Protective Effect of Selenium on Lipopolysaccharide-Induced Oxidative Damage in Dairy Cow Mammary Epithelial Cells (Postprint)

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Abstract

This study aimed to investigate the protective effect of selenium (Se) on lipopolysaccharide (LPS)-induced oxidative damage in bovine mammary epithelial cells (BMEC) and its underlying mechanism. Third-passage BMEC in adherent culture were randomly divided into 8 groups, with 6 replicates per group and one culture well per replicate. The control (CON) group was cultured in basal culture medium without Se and LPS for 30 h; the LPS group and six Se protection groups were supplemented with different levels of Se (0, 10, 20, 50, 100, 150, and 200 nmol/L) in basal culture medium, and after 24 h of culture, 1 g/mL LPS was added as an exogenous stimulus for 6 h. The results showed: 1) Compared with the CON group, the relative proliferation rate of BMEC in the LPS group decreased significantly ($P<0.05$), the activities of glutathione peroxidase (GPx), thioredoxin reductase (TrxR), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) all decreased significantly ($P<0.05$), and the gene and protein expression levels of GPx1 and TrxR1, as well as selenoprotein P (SelP) content, were also significantly downregulated ($P<0.05$); whereas the nitric oxide (NO) content, inducible nitric oxide synthase (iNOS) activity and its gene and protein expression levels, the contents and gene expression levels of inflammatory cytokines tumor necrosis factor- (TNF-), interleukin-1 (IL-1), and interleukin-6 (IL-6), reactive oxygen species (ROS) activity, and malondialdehyde (MDA) content all increased significantly ($P<0.05$) in the LPS group, and the gene expression levels of mitogen-activated protein kinase (MAPK) signaling pathway-related factors p38 mitogen-activated protein kinase (p38MAPK), c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase 1/2 (ERK1/2) showed similar changes. 2) Compared with the LPS group, with increasing Se supplementation levels in the Se protection groups, the relative proliferation rate, activities of T-SOD, CAT, GPx, and TrxR, T-AOC, and gene and protein expression levels of GPx1 and TrxR1 all showed

a trend of increasing first and then decreasing; whereas NO content, iNOS activity and its gene and protein expression levels, contents and gene expression levels of inflammatory cytokines TNF- , IL-1, and IL-6, gene expression levels of MAPK signaling pathway-related factors ERK1/2, JNK, and p38MAPK, ROS activity, and MDA content showed a trend of decreasing first and then increasing; Se at 20–100 nmol/L exhibited better protective effects, and overall, 50 nmol/L Se showed the best protective effect. The results suggest that Se can enhance the antioxidant function of BMEC and exerts a protective effect against LPS-induced cellular oxidative damage, the mechanism being that Se enhances TrxR activity thereby inhibiting the activation of the MAPK signaling pathway and ultimately reducing the massive release of NO, but excessive levels of Se can cause cellular damage. Se at 20–100 nmol/L in the culture medium exhibited better protective effects, with 50 nmol/L Se being the most effective.

Full Text

Protective Effects of Selenium on Bovine Mammary Epithelial Cells Oxidative Damaged by Lipopolysaccharide

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Abstract

This experiment was conducted to investigate the protective effects of selenium (Se) on bovine mammary epithelial cells (BMEC) oxidative damaged by lipopolysaccharide (LPS) and the underlying mechanism. Third-generation BMEC were randomly divided into 8 groups with 6 replicates per group and 1 culture well per replicate. The control (CON) group was cultured in basal medium without Se or LPS for 30 h. The LPS group and six Se protection groups were cultured in basal medium supplemented with different Se levels (0, 10, 20, 50, 100, 150, and 200 nmol/L) for 24 h, followed by treatment with 1 μg/mL LPS for 6 h as an exogenous stimulus. The results showed: 1) Compared with the CON group, the LPS group exhibited a significantly decreased relative proliferation rate ($P<0.05$), reduced activities of glutathione peroxidase (GPx), thioredoxin reductase (TrxR), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) ($P<0.05$), and downregulated gene and protein expression of GPx1 and TrxR1 as well as selenoprotein P (SelP) content ($P<0.05$). Conversely, the LPS group showed significantly elevated nitric oxide (NO) content, inducible nitric oxide synthase (iNOS) activity and its gene and protein expression, inflammatory cytokines [tumor necrosis factor- (TNF-), interleukin-1 (IL-1), and interleukin-6 (IL-6)] content and their gene expression, reactive oxygen species (ROS) activity, and malondialdehyde (MDA) content ($P<0.05$). Gene expression of mitogen-activated protein kinase (MAPK)

signaling pathway-related factors [p38 MAPK, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase 1/2 (ERK1/2)] showed similar changes. 2) Compared with the LPS group, Se protection groups showed dose-dependent effects: relative proliferation rate, T-SOD, CAT, GPx, and TrxR activities, T-AOC, and GPx1 and TrxR1 gene and protein expression increased initially and then decreased with increasing Se supplementation, while NO content, iNOS activity and expression, inflammatory cytokines (TNF- , IL-1, IL-6) content and expression, MAPK pathway-related gene expression, ROS activity, and MDA content decreased initially and then increased. Se concentrations of 20–100 nmol/L demonstrated better protective effects, with 50 nmol/L Se showing the optimal protection overall. These results indicate that Se enhances BMEC antioxidant function and protects against LPS-induced oxidative damage by increasing TrxR activity, thereby inhibiting MAPK signaling pathway activation and ultimately reducing excessive NO release. However, excessively high Se levels can cause cellular damage. Se at 20–100 nmol/L in culture medium provides effective protection, particularly at 50 nmol/L.

Keywords: selenomethionine; lipopolysaccharide; bovine mammary epithelial cell; oxidative damage; protective effect

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Bovine mammary epithelial cells (BMEC) are the primary site for milk component synthesis and secretion, and their biosynthetic capacity determines the lactation potential of the mammary gland. During lactation, BMEC accumulate large amounts of oxygen free radicals due to high metabolic rates, which is a major cause of cellular oxidative damage, reduced antioxidant function, and consequent metabolic diseases such as mastitis and decreased milk quality. Selenium (Se), a trace element, exerts its biological functions primarily through selenoprotein synthesis and significantly promotes antioxidant and immune functions in dairy cows. Previous studies have indicated that appropriate Se supplementation above dietary recommendations can further enhance antioxidant function, though the underlying mechanisms remain unclear. Therefore, in-depth investigation of Se' s alleviating effects on mammary oxidative stress and its mechanisms is theoretically and practically significant for scientific Se supplementation, ensuring mammary health, and improving milk quality.

Nitric oxide (NO) is a gaseous signaling molecule generated through multi-step redox reactions catalyzed by inducible nitric oxide synthase (iNOS) using L-arginine as substrate. Research has confirmed that NO has bidirectional regulatory functions. On one hand, appropriate NO levels participate in various physiological activities and exhibit antibacterial and antitumor effects, protecting animals from environmental infections. On the other hand, excessive NO production leads to reactive nitrogen species formation, disruption of cell signaling pathways, and induction of oxidative stress and inflammatory responses. Studies have shown that selenomethionine can downregulate interleukin-1 (IL-1) gene expression in mouse chondrocytes, thereby inhibiting excessive production of NO and prostaglandin E2. Ferret et al. reported that the thioredoxin (Trx)

system protects human mononuclear macrophages from NO-induced damage, with cellular susceptibility to NO injury negatively correlated with gene expression of thioredoxin reductase (TrxR) and Trx. Obata et al. demonstrated that p38 MAPK signaling pathway activation promotes gene expression of various cytokines including TNF- , IL-1, and IL-6 in mononuclear macrophages, and increases iNOS gene expression, resulting in excessive NO production. Apoptosis signal-regulating kinase-1 (ASK-1) is an upstream activator of the MAPK signaling pathway; when reduced Trx converts to its oxidized form, ASK-1 becomes activated, thereby activating the MAPK signaling pathway. Both in vivo and in vitro studies have shown that Se supplementation can significantly increase TrxR activity in dairy cow blood and BMEC, improve antioxidant function, and reduce oxidative damage. These findings suggest that Se may protect BMEC from oxidative damage by enhancing selenoprotein TrxR activity to regulate the MAPK signaling pathway, thereby influencing cytokine IL production and subsequently modulating NO generation. However, related research is scarce, and the exact mechanism requires further investigation. The LPS-induced mastitis model in dairy cows is internationally recognized as a primary pathological model for studying mastitis. Our research group has successfully established an oxidative stress model in BMEC using LPS as a stressor to induce excessive NO production. Therefore, this experiment used LPS to induce oxidative stress in BMEC, focusing on the relationship between TrxR activity, the MAPK signaling pathway, and NO, to investigate the protective effects of different Se levels against cellular damage and the potential mechanism, providing a theoretical basis for improving mammary antioxidant function, scientific Se supplementation, and ensuring mammary health.

1.1 BMEC Culture

BMEC were obtained using collagenase digestion. Mammary tissue was collected from three healthy cows at Beiya Halal Cold Storage in Hohhot, Inner Mongolia. The outer tissue was removed, and deep tissue samples were sequentially washed three times with phosphate-buffered saline (PBS) containing 3× antibiotics, once with 75% ethanol, and three times with PBS containing 1× antibiotics. Approximately 1 mm^3 pieces were cut from the deep tissue and placed in 5 mL centrifuge tubes. After mincing thoroughly, an equal volume of 0.5% collagenase II was added, and the mixture was digested for 1 h in a CO incubator (HF240, HealFORS) at 37°C with 5% CO . The digested solution was filtered through an 80-mesh cell strainer, and the filtrate was collected in a 15 mL centrifuge tube and centrifuged; the supernatant was discarded. The pellet was resuspended in PBS, centrifuged again, and the supernatant was discarded. The final pellet was resuspended in culture medium and seeded into 25 cm^2 culture flasks, then incubated in a CO incubator. When primary cell confluence reached 80–90%, cells were purified and passaged. After identification by fluorescence immunocytochemical staining, purified BMEC were continuously cultured to the third generation and seeded into appropriate culture vessels according to experimental design. Cells were cultured in complete medium at 37°C with 5%

CO . When cell confluence reached 80–90%, the old medium was removed and replaced with fresh serum-free medium for starvation culture for 24 h before subsequent experiments.

1.2 Experimental Design

LPS (L4391) and selenomethionine (Se source, purity 98%, S3132) were purchased from Sigma-Aldrich. An oxidative damage model was established by inducing BMEC with LPS; the treatment time and concentration were determined based on previous research by Gao Ruifeng. Third-generation BMEC were randomly divided into 8 groups with 6 replicates per group and 1 culture well per replicate. The control (CON) group was cultured in basal medium without Se or LPS for 30 h. The LPS group was cultured in basal medium for 24 h, then treated with 1 μg/mL LPS for 6 h. Se protection groups were cultured in basal medium supplemented with 10 (LSe10), 20 (LSe20), 50 (LSe50), 100 (LSe100), 150 (LSe150), or 200 nmol/L Se (LSe200) for 24 h, followed by treatment with 1 μg/mL LPS for 6 h. The basal medium contained 115,588.99 nmol/L methionine; therefore, selenomethionine supplementation had minimal impact on the basal methionine concentration.

1.3.1 Relative Proliferation Rate

BMEC relative proliferation rate was determined using the MTT assay. Third-generation BMEC suspension was seeded in 96-well plates and cultured according to experimental design. After treatment, 20 μL of MTT (5 mg/mL) was added to each well and incubated for 4 h. The supernatant was removed, plates were dried, and 100 μL of dimethyl sulfoxide was added to each well. After shaking for 10 min using a microplate reader (Synergy 4, BioTek), the optical density at 490 nm (OD) was measured. Relative proliferation rate (%) = (OD of treatment group / OD of CON group) × 100.

1.3.2 Antioxidant Function and Inflammatory Factors

Third-generation BMEC suspension was seeded in 60 mm culture dishes and cultured for 30 h according to experimental design. Cell culture supernatant was collected in 1.5 mL Eppendorf tubes and centrifuged at 15,455×g for 10 min; the supernatant was used for antioxidant function and inflammatory factor measurements. Total antioxidant capacity (T-AOC) was measured by ammonium molybdate colorimetry, T-SOD activity by xanthine oxidase method, CAT activity by colorimetry, and iNOS activity and SelP, NO, IL-1, IL-6, and TNF- contents by enzyme-linked immunosorbent assay (ELISA) according to kit instructions (R&D Systems, USA).

After removing the culture supernatant, cell culture dishes were placed on ice, washed twice with 1 mL PBS, and lysed with 1 mL animal cell lysis buffer on ice for 30 min. Adherent cells were scraped, collected in 1.5 mL Eppendorf tubes, and centrifuged at 4°C, 15,455×g for 10 min; the supernatant was used for

intracellular antioxidant function measurements. GPx activity was measured by dithiobisnitrobenzoic acid method and MDA content by thiobarbituric acid method according to kit instructions (Nanjing Jiancheng Bioengineering Institute, China). ROS and TrxR activities were measured by ELISA according to kit instructions (R&D Systems, USA). Intracellular GPx activity and MDA content were normalized to total protein content, which was determined by bicinchoninic acid (BCA) method using a kit from Beyotime Biotechnology (Beijing, China).

1.3.3 Intracellular Gene Expression

Using -actin as the reference gene, real-time quantitative PCR was used to determine relative expression of GPx1, GPx4, SelP, TrxR1, iNOS, IL-1, IL-6, TNF- , p38MAPK, JNK, and ERK1/2 genes.

1.3.4 Intracellular Protein Expression

Using -actin as the reference protein, Western blot was used to determine protein expression of GPx1, TrxR1, and iNOS in BMEC, with 3 replicates per treatment. Main procedures: 30 μg protein samples were mixed with 5× loading buffer at a 4:1 volume ratio, denatured for 5 min, and loaded onto gels. Proteins were separated by electrophoresis through stacking gel (80 V, 30 min) and separating gel (120 V, 90 min), followed by membrane transfer (100 V, 50 min). Membranes were washed three times with Tris-buffered saline with Tween (TBST) for 5 min each, blocked at room temperature for 1 h, then washed once with TBST for 5 min. Membranes were incubated overnight at 4°C with primary antibodies: rabbit anti- -actin polyclonal antibody (20536-1-AP, Proteintech, 1:2,000 dilution), rabbit anti-GPx1 polyclonal antibody (ab22604, Abcam, 1:1,000 dilution), rabbit anti-TrxR1 polyclonal antibody (ab16840, Abcam, 1:500 dilution), and rabbit anti-iNOS polyclonal antibody (NBP1-97471, Novus Biological, 1:1,000 dilution). After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (04-15-06, KPL, 1:1,000 dilution) at room temperature for 1 h. Bands were visualized using an ECL chemiluminescence kit and imaged with a gel imaging system (ImageQuant RT ECL, GE). ImageJ software was used for densitometric analysis. Target protein expression was calculated as: target protein expression = gray value of target protein / gray value of reference protein.

1.4 Data Processing and Analysis

Experimental data were analyzed for significance using analysis of variance (ANOVA) in SAS 9.0 statistical software. $P < 0.05$ was considered statistically significant, and $0.05 \quad P < 0.10$ indicated a trend toward significance.

2.1 Cell Morphology, Relative Proliferation Rate, Antioxidant Function, and Inflammatory Factor Content

As shown in and [Figure 1: see original paper], compared with the CON group, the LPS group exhibited altered cell morphology with indistinct boundaries and obvious shrinkage, a significantly decreased relative proliferation rate ($P<0.05$, reduced by 23.57%), and significantly reduced activities of T-SOD, CAT, GPx, and TrxR, T-AOC, and SelP content ($P<0.05$). Conversely, ROS and iNOS activities, NO, MDA, and inflammatory cytokines (TNF- , IL-1, and IL-6) contents were significantly increased ($P<0.05$).

Compared with the LPS group, Se protection groups LSe20, LSe50, LSe100, and LSe150 showed alleviated cell shrinkage and significantly increased relative proliferation rates ($P<0.05$), with LSe50 being the highest among Se protection groups and significantly higher than other treatment groups ($P<0.05$). LSe10 and LSe200 showed no significant changes ($P>0.05$). T-AOC, CAT, and TrxR activities in LSe20, LSe50, and LSe100 groups were significantly higher than in the LPS group ($P<0.05$), with LSe50 being the highest among Se protection groups, while LSe10, LSe150, and LSe200 showed no significant differences ($P>0.05$). GPx activity in LSe50, LSe100, and LSe150 groups was significantly higher than in the LPS group ($P<0.05$), with LSe100 being the highest among Se protection groups; LSe10, LSe20, and LSe200 showed no significant differences ($P>0.05$). All Se protection groups showed higher T-SOD activity than the LPS group, with LSe50 being the highest. SelP activity in LSe50 was significantly higher than in the LPS group ($P<0.05$), while other Se protection groups showed no significant differences ($P>0.05$). MDA content and ROS activity in all Se protection groups were significantly lower than in the LPS group ($P<0.05$), with LSe50 showing the lowest values. NO content and iNOS activity showed similar trends, with the lowest NO content in LSe100 and lowest iNOS activity in LSe50; LSe200 showed no significant differences from the LPS group ($P>0.05$). TNF- and IL-1 contents in LSe50 and LSe100 groups, and IL-6 content in LSe20 and LSe50 groups, were significantly lower than in the LPS group ($P<0.05$), while other Se protection groups showed no significant differences ($P>0.05$).

2.2 Gene and Protein Expression of Antioxidant Enzymes and Inflammatory Factors

As shown in and [Figure 2: see original paper], compared with the CON group, the LPS group showed significantly decreased gene and protein expression of GPx1 and TrxR1 ($P<0.05$), while iNOS gene and protein expression, inflammatory cytokines (TNF- , IL-1, and IL-6), and MAPK signaling pathway-related factors (p38MAPK, JNK, ERK1/2) showed the opposite pattern with significantly increased expression ($P<0.05$).

Compared with the LPS group, GPx1 and TrxR1 gene expression in LSe20, LSe50, and LSe100 groups, and SelP gene expression in LSe50, LSe100, LSe150,

and LSe200 groups were significantly increased (P<0.05), with LSe50 showing the highest TrxR1 and SelP expression among Se protection groups. GPx1 and TrxR1 protein expression in all Se protection groups was significantly higher than in the LPS group (P<0.05), with the highest values also in LSe50. IL-1 gene expression and iNOS gene and protein expression in LSe20, LSe50, and LSe100 groups were significantly lower than in the LPS group (P<0.05), with LSe50 showing the lowest values among Se protection groups; however, IL-1 and iNOS gene expression in LSe200 was significantly increased (P<0.05). TNF- gene expression in all Se protection groups was significantly lower than in the LPS group (P<0.05), with LSe50 showing the lowest value. MAPK signaling pathway-related gene expression showed similar trends: p38MAPK (LSe20, LSe50, LSe100, LSe150) and ERK1/2 (LSe20, LSe50, LSe100) gene expression were significantly lower than in the LPS group (P<0.05), with LSe50 showing the lowest values among Se protection groups. JNK gene expression in all Se protection groups was significantly lower than in the LPS group (P<0.05), with LSe100 showing the lowest value.

3.1 LPS-Induced Oxidative Damage in BMEC

Cell relative proliferation rate, antioxidant enzyme activities, and inflammatory factor contents are primary indicators of cellular oxidative stress status. Under normal conditions, antioxidant enzymes scavenge excess free radicals, maintaining them at physiological levels for signal transduction. However, when cells are exposed to exogenous stimuli such as LPS, large amounts of free radicals are generated and accumulate, directly damaging DNA, proteins, and organelles, promoting apoptosis, and initiating inflammatory mediator production. In this study, LPS induction decreased relative proliferation rate by 23.57% compared with the CON group, significantly reduced activities of T-SOD, GPx, TrxR, and CAT, while significantly increasing inflammatory cytokines (TNF- , IL-1, IL-6) content and expression, MDA content, ROS activity, NO content, iNOS activity and expression, indicating that LPS induced oxidative damage in BMEC. Studies have reported that LPS is recognized by Toll-like receptor 4, transmitting signals to the cytoplasm and activating the MAPK signaling pathway. p38 pathway activation increases iNOS gene expression, producing excessive NO, which leads to reactive nitrogen species formation, disruption of cell signaling pathways, and uncontrolled systemic inflammation, causing tissue damage. MAPK pathway activation can induce NF- B pathway activation, which promotes massive inflammatory cytokine production, leading to excessive NO release. In turn, excessive NO induces ERK1/2, p38MAPK, and JNK activation and further stimulates NF- B activation, creating a vicious cycle that ultimately results in inflammatory responses and tissue damage. Thus, LPS-induced oxidative damage occurs through MAPK pathway activation, inflammatory cytokine production, iNOS overexpression, and excessive NO generation. This study found that LPS increased inflammatory cytokine content and expression while activating the MAPK pathway with significantly elevated expression of p38MAPK, JNK, and ERK1/2, further confirming that LPS-induced oxidative damage in BMEC is

associated with excessive NO production.

3.2 Protective Effects and Mechanisms of Se Against LPS-Induced Oxidative Damage

This study demonstrated that Se protection groups showed increased TrxR and GPx activities, upregulated TrxR1 and GPx1 gene and protein expression, decreased MDA content, ROS activity, NO content, iNOS gene and protein expression, and downregulated inflammatory cytokine content and expression compared with the LPS group, indicating that Se improves cellular antioxidant function and protects BMEC from LPS-induced oxidative damage. As a component of antioxidant enzymes such as TrxR and GPx, Se exerts antioxidant protective effects by scavenging free radicals and preventing oxidative stress reactions in macromolecules. Therefore, Se' s protective mechanism against BMEC oxidative damage is related to enhanced selenoprotein activity. Excessive NO production is a primary cause of cellular damage. Zhao et al. reported that Se deficiency significantly reduced TrxR gene expression while increasing iNOS gene expression and NO content in chicken pancreas. Studies have shown that pro-inflammatory cytokine IL-1 can induce increased iNOS activity and expression, promoting massive NO synthesis and exacerbating inflammation. Research in mouse macrophages indicated that Se protects against oxidative stress by reducing inflammatory cytokine gene and protein expression, decreasing cytokine release, and improving cellular antioxidant and anti-inflammatory functions. This study in BMEC yielded similar results: Se protection groups reduced TNF- , IL-1, and IL-6 content and expression, and combined with changes in antioxidant function, free radical content, and cell viability, demonstrated that Se' s protective effect against BMEC oxidative damage is associated with reduced inflammatory cytokine expression, decreased iNOS activity, and reduced NO generation.

In inflammatory responses, increased expression of cytokines IL-1 , IL-6, and TNF- is associated with enhanced MAPK signal transduction. Se may regulate cytokine expression and restore immune homeostasis by modulating selenoprotein expression to inhibit MAPK pathways involved in cell signal transduction. The MAPK family includes p38MAPK, JNK, and ERK, which are activated by corresponding upstream kinases. ASK-1 is an important MAPK family member and upstream activator of the MAPK pathway; reduced Trx binds to the N-terminal region of ASK-1, inhibiting its activity. When Trx is converted to its oxidized form due to decreased TrxR activity, ASK-1 becomes activated, thereby activating JNK and p38MAPK in the MAPK pathway. In vitro macrophage studies have shown that LPS induces MAPK pathway activation, increasing ROS and NO production, while Se supplementation significantly reduces LPS-induced H O generation and p38 activation, decreasing proinflammatory factors such as NO. Liu Chen established an NO toxicity model using Trx-transfected mouse neuroblastoma cells to study the relationship between Trx expression and NO, finding that Trx protects cells from NO-induced

damage, possibly because Trx S-subunits can bind NO, reducing NO content, scavenging oxygen free radicals, and improving cellular antioxidant capacity. Our previous research indicated that Se supplementation significantly enhances TrxR activity in dairy cow blood, promoting antioxidant function. This study found that Se protection groups showed upregulated TrxR activity and TrxR1 gene and protein expression, while downregulating MAPK pathway-related gene expression, suggesting that after LPS injury, Se may protect BMEC from oxidative damage by upregulating selenoprotein TrxR1 expression, increasing TrxR activity, inhibiting MAPK pathway activation, suppressing IL-1 production, reducing iNOS activity, and decreasing NO release. However, this study did not perform TrxR1 gene silencing or overexpression, requiring further validation.

Appropriate Se levels can promote cell proliferation and enhance antioxidant and immune functions, whereas high Se levels inhibit cell proliferation, weaken antioxidant and immune functions, and may even cause pathological damage. This study also found that Se' s protective effect against BMEC oxidative stress is dose-dependent: 10 nmol/L Se showed no significant protective effect, 20–100 nmol/L Se demonstrated better protection, with 50 nmol/L Se showing optimal effects. However, as Se levels increased further, protective effects gradually diminished; notably, 200 nmol/L Se not only lacked protective effects but also caused cellular damage. This may be because Se can substitute for sulfur atoms in sulfur-containing compounds, inhibiting various enzymes and sulfur-containing amino acids while suppressing antioxidant processes, resulting in toxicity. Se toxicity may also result from disruption of normal metal elements and trace elements, which in turn affects metabolic pathways and cascade reactions, accompanied by inflammatory responses, ultimately leading to oxidative stress. Although Se has protective effects, its toxic range is narrow, with toxic doses approximately 3–5 times the nutritional requirement. Therefore, reasonable control of Se supplementation levels is a prerequisite for Se to exert its antioxidant and anti-inflammatory effects.

Conclusions

1. Se improves BMEC antioxidant function and protects against LPS-induced oxidative damage by enhancing TrxR activity, thereby inhibiting MAPK signaling pathway activation and ultimately reducing excessive NO release. However, excessively high Se levels can cause cellular damage.
2. Se concentrations of 20–100 nmol/L in culture medium provide effective protection, with 50 nmol/L Se showing the optimal effect.

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Note: Figure translations are in progress. See original paper for figures.