Original Article
Vincamine prevents lipopolysaccharide induced inflammation and oxidative stress via thioredoxin reductase activation in human corneal epithelial cells

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Abstract: Lipopolysaccharide (LPS) induced keratitis is a progressive infectious ocular disease in which innate inflammatory responses often cause clinical tissue damage and vision loss. In this study, the potential protective effects of vincamine, a plant alkaloid used clinically as a peripheral vasodilator, against LPS induced inflammation and oxidative stress were investigated on human corneal epithelial cells (HCECs). HCECs were treated with LPS and vincamine at various concentrations. Cell viability, reactive oxygen species (ROS) levels, and the gene expression levels of interleukin-6 (IL-6), IL-8, IL-1β, TNF-α, transforming growth factor-β (TGF-β) in HCECs, were assessed. The antioxidant potential of vincamine was evaluated by measuring the levels of malondialdehyde (MDA), total antioxidant capacity (T-AOC), and superoxide dismutase (SOD). The effects of vincamine on intracellular activities of thioredoxin reductase (TrxR) as well as other anti-oxidant proteins were also investigated in LPS treated HCECs. The results showed that vincamine protected HCECs from LPS induced cell viability reduction and ameliorated the inflammation. Vincamine exhibited a strong antioxidant activity, decreasing ROS levels and regulating the levels of SOD, T-AOC and MDA. Vincamine also exerted anti-inflammatory activities by decreasing IL-6, IL-8, IL-1β, TNF-α, TGF-β expression. Intracellular TrxR activity was significantly activated by vincamine. These findings suggest that vincamine exerts positive effects against LPS induced oxidative stress and inflammation and may be useful in protecting corneal epithelial cells from LPS induced keratitis.

Keywords: Vincamine, lipopolysaccharide, human corneal epithelial cells, oxidative stress, inflammation, thioredoxin reductase

Introduction

Microbial keratitis is a common ocular infection caused by bacteria, fungi, viruses or parasites and is the second most significant cause of monocular blindness, particularly in certain developing countries and, generally, in the tropics [1]. Clinically, this infection requires aggressive antimicrobial management to eliminate the causative organisms, suppress destructive reactions, and restore normal ocular structure and vision [2, 3]. However, despite timely and correct therapeutic strategies, infective keratitis remains clinically challenging, in which approximately 50% of eyes have poor visual outcomes [4, 5], because conventional therapies, such as anti-biotic treatment, often fail to control the tissue damage caused by excessive

local inflammation, even if viable bacteria are cleared from the cornea [6]. Hence, in addition to antibiotic treatment, it is also important to develop new therapeutic modalities to control the inflammatory response in microbial keratitis. Lipopolysaccharide (LPS) is one of the most common causes of microbial keratitis to eyes [7]. As a well-characterized pathogenassociated molecular pattern found in the outer leaflet of the outer membrane of the bacteria, LPS induced keratitis is a rapidly progressive infectious ocular disease [8]. A number of key factors were found in the pathogenesis of LPS induced injury including inflammation and oxidative stress. Previous studies have shown that inflammatory cells were recruited to the cornea to produce various pro-inflammatory cytokines (e.g., IL-6 and IL-1β) and modulate anti-inflam-
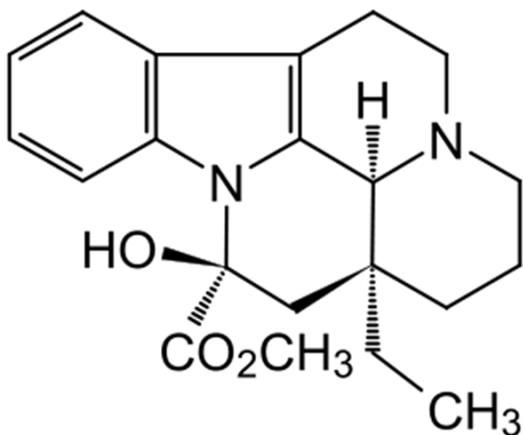
Figure 1. Chemical structure of vincamine.

matory cytokines (e.g., IL-10 and TGF-β) to regulate antibacterial immunity [9-11]. However, if uncontrolled, these inflammatory mediators often elicit an overly robust response, resulting in bystander tissue damage. Thus, tight regulation of innate immune response, especially pro-inflammatory and anti-inflammatory responses, is critical for the resolution of LPS induced bacterial keratitis [12]. Cellular redox homeostasis is normally maintained by a delicate balance between reactive oxygen species (ROS) generation and antioxidant defenses but when this balance is disrupted, the overproduction of ROS elicits DNA, protein and lipid oxidative damage and induces pro-inflammatory cytokines, leading to LPS mediated corneal inflammation [13-15]. ROS also play a role in the pathogenesis of glaucoma, stimulating apoptotic and inflammatory pathways [16].

Cytosolic thioredoxin (Trx), thioredoxin reductase (TrxR) and nicotinamide adenine dinucleotide phosphate (NADPH) comprise the mammalian Trx system, which plays powerful roles in defense mechanism against oxidative stress, nitrosative stress and in redox regulation [17, 18]. Trx is a small redox-active protein that is ubiquitously present in mammalian and is one of the defense proteins induced in response to various oxidative stress conditions [19, 20]. The reduction of oxidized Trx by NADPH is catalyzed by seleno protein TrxR. TrxR may catalyze the NADPH-dependent reduction of H_2O_2, lipid hydroperoxides and dehydroascorbate as well [21]. In addition to its potent anti-oxidative effect, Trx system also has anti-inflammatory properties, mainly because of its ability to inhibit neutrophil chemotaxis to inflammatory

sites and to suppress the expression and activation of the macrophage migration-inhibitory factors [22, 23]. Because of its desirable anti-oxidative and anti-inflammatory properties, Trx system could be a new and potentially effective therapeutic target for anti-inflammation and anti-oxidant.

Herbal medicines have been proven to be a major source of novel agents with various pharmaceutical activities [24-28]. Vincamine, the chemical structure of which is given in **Figure 1**, is an indole alkaloid of clinical use against the brain sclerosis, as well as in post-operative states of the central nervous system [29]. Vincamine seems to act as an oxygen vector in living cells. Also, it has been proposed for the treatment of drepanocytosis (sicklemia) [30]. In addition, vincamine possesses a selective vasoregulator action on the microcapilar circulation, especially in the brain. Vincamine is a peripheral vasodilator that increased cerebral blood flow and used as a nootropic agent to combat the effect of aging [31]. Vincamine has been shown to be a cerebral metabolic enhancer through its effect on ATP production, efficient utilization of glucose and oxygen, while at the same time providing increased protection against ischemia and hypoxia [32]. Vincamine enhanced dopaminergic, serotonergic, and noradrenergic functions probably through its antioxidant capacity, comparably to vitamin E [33-35].

In the present study, the effects of vincamine on HCECs were investigated. Here we showed that vincamine presented potent protective effects against LPS induced oxidative damage and inflammation in HCECs. The underlying regulatory mechanisms associated with the potential anti-inflammatory and anti-oxidant effects of vincamine were also investigated.

Materials and methods

Reagents

Vincamine, *Pseudomonas aeruginosa* LPS, glutamine, fetal bovine serum (FBS), trypsin, 2',7'-dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture media and reagents were purchased from Invitrogen (Carlsbad, CA). Dulbecco's Modified Eagle's medium (DMEM) was obtained from Gibco BRL (Grand Island,

Table 1. The primers used for amplification of gene by PCR

Genes	Primer Sequence
IL-6	F: 5'-TGGCTGAAAAAGATGGATGCT-3'
	R: 5'-TCTGCACAGCTCTGGCTTGT-3'
IL-8	F: 5'-TTGGCAGCCTTCCTGATTTC-3'
	R: 5'-TGGTCCACTCTCAATCACTCTCA-3'
IL-1β	F: 5'-CCTGTCCTGCGTGTTGAAAGA-3'
	R: 5'-GGGAACTGGGCAGACTCAAA-3'
TNF-α	F: 5'-TGTAGCCCATGTTGTAGCAAACC-3'
	R: 5'-GAGGACCTGGGAGTAGATGAGGTA-3'
TGF-β	F: 5'-CGCCAGAGTGGTTATCTTTTGA-3'
	R: 5'-CGGTAGTGAACCCGTTGATGT-3'
β-actin	F: 5'-TGGAACGGTGAAGGTGACAG-3'
	R: 5'-GGCTTTTAGGATGGCAAGGG-3'

NY, USA). Fetal calf serum (FCS) and RPMI 1640 medium were purchased from HyClone (USA). The antibodies to TrxR, TrX, GR, GPx and GAPDH were purchased from Cell Signaling Technology (USA). Other routine laboratory reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of vincamine

Vincamine was dissolved in sterile PBS to a stock concentration of 0.1 M, and stored at 4°C in the dark to be used within 2 days after preparation.

Cell line and cultures

HECEs were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM supplemented with 2 mM glutamine, 10% fetal bovine serum, and antibiotics, incubated in 5% CO_2 incubator at 37°C. Cells were passaged at a 1:3 ratio with trypsin, every 5 to 7 days. In indicated experiments, cells (2×10^5) were seeded into 6-well culture dishes and allowed to grow 3-4 days until approximately 90% confluent. Preliminary experiments were performed to identify the suitable LPS concentration able to induce a significant ROS production with no cytotoxic effects. In preliminary experiments, LPS and vincamine were also tested for cytotoxicity as described below.

Cell viability assay

Cell viability was quantified by Cell Counting Kit-8 (CCK-8) (Beyotime, China) assay as previ-

ously described [36, 37]. In brief, HCECs were seeded into 96-well plates at a density of 2×10^3 cells per well. After incubation overnight, cells were treated as indicated concentration of LPS or vincamine and assessed by CCK-8 assay at 6 and 24 h respectively. 10 µL of CCK-8 reagent was added to each well and incubated for 1 h. The difference in absorbance between 450 and 630 nm was measured by a microplate reader (BioTek, Winooski, VT, USA) as an indicator of cell viability. Independent experiments were done in triplicate. IC_{50} values were calculated as the concentration of compound that inhibited the viability of cells by 50% as compared with control cells grown in the absence of LPS or vincamine.

Treatment of HCECs with LPS and vincamine

The HCECs (1.0×10^5 cells/mL) were seeded into 6-well plates and allowed to attach and grow overnight. They were then replaced with 1 mL of growth medium containing 0.5, 1, 5, 10, 20, 50 and 100 µg/mL of LPS and 1 mL of DMEM supplemented with 5% (v/v) FBS as a blank control group for 24 h, respectively. The experimental groups are listed as follows: control, LPS (10 µg/mL), vincamine (20 µM) + LPS (10 µg/mL), vincamine (40 µM) + LPS (10 µg/mL), and vincamine (80 µM) + LPS (10 µg/mL).

RNA extraction and quantitative real-time PCR

To investigate the change of the mRNA expression of inflammatory factors including interleukin-6 (IL-6), IL-8, IL-1β, TNF-α, transforming growth factor-β (TGF-β) in HCECs, RT-PCR analyses were performed. Total RNA was extracted from cells using an MN-total RNA isolation kit (Machereynagel, Germany) according to the manufacturer's instructions. First-strand cDNA were synthesized using the transcriptor first strand cDNA synthesis kit (Roche, Switzerland). The primers used to amplify IL-6, IL-8, IL-1β, TNF-α, TGF-β and β-actin are described in **Table 1**. Real-time studies were carried out using the SYBR Premix Ex TaqTM kit (TaKaRa, Japan) with β-actin as the reference gene.

Measurement of oxidative stress

Oxidative stress was assessed by measuring malondialdehyde (MDA), total antioxidant capacity (T-AOC), and superoxide dismutase (SOD) levels. HCECs cultured in six-well plates (4×10^4 cells/well) for 24 h were treated with vari-
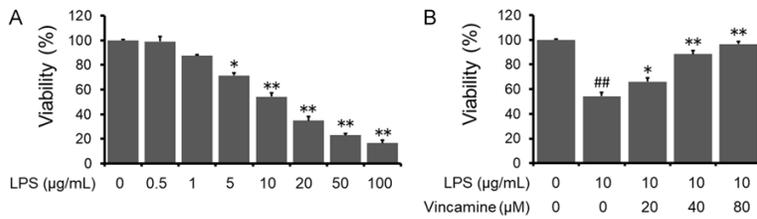
Figure 2. Vincamine increased the viability of LPS treated HCECs. A. Effect of LPS on cell viability by CCK-8 assay in HCECs. Viability of untreated control cells was 100% and it decreased with increase in LPS concentration administered for 24 h. The results are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01 as compared with the control cells. B. Effect of vincamine on cell viability by CCK-8 assay in LPS treated HCECs at 24 h. Viability of HCECs was increased after vincamine administration in a dose-dependent manner. The results are expressed as mean ± SD (n = 6). #P < 0.05, as compared with the control cells. *P < 0.05, **P < 0.01 as compared with the LPS treated cells.

ering Institute, Nanjing, China) following the manufacturer's instructions.

Measurement of ROS Production

To determine whether vincamine affects ROS generation, the contents of intracellular ROS was measured using the ROS assay kit (Beyotime Biotechnology, Haimen, China). The oxidation of DCFH-DA to 2',7'-dichlorofluorescein (DCF) was used to estimate the content of ROS. The cells of the experimental groups in 6-well culture dishes were incubated with DCFH-DA (1:5000, v/v) for 20 min and washed three times by serum-free DMEM, with group used as the positive control. The production of ROS was determined using a ROS Assay Kit in accordance with the manufacturer's instructions (Beyotime, Shanghai, China).

Western blot analysis

After treatments, HCECs were harvested, and homogenized in 200 μL RIPA lysis buffer. Then were extracted and the protein concentration was determined by Lowry method. Protein lysates (40 μg) from each sample were subjected to SDS-PAGE on 10% acrylamide gel and the separated proteins transferred to a PVDF

Figure 3. Effects of vincamine on the production of ROS, SOD, MDA, and T-AOC in LPS treated HCECs. LPS treatment significantly increased the values of the intracellular ROS and MDA levels in the HCECs. The T-AOC, and SOD levels were decreased significantly. However, vincamine administration reduced the values of the intracellular ROS and MDA, while increased the levels of T-AOC, and SOD in a dose dependent manner. All data were shown as mean ± SD of eight rats. #P < 0.05 vs. control group, *P < 0.05 vs. LPS treated group.

ous concentrations of vincamine and LPS. HCECs were then digested with trypsin and washed twice in PBS. Thereafter, cells were suspended in 500 μL of PBS and lysed by ultrasonication in the presence of protease inhibitor before centrifugation at 4,000 rpm for 5 min. The supernatant was collected for analysis. Supernatant protein concentrations were measured using a Bradford protein assay kit from KeyGen Biotech (Nanjing, China). The levels of MDA, T-AOC, and SOD were measured using appropriate kits (Jiancheng Bioengine-

membrane. After transfer, the membranes were blocked with 5% non-fat dry milk in TBS for 1 h at room temperature, then the membranes were incubated with primary antibodies to TrxR, TrX, GR, GPx, and GAPDH overnight at 4°C followed by secondary horseradish peroxidase-labeled antibody (1:2000). The bound antibodies were visualized using the ECL blotting detection system. The relative expression of proteins was quantified densitometrically with the software ImageJ and calculated according to the reference bands of GAPDH.

Determination of TrxR and Trx activity in cell lysates by insulin reduction assay

Freshly collected cell lysates were used to determine cellular Trx and TrxR activities as described previously [38]. Briefly, to measure TrxR activity, in each well of a 96-well plate, 25 *µg* of cell lysate was incubated in a final volume of 50 *µL* containing 85 mM Hepes (pH 7.6), 0.3 mM insulin, 10 *µM* Trx, 2.5 mM EDTA and 660 *µM* NADPH for 40 min at 37°C. 200 *µL* of 1 mM DTNB in 6 M guanidine-HCl, 200 mM Tris-HCl pH 8.0 solution was added to quench the reaction. The amount of free thiols generated from insulin reduction was determined by DTNB reduction at 412 nm using the VersaMax microplate reader. To measure Trx activity, procedures were carried out similarly to those for determining cellular TrxR activity, except that the cell lysates were incubated with TrxR in place of Trx. Controls containing lysates and all reaction reagents except TrxR for each lysate sample were also set up. For each sample, Trx or TrxR activity was calculated as the absorbance at 412 nm subtracted from that of the corresponding control and expressed as a percentage of the activity measured in DMSO-treated cells.

Determination of GPx activity in cell lysates by GPx activity assays

For determination of cellular GPx activity, vincamine (20, 40, 80 *µM*) was incubated with lysates of HCECs (25 *µg* protein), 20 nM GR, 1 mM GSH and 200 *µM* NADPH in a volume of 100 *µL* phosphate buffer (0.1 M sodium phosphate, 2 mM EDTA pH 7.5) for 1 h at room temperature. H_2O_2 solution in phosphate buffer was added to initiate the reaction (final concentration 1.5 mM). NADPH consumption was monitored at 340 nm using the VersaMax microplate reader. The results were calculated based on change in absorbance in the initial 3 min and presented as a percentage of GPx activity of drug-treated sample over that of DMSO-treated sample.

Determination of GR activity in cell lysates by glutathione reduction assay

To determine cellular GR activity, 25 *µg* of cell lysate was mixed with a solution of GSSG and NADPH in phosphate buffer to a final volume of 200 *µL* (final GSSG and NADPH concentra-

tions 1 mM and 200 *µM* respectively). The enzyme activity was determined by measuring the decrease in absorbance at 340 nm for 10 min at 37°C and expressed as a percentage of the enzyme activity of that of the DMSO-treated sample.

Statistical analysis

The data were expressed as means ± standard deviation (SD). T-test was used to compare the difference between the two groups. Statistical analyses between three or more groups were analyzed by one-way analysis of variance (ANOVA) by using SPSS software version 16.0 (IBM, Armonk, NY). Values for *P* < 0.05 were considered statistically significant.

Results

Effect of the vincamine on cell viability in LPS treated HCECs

Figure 2A shows the relationship between LPS concentration and percentage of cell survival relative to the control. Exposure of HCECs to various concentrations of LPS resulted in a concentration-dependent decrease in cell viability. Specifically, after a 24 h exposure with 10 µg/mL LPS, about 52.2% of the viability was remained, while in the cells exposed to 20, 50 and 100 µg/mL LPS, the relative viability was 32.3, 20.1 and 14.6%, respectively (**Figure 2A**). The results in **Figure 2B** show that vincamine (20, 40 and 80 µM) administration exerted a significant, concentration-dependent protective effect.

Vincamine reduced ROS levels in LPS treated HCECs

ROS levels were significantly increased in LPS treated HCECs (**Figure 3**). Vincamine significantly reduced ROS level in a dose-dependent manner (**Figure 3**). ROS levels in the vincamine 40 and 80 µM groups were significantly lower than the level in the model control group (*P* < 0.01).

Vincamine attenuated the oxidative stress in LPS treated HCECs

As shown in **Figure 3**, compared to untreated control HCECs, the intracellular MDA levels were significantly elevated in LPS treated cells,

Figure 4. Effects of vincamine on inflammatory mediators in LPS treated HCECs. Cytokines including IL-6, IL-8, IL-1β, TNF-α, and TGF-β in LPS treated HCECs were determined using ELISA. All these cytokines were significantly increased by LPS treatment. However, vincamine reduced the values of these cytokines significantly in a dose-dependent manner. All the data were shown as mean ± SD of eight rats. ##$P < 0.01$ vs. control group, *$P < 0.05$, **$P < 0.01$ vs. LPS treated group.
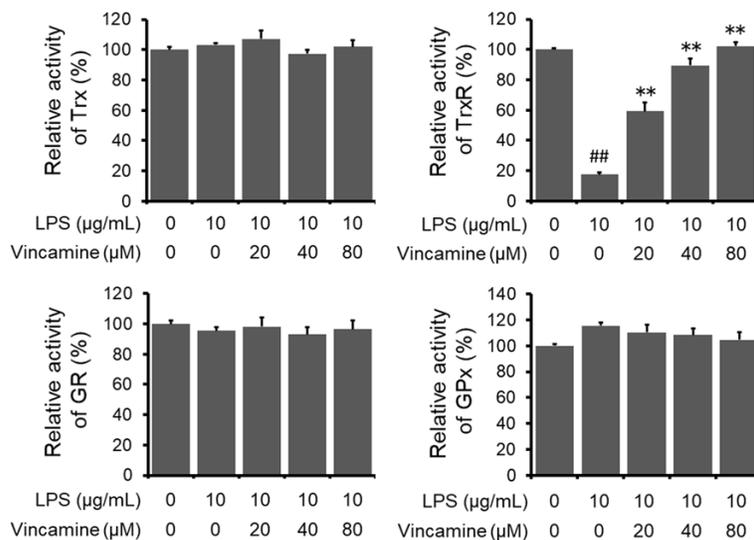
Figure 5. Effects of vincamine on the intracellular activities of redox proteins. Dose-dependent effects of vincamine on TrxR, GR, Trx and GPx activities in LPS treated HCECs. Lysates of cells treated with indicated concentrations of vincamine for 24 h were assessed for activities of TrxR, GR, Trx and GPx. Enzyme activities were expressed as a percentage of those in DMSO-treated cells. All data points are means ± SD of two to four independent experiments. *Statistically significant difference ($P < 0.05$) in enzyme activity as compared to DMSO control.

SOD were increased in a dose-dependent manner.

Effects of vincamine on the expressions of IL-6, IL-8, IL-1β, TNF-α and TGF-β mRNA in LPS treated HCECs

As shown in **Figure 4**, the RT-PCR results revealed that treatment with LPS could significantly increase the mRNA expression of inflammatory factors IL-6, IL-8, IL-1β, TNF-α and TGF-β. Vincamine treatment in HCECs elicited a significant reduction of IL-6, IL-8, IL-1β, TNF-α and TGF-β, compared to LPS treated cells.

Vincamine activated the activity of TrxR in HCECs

The effect of vincamine on Trx, TrxR, GR and GPx in LPS treated HCECs was evaluated. After 30 min of incubation in the presence of NADPH, the activation of mammalian TrxR by LPS, LPS + vincamine was evaluated in the DTNB reduction assay. As shown in **Figure 5**, LPS significantly inhibited the intracel-

while T-AOC, and SOD levels were decreased significantly. In contrast, after vincamine administration, the levels of MDA and were significantly reduced while the levels of T-AOC, and
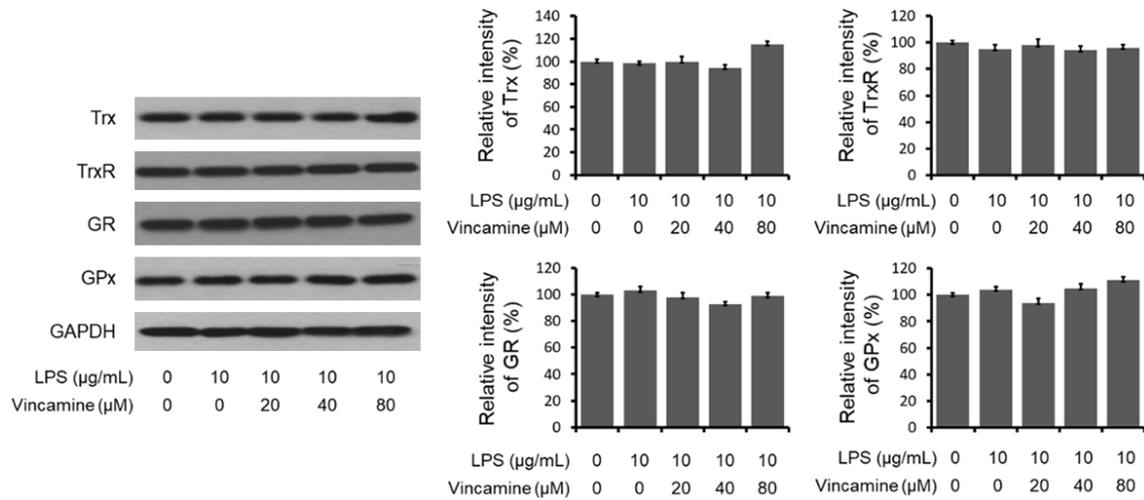
Figure 6. Effects of vincamine on the expression levels of redox proteins. Effect of vincamine on the expression levels of TrxR, GR, Trx and GPx in LPS treated HCECs were analyzed by western blotting. Cells were treated with LPS and various concentrations of vincamine, then the levels of TrxR, GR, Trx and GPx were detected by Western blot. The relative expression of proteins was quantified densitometrically with the software ImageJ and calculated according to the reference bands of GAPDH.

lular activity of TrxR, while vincamine rescued TrxR activity in a dose-dependent manner. However, the intracellular activities of Trx, GR and GPx were neither inhibited nor activated by both LPS and vincamine. These results suggested that TrxR activation could potentially serve as an underlying mechanism for at least part of the anti-oxidant effects of vincamine.

The effect of vincamine on the expression levels of Trx, TrxR, GR and GPx in HCECs

The effect of vincamine on the expression levels of Trx, TrxR, GR and GPx in LPS and vincamine treated HCECs was evaluated. Western blotting analysis revealed that the expression levels of Trx, TrxR, GR and GPx were not affected either by LPS or by vincamine administration, as shown in **Figure 6**. These results suggested that the activation of TrxR activity is not related to the expression changes of this enzyme.

Discussion

Eye infection is one of the major causes of visual impairment and blindness. Well-conserved structural motifs of different microorganisms including LPS of the gram-negative bacteria can mediate innate immune responses leading to either activation or suppression of inflammatory processes and eventually cell death. LPS administration increased the number of apoptotic cells in corneal injury models and induced the expression of autophagic related genes. LPS, through its receptor, toll-like receptor 4 (TLR4), can induce cell migration and proliferation.

Dietary phytochemicals consist of a wide variety of biologically active compounds that are ubiquitous in plants, many of which have been reported to have pharmaceutical properties. Epidemiological studies have shown that natural components may play an important role in preventing human diseases [39, 40]. Among them, vincamine, which is abundant in *Vinca minor L.*, has been reported to have therapeutic potential for treating many human diseases [41, 42]. Vincamine is employed from 1970s in the therapy of cerebral metabolic and circulatory disorders, since it combines cerebrometabolic and hemodynamic properties [29, 30]. However, no anti-oxidative and anti-inflammation effects of vincamine on LPS treated cells were reported so far.

In the present work, we found that vincamine could protect HCECs from LPS induced injury. LPS decreased the viability of HCECs significantly, while vincamine treatment increased HCECs viability in a dose-dependent manner. Following LPS administration, the mRNA expression levels of IL-6, IL-8, IL-1β, TNF-α and

TGF-β in HCECs were significantly raised, which represented a stimulation of inflammatory responses [43]. Reduction of these inflammatory factors in HCECs by vincamine was also detected in a dose-dependent manner.

Accumulating evidence has indicated that oxidative stress is a potential cause of corneal inflammation, and there is an imbalance between the generation of ROS and the capacity to detoxify these intermediates [44]. In addition to looking at indicators of oxidative damage, we also examined the concentrations of several important antioxidants ROS, SOD, T-AOC and MDA levels in HCECs. SOD is an important member of the antioxidant enzymatic defense system and the level of T-AOC reflects the overall cellular endogenous antioxidative capability [45]. And MDA level reflects the degree of organic lipid peroxidation, which indicated the severity of damage to cell membranes [46]. It was shown that in the LPS treated cells, MDA and ROS levels were substantially increased while SOD and T-AOC levels were reduced. In contrast, vincamine remarkably decreased the levels of MDA and ROS, and simultaneously enhanced the levels of SOD and T-AOC. Those results suggested that vincamine could significantly suppress LPS-induced inflammation and oxidative stress, which might explain the protective mechanisms against microbial keratitis induced by LPS.

Trx system is a very important anti-oxidative system which has been reported many effects, such as regulating cellular reduction/oxidation (redox) status and cell proliferation/cell survival processes [47]. Trx system has also been reported to regulate the pathologic processes of several kinds of tumors [17], as well involved in cardiovascular disease, heart failure, stroke, inflammation, metabolic syndrome, and other diseases [48]. However, Trx's function in microbial keratitis has not been thoroughly investigated. In the present study, we found that vincamine activated the activity of TrxR without affecting the activities of GR, Trx, and GPx. Interestingly, the expression levels of all these proteins were not affected significantly by both LPS and vincamine. These results indicated that the intracellular TrxR activity was specificly activated by vincamine adminstration. Its selectivity towards cellular TrxR activation over related antioxidant enzymes GR, Trx and GPx suggested that vincamine showed

anti-oxidant activity via targeting TrxR. TrxR activation mediated by vincamine led to cellular Trx reduction, decreased oxidative stress and inhibition of inflammation.

Conclusions

In summary, our study provided direct evidence to prove that LPS induced inflammation and oxidative stress in HCECs. Both inflammatory mediators' production and oxidative stress could be significantly inhibited by vincamine, through an activation of TrxR acitivity. Further investigation should be carried out to identify the accurate mechanism of vincamine's impact on Trx system in microbial keratitis.

Disclosure of conflict of interest

None.

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