce and aggravate oxidative stress [5, 6]. Some results strongly suggest that TrxR is involved in regulating the synthesis of ARA and preventing inflammation and oxidative stress [7].

The antioxidant thioredoxin (Trx) system is composed of the selenoprotein TrxR, Trx, and NADPH. The oxidized

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thioredoxin is reduced by TrxR and NADPH. Under normal circumstances, the reduced form of Trx binds to the N-terminal region of apoptosis signal-regulating kinase-1 (ASK-1) and inhibits kinase activity. Change in the redox status of Trx induces ASK-1 activation and activates the mitogen-activated protein kinase (MAPK) pathway [8]. Randall et al. [9] reported that small interfering RNA (siRNA) silencing of TrxR significantly suppressed the ability of acrolein to activate c-Jun N-terminal kinase (JNK) and appeared to attenuate acrolein-dependent activation of extracellular signal-regulated kinase (ERK) and p38MAPK. cPLA$_2$, a substrate for MAPK, can be activated by phosphorylation, leading to ARA liberation [10]. These results suggest that the activity of TrxR is strongly linked with oxidative stress due to the influence of the MAPK signaling pathway on ARA metabolism.

Previous studies from our research group demonstrated that Se is effective in improving antioxidant status and increasing TrxR activity [11]. 2,4-Dinitrochlorobenzene(DNCB) is the unique inhibitor of TrxR [7], and an oxidative stress model of BMECs induced by DNCB has been established by our research group [12]. The present study was carried out to investigate the protective effect of Se on oxidative stress in BMECs caused by DNCB and to elucidate whether the underlying mechanism of the antioxidative effects of Se was due to an increase in TrxR activity modulating the MAPK signaling pathway and downregulating the release and metabolism of ARA.

Materials and Methods

BMEC Isolation and Primary Cell Culture

Mammary tissue was collected from healthy lactating Holstein cows. Samples of mammary tissue were trimmed of outer layers and placed in phosphate-buffered saline (PBS) supplemented with 300 U/mL of antibiotic-antimycotic solution. Minced tissues were sequentially washed three times with PBS supplemented with 300 U/mL of antibiotic-antimycotic solution, transferred to 75% ethanol for 30 s, and washed three times with PBS supplemented with 100 U/mL of antibiotic-antimycotic solution. The tissues were transferred into a culture dish, trimmed, and cut into pieces the size of rice grains. Pieces rich with acini were transferred into a 5-mL centrifuge tube with 0.05% collagenase type II (0.5%, Gibco BRL, Grand Island, NY) and minced thoroughly to separate BMECs from erythrocytes. The cell suspension was incubated for 1 h at 37 °C in a CO$_2$ constant temperature incubator (Heal Force Bio-meditech Holdings Ltd., China) and shaken every 20 min. The digestion suspension was filtered through an 80-mesh sieve, and the dispersed cells were collected by centrifugation at 400×g for 5 min. The precipitate

was suspended in PBS and then centrifuged again at 400×g for 3 min. After centrifugation, the cell pellet was resuspended in growth DMEM/F12 medium (5 mL) containing 10% fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO, USA), 5 μg/mL insulin transferrin Se (Sigma Chemical Co., St. Louis, MO, USA), 10 ng/mL epidermal growth factor (Sigma Chemical Co., St. Louis, MO, USA), 1 μg/mL hydrocortisone (Sigma Chemical Co., St. Louis, MO, USA), 5 μg/mL prolactin (Sigma Chemical Co., St. Louis, MO, USA), 2.5 μg/mL amphotericin B (Sigma Chemical Co., St. Louis, MO, USA), 100 IU/mL penicillin, 100 μg/mL streptomycin, and basal medium (15.6 g DMEM/F12 powder, 1000 mL ultra-pure water, and 2.4 g sodium bicarbonate). Cell counts were determined by cytometry (ECI Inc., Yokohama, Japan). BMECs were seeded into 25-cm^2 cell culture flasks at an approximate density. The cells were then incubated in an atmosphere of 5% CO$_2$ at 37 °C.

Seeding and Starvation

When the confluence of BMECs reached 80 to 90%, the BMECs were subcultured twice. Third-generation BMECs were seeded at approximate densities of 1×10^4, 1×10^5, 3×10^5, and 5×10^5 cells/mL into 96-well plates (200 μL per well), 6-well plates (2.5 mL per well), 60-mm culture dishes (3.5 mL per well), and 25-cm^2 culture flasks (3.5 mL per flask). Cells were incubated with DMEM/F12 growth medium supplemented with 10% FBS in an atmosphere of 5% CO$_2$ at 37 °C for 24 h. The medium was decanted when the confluence of BMECs reached 80 to 90%, and then the cells were serum starved for 24 h.

Experimental Design

A single-factor experimental design was used for this experiment. After cells were serum starved for 24 h, BMECs were randomly divided into four treatments, each consisting of six replicates. Group 1 was the control group (cultured in medium without selenomethionine and DNCB for 26 h), group 2 was the Se treatment group (cultured with 50 nmol/L selenomethionine without DNCB for 26 h), group 3 was the DNCB inhibition group (pretreated with the medium without selenomethionine for 24 h followed by treatment with 300 μmol/L DNCB for 2 h), and group 4 was the Se prevention group (pretreated with 50 nmol/L selenomethionine for 24 h followed by treatment with 300 μmol/L DNCB for 2 h). The dose and processing time of Se and DNCB are based on previous research [12, 13].

Cell Viability Assay

Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salt (MTT) method

Table 1 Effect of Se on the proliferation of cells damaged by DNCB

Items	Control	Se treatment	DNCB inhibition	Se prevention	SEM	P value
RGR (%)	100.00b	123.20a	74.30c	98.60b	2.69	< 0.01

Means within a row without the same lowercase letters differ significantly (*P* < 0.05)

RGR relative growth rate, *SEM* standard error of the mean

adapted from Kim et al. [14]. After culture for 24 h, 20 μL of 5 mg/mL MTT solution was added to each well. BMECs in the 96-well plate were then cultured for 4 h, the medium was decanted, 100 μL dimethyl sulfoxide (DMSO; Sigma Chemical Co., St. Louis, MO, USA) was added to each well, and the plate was shaken for 10 min and then analyzed with an automatic microplate reader (Synergy H4, BioTek Co., Japan) at 490 nm. Cell viability of the control group was expressed as 100%. Cell viability of the treatment group was expressed as percent (%) viability = OD treatment group / OD control group * 100.

Measurement of Antioxidant Parameters

The cell culture medium was collected in 1.5-mL centrifuge tubes and centrifuged at 10000×*g* for 5 min at 4 °C. The supernatant was transferred into new tubes and stored at − 80 °C for the analysis of SelP content. SelP content was measured by radioimmunoassay.

After the cells were cultured for 24 h in 60-mm culture dishes, the medium was decanted, and the culture dishes were washed two times with PBS and placed on ice. Cells were scraped and lysed for 30 min in 600 μL of lysis buffer and centrifuged at 10000×*g* for 10 min at 4 °C. The cell suspension was collected into 1.5-mL Eppendorf tubes and stored at − 80 °C for analysis of TrxR and glutathione peroxidase (GPx) activity and ROS and malondialdehyde (MDA) content. The TrxR activity was measured using a 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reduction assay. The GPx activity was measured using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. ROS levels were analyzed using commercial ELISA kits (Neobioscience Co., Beijing, China). MDA concentrations were measured by thiobarbituric acid positive reactant (TBAR) assay. The absorbance was measured using a spectrophotometer at various wavelengths.
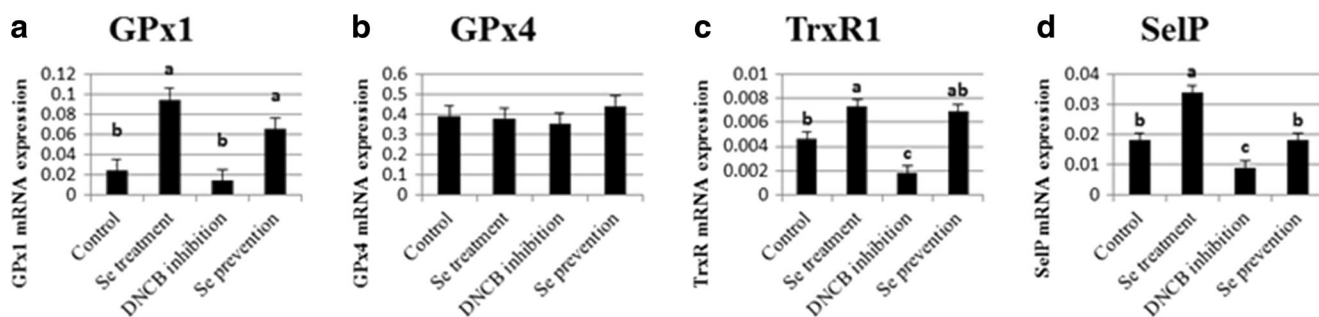
Relative Expression of Selenoprotein mRNA in BMECs

Total RNA extraction was carried out according to the manufacturer's instructions for the RNAiso™ Plus extraction kit (Takara Bio Inc., Dalian, China). After the cells were cultured for 24 h in 6-well plates, the medium was decanted, plates were washed twice with PBS, and 1 mL TRIzol Plus lysate was added to each well and repeatedly pipetted for thorough
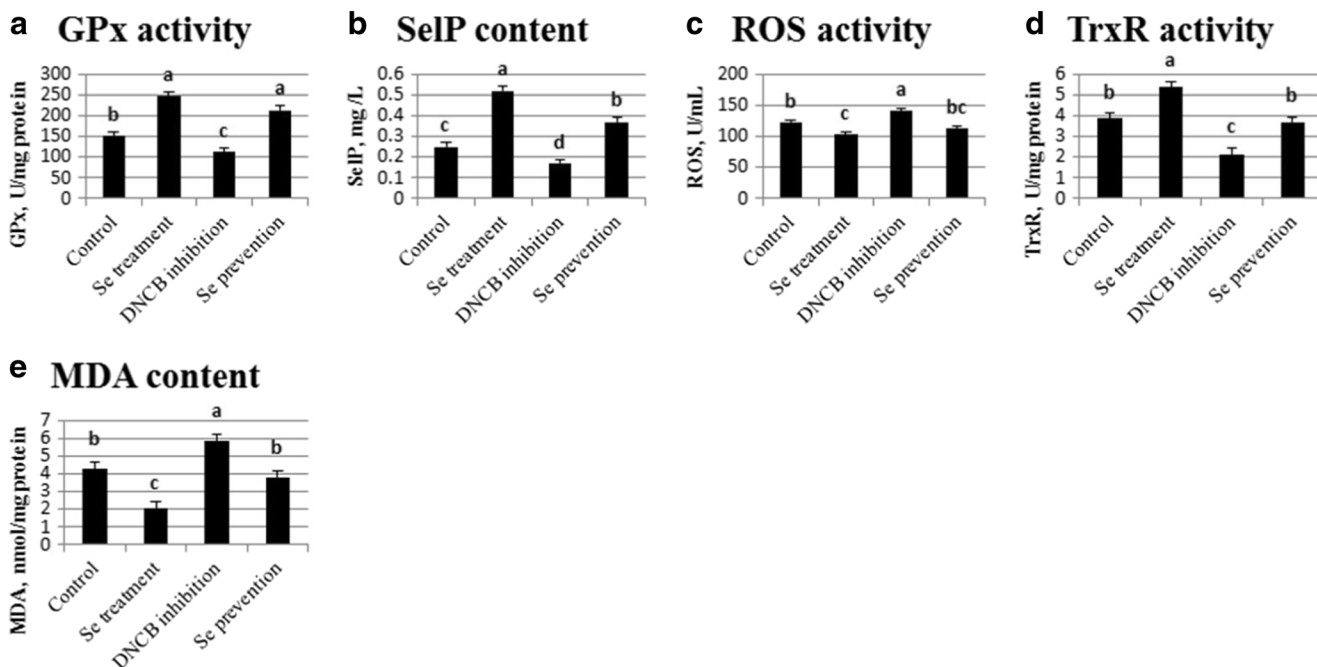
Fig. 1 Effect of Se on antioxidative parameters of cells damaged by DNCB. Data given are mean ± SEM. **a**, **b** Values with different letters differ significantly at *P* < 0.05. *GPx* glutathione peroxidase; *TrxR* thioredoxin reductase; *SelP* selenoprotein P; *ROS* reactive oxygen species; *MDA* malondialdehyde

Fig. 2 Effect of Se on the mRNA expression of selenoproteins in cells damaged by DNCB. Data given are mean ± SEM. **a**, **b** Values with different letters differ significantly at $P < 0.05$. *GPx* glutathione peroxidase; *TrxR* thioredoxin reductase; *SelP* selenoprotein P

lysis of the cells. The cell suspension was collected in 1.5-mL Eppendorf tubes.

The complementary DNA (cDNA) probes were created using a PrimeScript™ reverse transcription kit (Takara Bio. Inc., Dalian, China) according to the manufacturer's instructions. The PCR primers were designed using Oligo and Premier software based on data from GenBank. Relative messenger RNA (mRNA) expressions of glutathione peroxidase 1 (GPx1), GPx4, thiorexin reductase1 (TrxR1), and SelP in BMECs were determined by fluorescence-based real-time PCR (SYBR Green I) using a SYBR Premix Ex Taq™ kit (Takara Bio Inc., Dalian, China), according to the manufacturer's instructions, with β-actin as the internal reference gene. The objective gene relative expression was calculated by the $2^{-\triangle\triangle Ct}$ method. PCR products were electrophoresed on 2% agarose gels (100 V, 20 min), photographed under UV light, and compared with a DNA marker. Sequence analysis was carried out by Shanghai Sangon Biotech.

GPx1 and TrxR1 Western Blots

After the cells were cultured for 24 h in 25-cm^2 culture flasks, the medium was decanted, and the culture flasks were washed twice with PBS. Cells were scraped, lysed for 5 min in RIPA lysis at 4 °C, and centrifuged at 10000×g for 10 min at 4 °C. The cell suspension was

collected in 1.5-mL Eppendorf tubes and used for protein expression of related indices in cells. Protein expression of GPx1 and TrxR1 in cells was determined by Western blotting with the internal reference gene β-actin. Cell lysates containing 30 μg of protein were boiled in one-fourth volume of four times sample loading buffer at 95 °C for 5 min and separated on 12% SDS-PAGE with a 5% stacking gel in electrophoretic buffer. Proteins were electrotransferred to a polyvinylidene fluorine membrane in electrophoresis buffer at 100 V for 50 min. Membranes were blocked with blocking buffer for 1 h at room temperature. The membrane was then washed three times for 2 min with PBST and incubated with diluted polyclonal rabbit anti-β-actin (1:2000; Protein Tech Co., Ltd., Shanghai, China), polyclonal rabbit anti-GPx1 (1:1000; Abcam Co., Cambridge, MA, USA), and polyclonal rabbit anti-TrxR1 (1:500; Abcam Co., Cambridge, MA, USA) overnight at 4 °C. The membrane was then washed three times for 5 min with PBST. Then, the membrane was incubated with goat anti-rabbit IgG (1:1000; KPL Co., Gaithersburg, MD, USA) for 1 h at room temperature and washed three times for 8 min with PBST. Color development was carried out with the ECL ultra-sensitive luminescence kit according to the manufacturer's instructions. The membrane was kept in the dark for 2 min and then photographed with an ImageQuant (GE Healthcare Co., Fairfield, Connecticut, USA). The image intensity

Fig. 3 Effect of Se on protein expression of selenoproteins in cells damaged by DNCB. Data given are mean ± SEM. **a**, **b** Values with different letters differ significantly at $P < 0.05$. *GPx* glutathione peroxidase; *TrxR* thioredoxin reductase
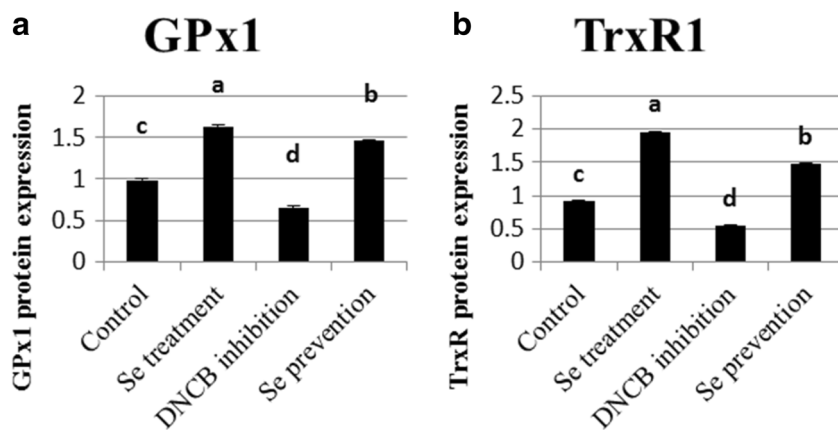
was analyzed with Quantity One software (Bio-Rad Laboratories Inc., Hercules, CA, USA), and the relative expression of the target protein was calculated (relative expression of target protein = objective protein gray value / reference protein gray value).

ARA-Related Metabolism Indicators and Gene Expression

The activity of $cPLA_2$, 5-lipoxygenase (5-LOX), and cyclooxygenase-2 (COX-2) in cells and the content of ARA, 15-hydroperoxyeicosatetraenoic acid (15-HPETE), 15-hydroxyeicosatetraenoic acid (15-HETE), leukotriene B4 (LTB4), and (prostaglandin E2 (PGE2) in culture solution were analyzed using ELISA kits according to the manufacturer's instructions. The values of the above indicators were calculated based on the corresponding standard curves.

ASK-1 Activity and Phosphorylation Levels of ERK1/2, JNK, and p38MAPK

The phosphorylation levels of ERK1/2, JNK, and p38MAPK in cells were analyzed using ELISA kits according to the manufacturer's instructions (eBioscience Co., Ltd., Kobe, Japan). ASK-1 activity in cells was measured using ELISA kits according to the manufacturer's instructions (MyBioSource Co., San Diego, USA). The absorbance was measured at 450 nm and correlated to the standard curve of ASK-1 activity in cells.

Statistical Analysis

The data were analyzed using one-way ANOVA and Duncan multiple comparison tests implemented in the SAS software (SAS Version 9.0, SAS Institute, Cary, NC). The linear and quadratic effects of Se level on all the indices were analyzed using regression analysis. Differences were considered to be significant when $P < 0.05$, and a statistical trend was indicated when $0.05 < P < 0.1$.

Results

Effect of Se on the Proliferation of Cells Damaged by DNCB

To determine the protective effects of Se on DNCB-induced cytotoxicity in BMECs, the cells were pretreated with Se for 24 h and exposed to DNCB for an additional 2 h. Treatment of BMECs with 300 μmol/L DNCB for 2 h resulted in an approximately 25.7% loss of cellular viability compared with the control cells (Table 1). However, the cytotoxic effect of DNCB was blocked by pretreatment with Se. Compared with the DNCB inhibition group, cell proliferation increased significantly in the Se prevention group ($P < 0.01$).

### Effect of Se on the Antioxidative Parameters of Cells Damaged by DNCB

GPx and TrxR activity and SelP content increased in the Se treatment group but decreased in the DNCB inhibition group ($P < 0.01$) as compared with the control group (Fig. 1). These same parameters were significantly higher in the Se prevention group than in the DNCB inhibition group ($P < 0.01$). As expected, increased ROS and MDA generation was detected in BMECs after stimulation with DNCB alone. However, pretreatment with Se significantly reduced DNCB-induced ROS and MDA production ($P < 0.01$, $P = 0.0003$).

Effect of Se on the mRNA Expression of Selenoproteins in Cells Damaged by DNCB

The addition of Se increased mRNA expression of GPx1, TrxR1, and SelP compared to the control group, while mRNA expression of TrxR1 and SelP decreased significantly in the DNCB inhibition group ($P = 0.0004$, $P = 0.0004$, $P < 0.01$; Fig. 2). Pretreatment with Se significantly reversed the DNCB-induced decrease in the mRNA expression of GPx1, TrxR1, and SelP increase ($P = 0.0004$, $P = 0.0004$, $P < 0.01$). However, the mRNA expressions of GPx4 not differ between groups ($P = 0.54$).

Effect of Se on Protein Expression of Selenoproteins in Cells Damaged by DNCB

Western blot analysis indicated that protein expressions of GPx1 and TrxR1 were higher in the Se treatment group than those in the control group and lower in the DNCB inhibition group than those in the control ($P < 0.01$). There was an increase in protein expression of GPx1 and TrxR1 in the Se prevention group compared to those in the DNCB inhibition group ($P < 0.01$) (Figs. 3, 4, and 5).
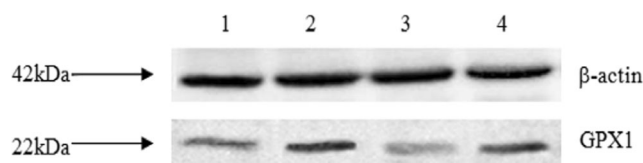
Fig. 4 The effect of selenium on GPx1 protein expression in cells damaged by DNCB. Group 1 was the control group, group 2 was Se treatment group, group 3 was DNCB inhibition group, group 4 was Se prevention group
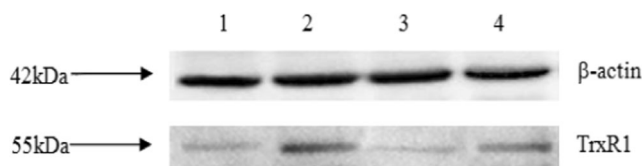
Fig. 5 The effect of selenium on TrxR1 protein expression in cells damaged by DNCB. Group 1 was the control group, group 2 was Se treatment group, group 3 was DNCB inhibition group, group 4 was Se prevention group

Effect of Se on ARA Release and Metabolism in Cells Damaged by DNCB

There were no differences in $cPLA_2$ activity or ARA content between the Se treatment group and the control group, but an increase was observed in the DNCB inhibition group ($P = 0.0006$; $P = 0.0019$). There was a decrease in $cPLA_2$ activity and ARA content in the DNCB inhibition group compared to those in the Se prevention group ($P = 0.0006$; $P = 0.0019$) (Fig. 6).

LTB4 and 15-HPETE content and 5-LOX activity in cells were lower in the Se treatment group than in the control group but higher in the DNCB inhibition group as compared with those in the controls ($P = 0.0025$; $P = 0.0005$; $P < 0.01$). There was a decrease in LTB4 and 15-HPETE content and 5-LOX activity in the DNCB inhibition group as compared with those in the Se prevention group ($P = 0.0025$; $P = 0.0005$; $P < 0.01$). Change in 15-HETE content exhibited the opposite pattern ($P = 0.0004$). COX-2 activity and PGE2 content were lower in the Se treatment group than in the

control group; however, there was no difference between the DNCB inhibition and control groups ($P = 0.0058$; $P = 0.0145$). Compared to the DNCB inhibition group, COX-2 activity and PGE2 content in the Se prevention group decreased ($P = 0.0058$; $P = 0.0145$) (Fig. 6).

Effect of Se on mRNA Expression of Enzymes Related to ARA Metabolism in Cells Damaged by DNCB

There was no significant change in the mRNA expression of $cPLA_2$, 5-LOX, and COX-2 between the control group and the Se treatment group, but there was an increase in the DNCB inhibition group compared with those in controls ($P < 0.01$). Compared with the DNCB inhibition group, mRNA expression of $cPLA_2$, 5-LOX, and COX-2 decreased in the Se prevention group ($P < 0.01$) (Fig. 7).

Effect of Se on Activities of Enzymes in the MAPK Pathway in Cells Damaged by DNCB

ASK-1 activity and JNK and p38MAPK phosphorylation levels did not change significantly with Se treatment but increased in the DNCB inhibition group ($P < 0.01$; $P = 0.0014$; $P = 0.0038$) as compared with those in the control group. ASK-1 activity and JNK and p38MAPK phosphorylation levels were lower in the Se prevention group than in the DNCB inhibition group ($P < 0.01$; $P = 0.0014$; $P = 0.0038$). The phosphorylation level of ERK1/2 did not differ significantly among groups (Table 2).
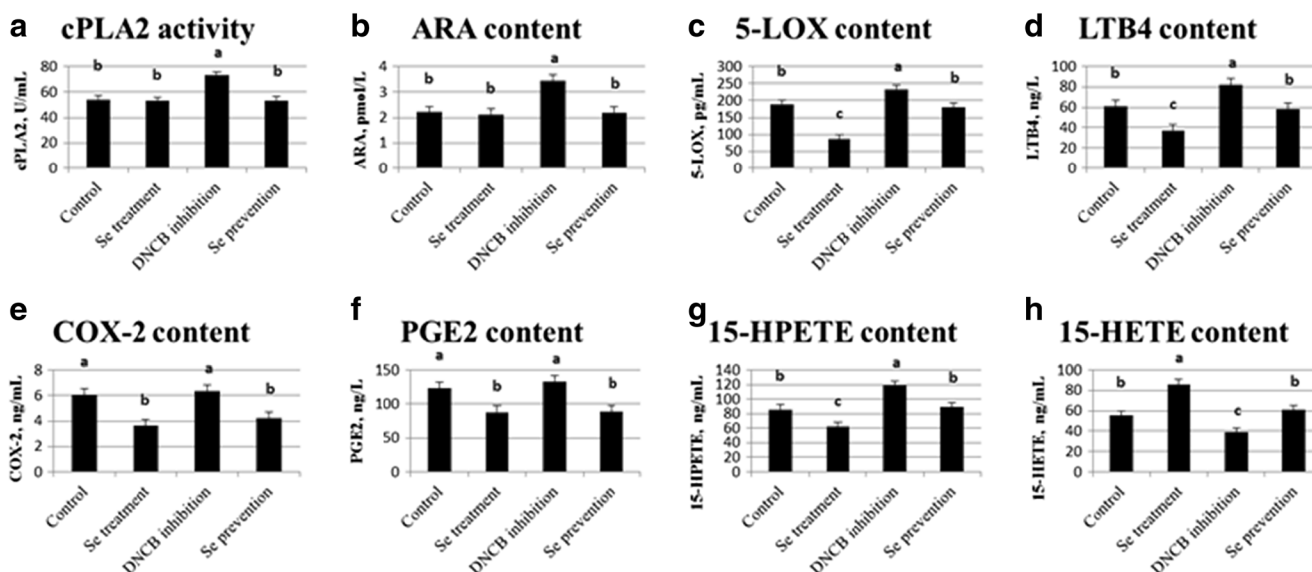
Fig. 6 Effect of Se on the parameters related with ARA release and metabolism in cells damaged by DNCB. Data given are mean ± SEM. **a, b** Values with different letters differ significantly at $P < 0.05$. *cPLA2* cytosolic phospholipase A2; *ARA* arachidonic acid; *5-LOX* 5-

lipoxygenase; *LTB4* leukotriene B4; *COX-2* cyclooxygenase-2; *PGE2* prostaglandin E2; *15-HPETE* 15-liydroperoxyeicosatetraenoic acid; *15-HETE* 15-hydroxyeicosatetraenoic acid
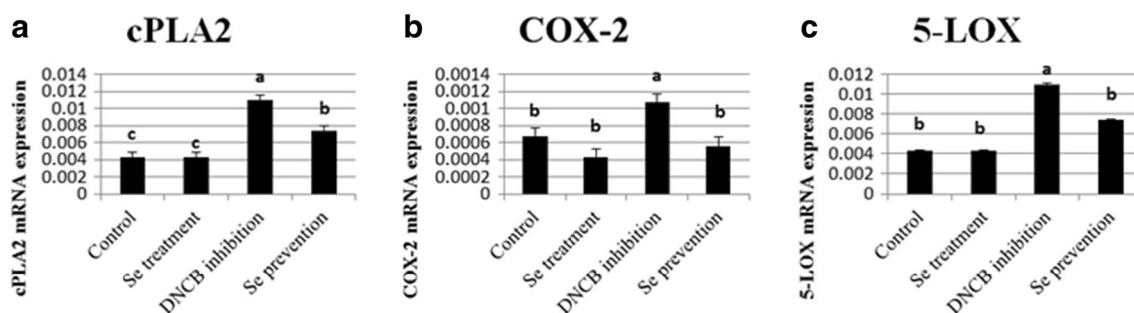
Fig. 7 Effect of Se on mRNA expression of enzymes related with ARA metabolism in cells damaged by DNCB. Data given are mean ± SEM. **a**, **b** Values with different letters differ significantly at $P < 0.05$. *cPLA2* cytosolic phospholipase A2; *COX-2* cyclooxygenase-2; *5-LOX* 5-lipoxygenase

Discussion

Changes in cell viability, and ROS and antioxidant indices, such as TrxR, GPx, SelP, and MDA, are the primary indicators indicating cell oxidative stress. Oxidative stress can lead to a decrease in SelP content and TrxR and GPx activity and a rise in MDA and ROS concentrations. DNCB is a unique inhibitor of TrxR and can increase intracellular ROS levels [7]. The results shown here indicate that the presence of DNCB significantly inhibited TrxR and GPx activity and SelP content in BMECs, but increased the release of ROS and MDA, suggesting that DNCB induced cell oxidative damage. The inhibition of TrxR by DNCB occurs through alkylation of the TrxR thiolate/selenolate active sites [15]. The inhibition of TrxR leads to activation of NADPH oxidase in the Trx system, which increases the production of ROS [16]. Our results also indicated that DNCB significantly downregulated both mRNA and protein expression of TrxR1. Conversely, the results from the Se prevention group showed an increase in TrxR activity and increased gene and protein expression. In addition, Se treatment stimulated TrxR activity as compared to control levels. These results supported the conclusion that Se treatment protects BMECs against DNCB-induced oxidative stress by enhancing TrxR activity and inhibiting the activation of the MAPK signaling pathway. The massive release of ARA and increased metabolism through the LOX pathway can induce and aggravate oxidative stress in cells [5, 6]. Activation of the MAPK signaling pathway can directly activate cPLA₂, resulting in a substantial release of ARA [10, 17]. ASK-1 is the upstream kinase of the MAPK signaling pathway and can

be activated when TrxR activity is attenuated or inhibited; the MAPK signaling pathway can then also be activated [8]. Randall et al. [9] found that siRNA silencing of TrxR1 significantly suppressed the ability of acrolein to activate JNK and appeared to increase p38MAPK and JNK phosphorylation levels in human bronchial epithelial cells. Kim et al. [14] suggested that the addition of Se to rat macrophages significantly increased the activity of TrxR and attenuated the activation of the MAPK signaling pathway by LPS. The study of Kurosawa et al. [7] showed that TrxR had a strong inhibitory effect on the activity of cPLA₂, which led to a reduction in the level of free ARA in murine sarcoma cells cultured in vitro. The results of the current experiment showed that, compared with controls, TrxR activity was significantly decreased in response to DNCB treatment, but the activity of ASK-1 and p38MAPK and JNK phosphorylation levels increased significantly. Similarly, cPLA₂ activity and gene expression and ARA content increased markedly. Metabolism-related indicators of ARA, such as 5-LOX activity and gene expression and concentrations of 15-HPETE and LTB4, showed a similar change. However, changes in the aforementioned parameters demonstrated the opposite trend in the Se prevention group. Research in bovine peripheral blood lymphocytes demonstrated similar results to those shown here [18]. Taken together, these results imply that Se protects BMECs from oxidative stress and that this effect is due to increased TrxR activity, which modulates the MAPK signaling pathway and downregulates the release of ARA.

Here, we did not silence the TrxR gene; therefore, the exact mechanism by which Se acts needs to be explored further. In

Table 2 Effect of Se on the activities of the related enzymes of MAPK pathway in cells damaged by DNCB

Items	Control	Se treatment	DNCB inhibition	Se prevention	SEM	*P* value
ASK-1 (ng/mL)	323.00c	302.67c	701.11a	529.00b	21.47	< 0.01
JNK (OD value)	0.370c	0.481bc	1.079a	0.734b	0.09	0.0014
p38MAPK (OD value)	0.574b	0.465b	1.068a	0.739b	0.10	0.0038
ERK1/2 (OD value)	0.238a	0.271a	0.286a	0.264a	0.02	0.5049

Means in a row without same lowercase letters significantly differ ($P < 0.05$)

ASK-1 apoptosis signaling kinase-1, *JNK* c-Jun N-terminal kinase, *p38MAPK* p38 mitogen-activated protein kinase, *ERK1/2* extracellular signal-regulated kinase1/2, *SEM* standard error of the mean

addition, Straif et al. [19] found that GPx1 can effectively inhibit the activity of 5-LOX in peripheral blood mononuclear cells and reduce the generation of 15-HPETE. We also observed an increase in GPx1 gene and protein expression in the Se prevention group. Therefore, it is likely that Se can reduce the release of ARA and the formation of lipid peroxidation by regulating GPx activity. However, there was no specific inhibition of GPx1 in this study, so further research on the role of GPx is also needed.

Conclusion

Selenoprotein TrxR plays a key role in protecting cells against oxidative damage. Enhancement of TrxR activity induced by Se treatment inhibits the activation of the MAPK signaling pathways, thereby reducing the release of ARA, and related metabolic parameters, and ultimately protecting cells from oxidative damage.

References

1. Sordillo LM, Aitken SL (2009) Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunop 128(1–3):104–109
2. Duntas LH (2006) The role of selenium in thyroid autoimmunity and cancer. Thyroid 16(5):455–460
3. Wei C (2010) The role of 15-lipoxygenase-1- and cyclooxygenase-2-derived lipid mediators in endothelial cell proliferation. University of Pennsylvania, Dissertations
4. Piotrowskatomala KK, Siemieniuch MJ, Szóstek AZ et al (2012) Lipopolysaccharides, cytokines, and nitric oxide affect secretion of prostaglandins and leukotrienes by bovine mammary gland epithelial cells. Domest Anim Endocrin 43(4):278–288
5. Jagnandan D, Church JE, Banfi B et al (2007) Novel mechanism of activation of nadph oxidase 5 calcium sensitization via phosphorylation. J Biol Chem 282(9):6494–6507
6. Schönfeld P, Wojtczak L (2007) Fatty acids decrease mitochondrial generation of reactive oxygen species at the reverse electron transport but increase it at the forward transport. B.B.A.-Biomembranes 1767(8):1032–1040
7. Kurosawa T, Nakamura H, Yamaura E et al (2009) Cytotoxicity induced by inhibition of thioredoxin reductases via multiple signaling pathways: role of cytosolic phospholipase a(2)alpha-dependent and -independent release of arachidonic acid. J Cell Physiol 219(3):606–616
8. Soga M, Matsuzawa A, Igchijo H (2012) Oxidative stress-induced diseases via the ASK-1 signaling pathway[J]. Int J Biochem Cell B 2012:1–6
9. Randall MJ, Spiess PC, Hristova M et al (2013) Acrolein-induced activation of mitogen-activated protein kinase signaling is mediated by alkylation of thioredoxin reductase and thioredoxin 1. Redox Bio 1(1):265–275
10. Lin AY (1993) Cpla2 is phosphorylated and activated by map kinase. Cell 72(2):269–278
11. Gong J, Ni L, Wang D et al (2014) Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in midlactation dairy cows. Livest Sci 170(1):84–90
12. Gong J, Yan SM, Shi BL et al (2015) Establishment of oxidative damage model of bovine mammary epithelial cells induced by dinitrochlorobenzene inhibiting thioredoxin reductase activity. Chin J Anim Nutr 27(12):3984–3990 (In Chinese)
13. Guo Y.M., Gong J., Shi B.L., et al. (2015). Effects of selenium on the selenoproteins synthesis and antioxidant parameters of bovine mammary epithelial cells. 4th International Symposium on Dairy Cow Nutrition and Milk Quality
14. Kim SH, Johnson VJ, Shin TY et al (2004) Selenium attenuates lipopolysaccharide-induced oxidative stress responses through modulation of p38 mapk and nf-kappab signaling pathways. Exp Biol Med 229(2):203–213
15. Cai W, Zhang L, Song Y et al (2012) Small molecule inhibitors of mammalian thioredoxin reductase. Free Radic Biol Med 52(2):257–265
16. Choi H, Tostes RC, Webb RC (2011) Thioredoxin reductase inhibition reduces relaxation by increasing oxidative stress and s-nitrosylation in mouse aorta. J Cardiovasc Pharmacol 58(5):522–527
17. Camps M, Nichols A, Arkinstall S (2000) Dual specificity phosphatases: a gene family for control of map kinase function. FASEB J 14(1):6–16
18. Cao YZ, Maddox JF, Mastro AM et al (1992) Selenium deficiency alters the lipoxygenase pathway and mitogenic response in bovine lymphocytes. J Nutr 122(11):2121–2127
19. Straif D, Werz O, Kellner R et al (2000) Glutathione peroxidase-1 but not -4 is involved in the regulation of cellular 5-lipoxygenase activity in monocytic cells. Biochem J 349(Pt 2):455–461