The pre-protective effect of vitamin A on LPS-induced oxidative stress of bovine mammary epithelial cells

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ABSTRACT
All-trans retinoic acid (RA, derivative of vitamin A) has been demonstrated to have anti-oxidative activity and is needed for the proper function of the immune system. In this study, the protective effects of RA against lipopolysaccharide (LPS)-induced oxidative stress on bovine mammary epithelial cells (BMECs) have been investigated. BMECs were divided into four equal groups with six replicates. The first group was used as a control without RA or LPS. The three experimental groups were treated with RA (1 μg/mL) alone, LPS (1 μg/mL) alone and RA plus LPS, respectively. The cells were treated for 30 h in all. Our results showed that cells in LPS-treated group have a significantly higher inflammatory cytokines concentration and NF-κBp50 and NF-κBp65 gene expression than the control (p < .01). Furthermore, compared with control, selenoproteins’ activity of glutathione peroxidase (GPx), thioredoxin reductase (TrxR), the concentration of SelP and the gene expression of Nrf2 were significantly decreased while the activity of inducible nitric oxide synthase (iNOS), the production of intracellular reactive oxygen species (ROS) and malonaldehyde (MDA) were elevated dramatically in LPS-treated group (p < .01). However, RA in combination with LPS enhanced the antioxidant enzyme activities of selenoprotein compared to LPS-treated group. In conclusion, RA ameliorated the LPS-induced BMECs damage by improving levels of antioxidant markers and reducing inflammatory cytokines contents, indicating that RA has the potential to counter measure the immunosuppressive condition on BMECs oxidative damage and to improve antioxidative function. However, further studies are still required to elucidate the molecular mechanisms underlying the protective effects of RA as an antioxidant.

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INTRODUCTION
The high-yielding dairy cattle is easier to experience the oxidative stress resulted from metabolic reactions, which lead to the decreased antioxidant function, milk production and quality, and the increased loss of economy (Sordillo and Aitken 2009). It was reported that supplementation of diet with higher doses of vitamin A (VA, 220 U/kg BW) higher than the current recommended dose (110 U/kg BW) could enhance selenoprotein glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) activity in dairy cows (Jin et al. 2014) and RA increased mRNA expression of GPx1 and TrxR1 in bovine mammary cells, indicating that VA might improve cell antioxidant capacity by regulating the biosynthesis of these selenoproteins (Bruzelius et al. 2010). Similar results were obtained from our previous study (Jin et al. 2016). But there are limited references in terms of the mechanism of improving antioxidant function with RA.

Nitric oxide (NO) induced by inducible nitric oxide synthase (iNOS) is a highly reactive free radical, and can be viewed as pro-inflammatory and cytotoxic agent under high concentration (Kielbik et al. 2013). Pro-inflammatory cytokine interleukin-1 (IL-1) is critical for the activation of iNOS in human astrocytes (Tarassishin et al. 2014), and typically, NF-κB induces gene expression of large number of immune molecules, including IL-1 (Kabe et al. 2005). In addition, numerous studies have also demonstrated that Nrf2 is critical for the activation of the cellular glutathione system and maintaining the redox state (Maes et al. 2012). Li et al. (2001) demonstrated that GPx1 over-expression could down-regulate NF-κB DNA binding and transcriptional activity. Specific antioxidant enzymes, such as GPx1 have strong inhibitory effects on IL-1-induced signal transduction in MCF-7 cells (Qiang and Engelhardt 2006). Our previous study indicated that vitamin A could decrease NO-induced...
oxidative stress by downregulating IL-1 content and increasing activities of selenoprotein GPx1 (Shi, Yan, et al. 2016). Therefore, we speculate that the protective mechanisms of RA on oxidative damage in BMECs can probably attribute to its inhibition effect on IL-induced iNOS protein expression, thus preventing NO from overproduction by improving GPx1 activity. However, there is little information on the study in BMECs. Certainly, the relationship between GPx1, NF-kB and Nrf2 is necessary for us to study in the future.

Talwar et al. (2017) found IL-1β production in response to LPS activation. It is well known that lipopolysaccharide (LPS), typically increases the production of pro-inflammatory cytokines in immune cells and promotes iNOS/NO biosynthesis (Pei and Wang 2014). Our previous research has established LPS-induced oxidative stress model of BMECs (Shi, Guo, et al. 2016). Therefore, the aim of this study was to investigate the pre-protective effect of RA on LPS-induced oxidative stress of BMECs, discussing the mechanism of RA to protect BMECs from oxidative injury by improving antioxidant capacity.

Material and methods

The protocol of the present experiment was approved by the Animal Care and Use Committee, Inner Mongolia Agricultural University, Hohhot, China.

Isolation of mammary epithelial cells

Bovine primary cells were isolated from the mammary glands of Holstein dairy cows at a local abattoir and cultured as previously described (Dheen et al. 2005). Briefly, several pieces of approximately 1 cm³ mammary gland tissues were removed aseptically, then washed with cold phosphate buffered solution (PBS) containing 100 U/mL penicillin and 100 µg/mL streptomycin. The mammary tissue fragments were minced with sterile scissors and digested by collagenase II (GIBCO, Grand Island, NY) at 37°C/14°C and 5% CO₂ for 1 h with shaking every 20 min. The digesta were filtered through 200 µm nylon mesh to remove large tissue fragments and the filtered liquid was centrifuged at 179 g for 5 min and the supernatant liquid was removed. The cell pellet was resuspended in the culture medium contained Dulbecco’s modified Eagle’s medium/F12 (DMEM/F12) (GIBCO) media supplemented with 10% foetal bovine serum (FBS) (GIBCO), 0.5% insulin, transferrin and sodium selenite solution (ITS Liquid Media Supplement, GIBCO), 4 µg/mL prolactin (Sigma, St. 96 Louis, MO), 1 µg/mL hydrocortisone (Sigma), 100 U/mL penicillin, and 100 µg/mL streptomycin (GIBCO) under 5% CO₂ and air at 37°C.

Cell proliferation assay

The proliferation potential of cultured cells was determined by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrasodiumbromide) assay. After treatment groups, the cells in 96-well plates washed twice with PBS, and then 20 µL MTT (5 mg/mL of PBS) was added to each well. The cells were then incubated at 37°C for 4 h, and DMSO (100 µL) was added to dissolve the formazan crystals and shaked 10 min. The optical density (OD) of each well at 490 nm was measured in a microplate reader (Bio-Tek, Vermont).

RA preparation and experimental design

The concentrations of RA in this study were chosen according to the one resulting after a dose response test on BMECs and the optimal RA dose was 1 µg/mL (Jin et al. 2016). Stock RA solution was prepared as follows: 100 mg of RA was dissolved in 5 mL dimethyl sulfoxide (DMSO) to attain the concentration of 20 mg/mL and dissolved in DMEM/F12 to obtain the final concentration of 1 µg/mL and then sterile-filtered; 1 mg LPS (Sigma) was prepared in DMEM/F12 at room temperature to reach the concentration of 1 µg/mL. The resulting solution was added to cell culture medium to get the desired concentration of 1 µg/L and then sterile-filtered before the experiments. The two working solutions were stored at −4°C before use. The BMECs were subjected to the following treatments. Cell plates were randomly divided into four groups including the control, RA-treated group, LPS-treated group and RA + LPS-treated together group. Each treatment was replicated six times. The control was treated without RA and LPS. The RA-treated group was treated with RA alone for 30 h; LPS-treated group were treated without RA for 24 h before treated with LPS for 6 h; the RA in combination with LPS treated group was administered LPS for 6 h after RA for 24 h. The LPS concentration (1 µg/L) and its reaction time (6 h) and the VA concentration (1 µg/mL) and its reaction time (30 h) were performed as pre-test (Jin et al. 2016; Shi, Guo, et al. 2016).
**NO, IL-1, IL-6, TNF-α, ROS, MDA content determination**

BMECs culture media was harvested after incubation with different treatment for 30 h for analysis the production of supernatant NO, IL-1, IL-6, TNF-α (RD, Minnesota) by Elisa, following their respective manufacturer’s instructions and using standard curves. Colour changes were determined at 450 nm. The inter-assay coefficient of variation was lower than 11%, and the intra-assay coefficient of variation was lower than 9%. Then, after washed twice in ice-cold PBS, the cells were lysed using 80807A-50 (TIANDZ, Beijing, China) and centrifuged at 1200 g for 10 min at 4 °C to evaluate the contents of intracellular ROS and MDA using the appropriate biochemical kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions.

**GPx, TrxR, SelP, iNOS activity determination**

The BMECs were harvested after incubation with different treatment groups for 30 h. The levels of SelP and iNOS activity in supernatant were detected by commercially available kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s protocol. Then, BMECs washed in twice in ice-cold PBS. Subsequently, the cells were treated with lysis buffer and incubated for 20 min in an ice bath. The lysate was centrifuged for 10 min at 1200 g at 4 °C, and the antioxidant parameters activities of GPx and TrxR in the supernatant were measured using a ELISA by commercial kits according to their respective manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), expect the iNOS (RD, San Francisco).

**RNA extraction and real-time PCR**

Total BMECs RNA was extracted with RNAprep pure Cell Kit (TIANGEN, Beijing, China) according to the supplier’s protocol. The RNA concentration was determined using microplate reader (Synergy H4 BioTek, Vermont) before reverse transcription polymerase chain reaction (RT-PCR). Approximately, 2 µL of total RNA was reverse-transcribed to cDNA in 20-µL reactions using MxPro-Mx3000P (Agilent, Beijing, China). Real-time PCR machine according to the manufacturer’s instructions. The primers were designed using Oligo software (National Biosciences, Plymouth, MN) and custom-synthesized (Sangon Biological Technologies, Shanghai, China). The mRNA expression levels were estimated by real-time PCR analysis using a Bio-Rad iCycler IQ5 detector system (Perkin Elmer-Applied Biosystems, Foster city, CA). The conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing temperatures at 60 °C for 30 s, extension at 72 °C for 20 s. Fold increment of an assayed gene was calculated by normalising its expression level to that of the glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene, which was used as an internal control. Each gene analysis was performed in six replications. Primer’s sequences of the targeted genes were listed in Table 1.

**Statistical analyses**

The data were analysed statistically with an ANOVA procedure according to the General Linear Model procedure of SAS software (SAS Version 9.0, SAS Institute, Cary, NC, SAS Institute 2002). Differences between the mean values of the different groups were determined using Tukey’s post hoc test. Data are presented in the tables as means with their standard errors of the mean. A value of $p < .05$ was regarded as significant, whereas difference were considered to be a statistical trend when $.05 < p < .10$.

**Results**

**Effects of RA on LPS-stimulated BMECs proliferation**

The effect of RA on BMECs proliferation was evaluated using the MTT assay. As shown in Figure 1, cells proliferation in RA-treated group was significantly higher than the control $(p < .01)$. After exposure to LPS alone, a markedly decrease occurred compared with the control $(p < .01)$. However, RA in combination with LPS significantly improved BMECs proliferation compared with the LPS group $(p < .01)$.

**Effect of RA on LPS-induced oxidation markers ROS, MDA, NO and proinflammatory cytokines concentration and their mRNA expression in BMECs**

Results showed that there were no significant effects of RA-treated group on ROS, MDA, NO, IL-1, IL-6, TNF-α production, as well as IL-1β, IL-6, TNF-α, NF-κBp50 and NF-κBp65 gene expression (Table 2) compared to control. The addition of LPS alone significantly increased indicators above than control group $(p < .01)$. However, there were a significant down-regulation in those oxidation markers $(p < .01)$ in LPS-stimulated BMECs which were pre-treated with the RA compared to LPS-treated
group, as well as the gene expression levels of IL-1β, IL-6, TNF-α, NF-jBp50 and NF-jBp65.

**Effect of RA on LPS-induced activation of iNOS, GPx, TrxR, SelP, Nrf2 and their mRNA levels**

As shown in Table 3, expression of iNOS, the enzyme responsible for NO production, was investigated in BMECs. In response to LPS-treated group, the activity and mRNA level of iNOS increased dramatically in mammary epithelial cells compared to the control. However, there is no significant difference in those indicators when BMECs incubated with RA compared with the control. Other parameters on mRNA expression such as GPx1, TrxR1 and SelP were significantly higher in RA-treated group than the control group except GPx4 and Nrf2, and activities of GPx and TrxR and SelP content were increased in RA-treated group than the control significantly. Results showed a significant decrease (p < 0.01) in selenoprotein enzymes activities of GPx and TrxR and SelP concentration in LPS-treated group compared to the control, and mRNA expression of TrxR, SelP and Nrf2 were significantly lower than control. In addition, the activity and gene expression of iNOS were increased dramatically in LPS-treated group. RA + LPS-treated group did significantly increased (p < 0.01) in activities of selenoprotein enzymes of GPx and TrxR and SelP content and their levels of mRNA expression and gene expression of Nrf2 except GPx4 gene expression compared to LPS-treated group. Surprisingly, iNOS activity and gene expression were markedly lower (p < 0.0001) in RA + LPS-treated group as displayed in Table 3 than LPS-treated group.

**Discussion**

Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms (Cadenas 1997), which caused a great deal of attention towards the field of antioxidants. Antioxidants have become of interest to scientists because they can protect the body from oxidative stress by eliminating free radicals. Reactive nitrogen...
species (RNS, e.g. NO) as one of kind of main free radicals during normal cellular metabolism playing a dual role to the body (Valko et al. 2006). Our previous studies have shown that overproduction of NO can indeed cause oxidative damage (Shi, Yan, et al. 2016). The results of Banan et al. (2000) and Valko et al. (2007) view are consistent with our findings. INOS up-regulation is predicted to lead to NO overproduction (Unno et al. 1997). The enzymatic antioxidant defence system includes mainly SOD, CAT, GPx and TrxR, and this system protects cells against ROS toxicity, NO damage and lipid peroxidation. MDA is a main product of lipid peroxidation (Hawkes and Alkan 2010). LPS is derived from the gram-negative bacteria cell wall, always used to build animal models due to its potent bioactivator of the immunological system. Especially in innate immunity, LPS activates the epithelial cells and alveolar macrophages to release numerous inflammatory mediators, such as IL-1β, TNF-α (Li et al. 2015). Therefore, LPS is an inducer specifically used to induce inflammatory factors including IL-1 in the study of inflammation (Zhang et al. 2014). The present results showed that oxidation markers ROS, MDA, NO and proinflammatory cytokines concentration were significantly higher after LPS stimulation, indicating that LPS induced the cellular oxidative stress. But, the addition of RA in advance ameliorated the LPS-induced BMECs damage by up-regulating the antioxidant enzyme activities of GPx and TrxR significantly. These results suggested that RA had the potential to counter measure the oxidative damage of BMECs and to improve antioxidative function. But the mechanism is not clear.

| Table 2. Effects of RA on LPS-induced oxidation markers ROS, MDA, NO and proinflammatory cytokines concentration and mRNA expression in BMECs. a |
| Parameters b | Control | RA | LPS | RA + LPS | SEM c | p value |
| Cytokines | | | | | | |
| ROS, fluorescence intensity/mL | 149.03 b | 131.15 c | 169.13 a | 155.28 b | 4.348 | <.0001 |
| MDA, nmol/mgPr | 1.31 b | 1.08 c | 3.77 a | 2.69 b | 0.208 | <.0001 |
| NO, umol/L | 16.33 b | 20.00 a | 42.67 a | 27.13 c | 1.049 | <.0001 |
| L-1, ng/mL | 11.37 b | 12.20 c | 43.67 a | 30.03 b | 0.628 | <.0001 |
| IL-6, pg/mL | 18.23 b | 18.06 c | 42.62 a | 28.42 b | 0.642 | <.0001 |
| TNF-α, ng/mL | 36.00 b | 50.50 a | 147.50 a | 71.67 b | 4.877 | <.0001 |
| Gene expression | | | | | | |
| IL-1β | 1.01 c | 1.09 c | 2.78 a | 1.92 b | 0.170 | <.0001 |
| IL-6 | 1.02 c | 1.31 c | 3.71 a | 2.19 b | 0.116 | <.0001 |
| TNF-α | 1.04 c | 1.47 c | 7.03 a | 3.20 b | 0.322 | <.0001 |
| NF-kB50 | 1.02 c | 0.99 c | 1.46 a | 1.17 b | 0.064 | <.0001 |
| NF-kB65 | 1.05 b | 1.10 b | 1.71 a | 1.14 b | 0.091 | <.0001 |

aBMECs: bovine mammary epithelial cells; LPS: lipopolysaccharide; RA: retinoic acid.
bControl: control-treated group without RA and LPS; IL-1: interleukin-1; IL-1β: interleukin-1β; IL-6: interleukin-6; LPS: LPS-treated group; MDA: malondialdehyde; NF-kB50: nuclear factor kB50; NF-kB65: nuclear factor kB65; ROS: reactive oxygen species; RA: RA-treated group; NO: nitric oxide; RA + LPS: RA plus LPS-treated group; TNF-α: tumour necrosis factor-alpha.
cSEM: standard error of the mean. Means in the same row not followed by the same letter differ significantly (p < .05).

| Table 3. Effects of RA on LPS-induced antioxidant indicators GPx, TrxR, SelP and iNOS activities and mRNA expression in BMECs. a |
| Parameters b | Control | RA | LPS | RA + LPS | SEM c | p value |
| Antioxidant indicators | | | | | | |
| GPx, U/mgPr | 129.42 b | 182.40 a | 94.99 c | 138.37 b | 9.940 | .0032 |
| TrxR, U/gPr | 2.79 b | 3.47 a | 2.15 b | 2.69 b | 0.119 | <.0001 |
| SelP, mg/L | 0.59 b | 0.77 a | 0.44 b | 0.66 b | 0.030 | <.0001 |
| INOS, U/mL | 14.25 c | 16.50 b | 30.67 a | 26.64 b | 0.488 | <.0001 |
| Gene expression | | | | | | |
| GPx1 | 1.02 b | 2.22 b | 0.68 b | 1.46 b | 0.129 | <.0001 |
| GPx4 | 1.01 a | 1.07 a | 0.89 a | 0.86 a | 0.081 | .2593 |
| TrxR1 | 1.05 b | 1.65 b | 0.63 b | 1.02 b | 0.103 | <.0001 |
| SelP | 1.03 b | 1.38 b | 0.59 b | 0.98 b | 0.093 | <.0001 |
| INOS | 1.07 c | 1.33 b | 4.92 a | 2.71 b | 0.353 | <.0001 |
| Nrf2 | 1.10 c | 1.57 b | 0.58 b | 1.75 a | 0.091 | <.0001 |

aBMECs: bovine mammary epithelial cells; LPS: lipopolysaccharide; RA: retinoic acid.
bControl: control-treated group without RA and LPS; GPx: glutathione peroxidase; GPx1: glutathione peroxidase1; GPx4: glutathione peroxidase4; LPS: LPS-treated group; INOS: inducible nitric oxide synthase; Nrf2: nuclear factor E2-related factor 2; RA: RA-treated group; RA + LPS: RA plus LPS treated group; SelP: selenoprotein P; TrxR: thioredoxinreductase; TrxR1: thioredoxinreductase1.
cSEM: standard error of the mean. Means in the same row not followed by the same letter differ significantly (p < .05).
Stimulating with LPS can up-regulate the production of NO (Pei and Wang 2014). Okada et al. (1999) found that LPS increased IL-1 production from mammary cells in dose-dependent manner and IL-1-induced expression of iNOS and production of NO have been inhibited by RA in human chondrocytes in the research of Huang et al. (2008). Our present research indicated that the activity and gene expression of iNOS and NO production were down-regulated in RA+LPS-treated group compared with LPS-treated group, as well as the transcriptional level of IL-1β, suggesting that RA decreased damage induced from LPS may be due to decreased IL production which lead to the decreased NO production finally. In addition, it is well known that LPS can activate NF-κB signalling pathway, which lead to the release of inflammatory cytokines including IL-1 (Wang et al. 2017). NF-κB belongs to the Rel family of transcriptions factors, which is sensitive to redox status (Baldwin 1996). The NF-κB is normally maintained in the cytoplasm with the inhibitor of NF-κB (IκB) molecule. Following a variety of extracellular stimuli such as LPS, genotoxic and oxidative stress, the IκB kinase (IKK) signalosome is activated. Then, the activated IKK phosphorylates and degrades IκB. As a consequence, the activated NF-κB complexes usually composed of p50 and p65 and translocated to the nucleus binds to the promoter region of the target genes and induced their expression (Hayden and Ghosh 2009). Therefore, the present results supported that the pre-protective effect of vitamin A on LPS-induced oxidative stress of BMECs may be associated with the inhibited NF-κB and decreased NO production.

Pivotal to antioxidant response is the transcription factor Nrf2. Under basal conditions, Nrf2 is mainly found sequestered in the cytosol as an inactive complex bound to a repressor molecule known as Kelch-like ECH-associating protein (Keap1). Oxidants can induce Nrf2 releasing from Keap1. The free Nrf2 translocates to the nuclear protein and binds to the antioxidant response element (ARE) sequences of the target gene promoters in conjunction with small Maf protein, result in up-regulation of antioxidant and phase II detoxifying enzymes, such as SOD, GPx and haem oxygenase-1 (HO-1) (Chen and Kunsch 2004). Nikulina et al. (2000) concluded that GSH, which is a cosubstrate for GPx, regulated IL-1-induced NO production in islets, purified cells and insulinoma cells by modulation of iNOS gene expression. It was also reported that selenoproteins could block NO and prostaglandin E2 (PGE2) production through modulation of iNOS and cyclooxygenases-2 (COX-2) gene expression in primary chondrocytes (Sanmartin et al. 2011). Li et al. (2001) found that GPx1 inhibits the activity of IKKα. Tran et al. (2017) suggest that Phencyclidine treatment activates nuclear translocation of NF-κB p65 and its DNA binding activity in mice, which are further activated by knockout of the GPx-1 gene, while these activations are attenuated by over-expression of the GPx-1 gene. The current research indicated that RA significantly improved Nrf2 gene expression and benefited for the activity and gene expression of its downstream antioxidant enzymes GPx including TrxR, SelP, but LPS treatment showed the opposite effect. In addition, RA+LPS-treated group increased the Nrf2 gene expression and GPx1 activities and its mRNA expression and decreased inflammatory cytokines contents and gene expression of NF-κBp50, NF-κBp65 and concentrations of NO as well as ROS compared to LPS-treated group. Thus, we can infer that RA ameliorated the LPS-induced BMECs damage possibly by promoting the activity and mRNA expressions of GPx1 through up-regulation the Nrf2 gene expression significantly, and then resulting in inhibited NF-κB activity and reduced IL-1β contents and NO production.

The High-yielding milk cows, especially during late perinatal and early lactation period, need to increase the proportion of concentrates in the diet to meet the rising demand of milk. However, feeding high proportion of concentrated feed is associated with the high risk of subacute ruminal acidosis (SARA) (Ametaj et al. 2009; Dong et al. 2011), which might lead to enhanced release of LPS in the rumen (Dong et al. 2011). In vivo, LPS produced in the digestive tract can be translocated into bloodstream, thus the concentration of blood LPS increases (Khafipour et al. 2009). The translocation of LPS into the systemic circulation stimulates the release of proinflammatory cytokines such as IL-1 as well as NO content (Bouchard et al. 1999), and at last results in the decrease of milk yield and milk protein. Khafipour et al. (2009) found that SARA challenge increased the LPS in blood plasma from non-detectable levels (<0.05 EU/mL) to 0.52 EU/mL in lactating dairy cows. It was reported that VA content and the activities of GPx and SOD of serum in the postpartum and early-lactation were lower than other periods, but MDA content showed the opposite results (Ren et al. 2008; Qin, 2017). The concentrations of VA were 0.211 μg/mL in the postpartum and 0.215 μg/mL in the early-lactation. And VA contents were 0.243 μg/mL, 0.271 μg/mL, 0.326 μg/mL in the mid-lactation, late-lactation, dry period, respectively. In vivo results also suggested that supplementation of the diet with 220 U of VA/kg of BW might increase the serum VA concentration from 0.28 to 0.32 μg/mL, enhance the activities of GPx, TrxR, SOD, CAT and T-AOC, and improve the antioxidant functions of dairy cows (Jin et al. 2014). This implies that the VA in the cow serum is insufficient in the postpartum and early-lactation, and accompanied
by low levels of antioxidant function. BMECs due to its unique physiological function of synthesis and secretion have become an important cell model to study the oxidative stress of bovine mammary. The current in vitro research indicated that RA (1μg/mL) could reduce the oxidative stress induced by LPS and discussed its underlying mechanism. Although the doses of RA and LPS determined by in vitro studies are higher to the serum VA and LPS contents in vivo, it can provide a theoretical basis for the scientific application of VA in dairy cow diet and the prevention on oxidative stress in cows. The overall results suggest that VA addition might be used as a potential therapeutic agent for inflammation-associated disorders.

Conclusions

In conclusion, the results of our present study clearly indicated that RA had excellent antioxidant capacity in vitro. Pretreatment with RA can provide protection against LPS-induced oxidative injuries in BMECs. These findings reveal a new protective mechanism of RA as a potent antioxidant. The results demonstrated that RA is an accessible source of natural antioxidants and can be used in feed and as a therapeutic agent. However, further studies are still required to elucidate the molecular mechanisms underlying the protective effects of RA as an antioxidant.

Disclosure statement

The authors are as follows: HY Shi, SM Yan, YM Guo, BL Shi and XY Guo declare that there are no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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