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Perillaldehyde Protects Against *Aspergillus fumigatus* Keratitis by Reducing Fungal Load and Inhibiting Inflammatory Cytokines and LOX-1

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ABSTRACT

Purpose: The purpose of this research was to explore the antifungal and anti-inflammatory effects of perillaldehyde (PAE) in *Aspergillus fumigatus* (*A. fumigatus*) keratitis and the underlying mechanism.

Methods: The biofilm formation, adherence assay, and propidium iodide uptake test were used to determine the possible mechanism of PAE in terms of antifungal effects *in vitro*. The severity of corneal infection was evaluated by clinical scores. The immunofluorescence staining (IFS) was adopted to detect the number of macrophages in infected corneas. Draize test was performed to assess the ocular toxicity of PAE. Real-time polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and Western blot reflected the expression of inflammatory cytokines and Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) in mice corneas and RAW264.7 cells.

Results: PAE was able to inhibit the formation of biofilm, reduce conidial adhesion, and damage the integrity of membranes to exert antifungal activity. In C57BL/6 mice models, PAE alleviated the severity of infected corneas, reduced the recruitment of macrophages and had low ocular toxicity. In addition, the mRNA and protein levels of TNF-α, CCL-2, and LOX-1 could be significantly decreased by the application of PAE after *A. fumigatus* infection *in vivo* and *in vitro*.

Conclusion: Our study indicated that PAE protected against *A. fumigatus* keratitis by reducing fungal load, accumulation of macrophages, and inhibiting the expression of inflammatory cytokines.

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KEYWORDS
Perillaldehyde; *Aspergillus fumigatus*; keratitis; macrophage; fungal inflammation; LOX-1

Introduction

Fungal keratitis (FK), which is an infectious ocular disease caused by fungal pathogens mainly containing *Aspergillus fumigatus* (*A. fumigatus*) and *Fusarium*, can cause severe visual damages and high blindness rate.[1] Plant-related corneal trauma, excessive use of contact lenses, and overuse of broad-spectrum antibiotics and corticosteroids all contribute to the increased occurrence of FK.[2–4] The existing clinical drugs have disadvantages including poor local corneal permeability, strong eye irritation, and high drug toxicity.[5] Therefore, it is urgent for us to find new compounds to fight against fungal infections in corneas.

Perillaldehyde (PAE) is the main component of essential oil derived from perilla plant (*Perilla frutescens*), which has valued us due to its extensive antifungal, anti-inflammatory functions in fighting against infectious diseases.[6] Both fungal virulence factors and excessive inflammation can lead to corneal tissue damage.[7] Fungal infections include the adhesion to host cells, the growth of hyphae, and the formation of biofilm, which lead to the invasion of fungi and the aggravation of diseases.[8–10] Researches revealed that PAE was able to inhibit the growth of *Candida albicans* (*C. albicans*) *in vitro* and it could also interfere with ergosterol biosynthesis, which can lead to the destruction of biofilm of *Aspergillus niger*.[11–13] Our previous studies also verified that PAE had an inhibitory effect on the growth of *A. fumigatus*[14]. In addition, the excessive secretion of inflammatory cytokines in FK can exacerbate corneal damage.[15] In latest studies, PAE was confirmed to decrease the number of immune cells like neutrophils and macrophages in mice infected with *C. albicans* and alleviate the overexpression of inflammatory factors in cerebral ischemia–reperfusion injury.[16,17]

Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), as one of the pattern recognition receptors (PRRs), belongs to the C-type lectin-like receptors structurally.[18] It is closely related to promote inflammation, and has been illustrated to be crucial in the secretion of inflammatory cytokines and the recruitment of inflammatory cells in FK.[19–21] Whether PAE could inhibit the expression of LOX-1 to exert anti-inflammatory effect in FK remains unknown.

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In this study, we confirmed that PAE could reduce the fungal load, exert an anti-inflammatory effect by inhibiting the expression of LOX-1, and the infiltration of macrophages in FK. Collectively, the results revealed that PAE might be a promising therapeutic approach for FK.

Materials and methods

Preparation of PAE solution

PAE (CAS-No.18031-40-8), purchased from TCI Co. Ltd. (Tokyo, Japan), was dissolved and diluted in dimethyl sulfoxide (DMSO). The preparation of stock solutions and working solutions with different concentrations were consistent with our previous experiments.[14]

Preparation of A. fumigatus

A. fumigatus strain 3.0772 was provided by General Microbiological Culture Collection Center (Beijing, China). The conidia were obtained by repeatedly washing the *A. fumigatus* malt agar slope inoculated with phosphate-buffered saline solution (PBS) containing 0.1% Tween 20. The conidia suspension is resuspended and centrifuged (12 000 rpm, 5 min) several times to reach the final concentration of 1×10^7 CFU/mL for subsequent experiments. *A. fumigatus* was inoculated in the Sabouraud liquid medium which contains 4% glucose and 1% Mycopeptone. The mycelia were ground, washed, centrifuged, and the supernatant was discarded. The obtained fungi were active, and we used 70% alcohol to inactivate them for the cells stimulation *in vitro*.[22]

Determination of the biofilm formation

An aliquot of 100 μL Sabouraud medium with *A. fumigatus* conidia suspension (2×10^5/mL) was incubated with different concentrations of agents containing DMSO or serial dilutions of PAE (0.2, 0.4, 0.6, 0.8, 1.0,1.2, and 1.8 mM) in 96 wells at 37 °C for 48 h, the groups with only medium were used as blank control. Then removed the medium and wells were washed three times with PBS. The biofilm was fixed by methanol after air drying and stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO). Washed by PBS to remove the unbound dye. The bound dye was released with 95% ethanol. Optical density (OD) was measured at 570 nm for three times.[13]

Fungal adherence to HCECs

The chambered slides (four/slide) inoculated with human corneal epithelial cells (HCECs) were incubated overnight. Then washed three times with PBS, and 1 mL fresh media was added to the slides with conidia suspension (2×10^5/mL) and DMSO (0.1%) or PAE (0.6 mM). After incubated for 3 h at 37 °C, each slide was washed three times with PBS. Then slides were stained by hematoxylin and eosin (HE). The number of spores adhering to HCECs could be counted and photographed by microscopy (Nikon, Tokyo, Japan, 400×).[23]

Propidium iodide (PI) uptake testing

The conidia suspension of *A. fumigatus* (2×10^6 CFU/mL) was incubated at 37 °C for 24 h in 6-well plates. Then, the hyphae were washed, centrifuged (12 000 rpm, 10 min), and transferred into a new 6-well plate. After incubated with fresh medium with DMSO (0.1%) or PAE (0.4, 0.6, and 0.8 mM) at 37 °C for 24 h, the hyphae were rinsing with PBS. Then added the propidium iodide (PI) solution to each well. Images could be photographed 15 min later by fluorescence microscope (Nikon Tokyo, Japan, 200×).[22]

Mice models of A. fumigatus keratitis

C57BL/6 mice (female, 8 weeks) were purchased from Jinan Pengyue Laboratory. The treatments of animals were in line with the ARVO Statement towards the Use of Animals in Ophthalmic and Vision Research. Mice were grouping according to experimental needs. The method of modeling was intrastromal injection by sterile microliter syringe (10 μL; Hamilton Corp, Bonaduz, GR, Switzerland). Mice were anesthetized by chloral hydrate at a concentration of 8%. The right eye of each mouse in the infection group was injected with 2.5 μL of *A. fumigatus* conidia suspension at a concentration of 2.5×10^6 CFU/mL. The left eye was considered as black control. Twenty-four hours after infection, experimental eyes were treated with subconjunctival injection with 5 μL PAE (6 mM), the control group was injected with 5 μL DMSO (1%) at the same time. We used a slit lamp to observe the eyes and took photos to measure the uniformity of the model. From two days post infection (p.i.), 5 μL PAE (6 mM) or DMSO (1%) treatment was adopted for topical eye drop three times a day. Pictures were also taken by slit lamp at three and 5 d after infection. The clinical score was used to assess the severity of infection of the mice corneas. Mainly evaluated from the following aspects including the area, degree, and shape of the ulcer. Each aspect was divided into a score of 0–4 according to the severity of the infection. Removed mice corneas for experiments according to the experimental design, including RT-PCR, Western blot, and ELISA. The eyeballs at 3 d p.i. were used for IFS[24].

Ocular toxicology test

The ocular toxicity of PAE was detected by Draize Eye Test. An aliquot of 5 μL PAE (4, 6, and 8 mM) or 1% DMSO was dropped into the conjunctival sac of right eyes of four mice three times a day for 5 d. The left eyes of the mice served as the control. Corneal fluorescein staining (CFS) was used to observe the changes by slit-lamp microscopy. The changes of the eyes we observed mainly included the degree of turbidity of the corneas, the extent of damage, the severity of iritis, whether the conjunctiva had congestion, edema, and secretions. Images could be photographed under a cobalt blue light 1, 3, and 5 d after the treatments of topical.[23,25,26]

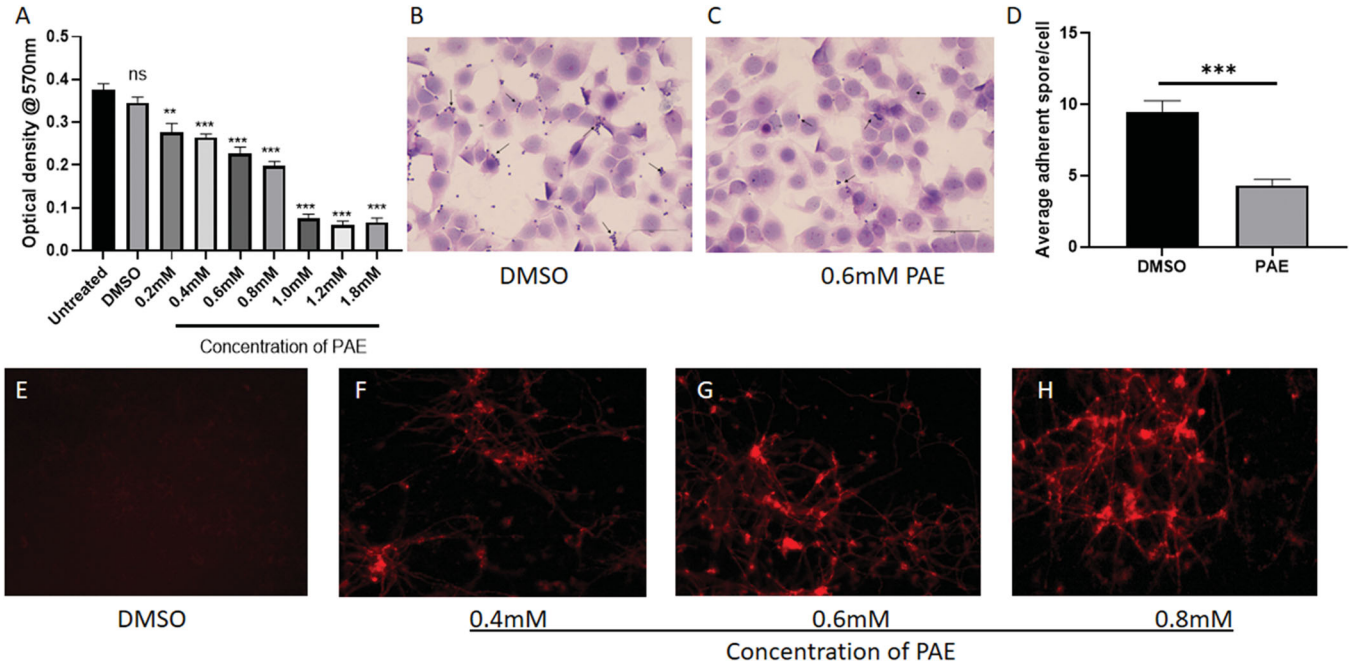

Figure 1. Mechanism of PAE against *A. fumigatus*. Biofilm formation could be inhibited by PAE in a dose-dependent manner compared with DMSO treated (A). HE staining images of HCECs treated with 0.1% DMSO (B) or 0.6 mM PAE(C) (magnification ×400, bar = 20μm) mixed with conidia suspension and the quantitative analysis of the number of spores attached on the surface of cells (D). The images of PI uptake testing showed *A. fumigatus* hyphae treated by 0.1% DMSO (E) and PAE at 0.4 mM (F), 0.6mM (G), 0.8 mM (H) (magnification ×200). (ns: no significance;*$p<.05$, **$p<.01$, ***$p<.001$).

HCECs and RAW264.7 cells culture and stimulation

The cultivation methods of HCECs and RAW264.7 cells were the same as before in our laboratory. As for *A. fumigatus* stimulation, cells were seeded in 12-well plates or 6-well plates according to the requirements and pretreated with PAE (600 μM) or DMSO (0.1%) for 4 h. Then the cells were stimulated with inactivated *A. fumigatus* hyphae, 8 h for Real-Time PCR, 24 h for ELISA, and Western blot.

RT-PCR

Total RNA of mice corneas and RAW264.7 cells was extracted by the RNAiso Plus kit (Takara, Dalian, China) and quantified, the cDNA was obtained through the reverse transcription kit, HiScript III RT SuperMix-cjj (Vazyme, Nanjing, China). The program of RT-PCR was performed referring to our previous experiments. The sequences of the oligonucleotide primers are as follows: mTNF-α F-ACCCTCACACTCAGATCATCTT and R-GGTTGTCTT TGAGATCCATGC, mCCL-2 F-CAGCAGGTGTCCCAAA GAAG and R-ATTTGGTTCCGATCCAGGTT, mLOX-1 F-AGGTCCT GTCCACAAGACTGG and R-ACGCCCCT GGTCTTAAAGAATTG, mβ-actin F-GATTACTGCTCTGG CTCCTAGC and R- GACTCATCGTACTC CTGCTTGC, which the β-actin was used as internal reference.

ELISA

Following the instructions of the ELISA kits purchased (BioLegend, San Diego, CA), samples of mice corneas ($n = 6$/group/time) at 0 and 3 d after infection were placed into centrifuge tubes with 500 μL PBS containing 1% PMSF (Solarbio, Beijing, China). Centrifuged for 10 min at 5000 g and extracted the supernatant for ELISA analysis. TNF-α and CCL-2 protein levels of each sample were detected in duplicate.

Western blot

Total protein was extracted by RIPA buffer (Solarbio) containing 1% phosphatase inhibitor cocktail (MCE) and 1% PMSF (Solarbio). All the tissues were lysed for 2 h and vibrated every 15 min. BCA kit (Solarbio) was used to detect the protein concentration. The protein was separated by SDS-PAGE and transferred onto PVDF membrane (Solarbio). After blocking for 2 h with blocking buffer (Elabscience, Wuhan, China), then incubate with the antibody. The primary antibodies against GAPDH (1:2000; Elabscience, Wuhan, China), LOX-1 (1:1000; Abcam, Cambridge, MA), need to be incubated overnight at 4 °C and the goat anti-rabbit secondary antibodies (1:2000; Elabscience, Wuhan, China) need to be incubated at 37 °C for 1 h. Then the blots could be visualized by enhanced chemiluminescence (ECL) (Thermo Fisher Scientific, Waltham, MA).

Immunofluorescence staining (IFS)

Removed the mice eyeballs of the normal group and the treatment groups 3 d after infection ($n = 3$/group/time). Embedded them into O.C.T. (Sakura Tissue-Tek, Torrance, CA) and frozened by liquid nitrogen. The thickness of the slices is 8–10 microns and fixed with acetone after drying. The slices were blocked by goat serum (1:10; Solarbio) for 30 min, then incubated with primary antibody F4/80 (1:100; Abcam, Cambridge, MA) at 4 °C, overnight. After rinsing with PBS,

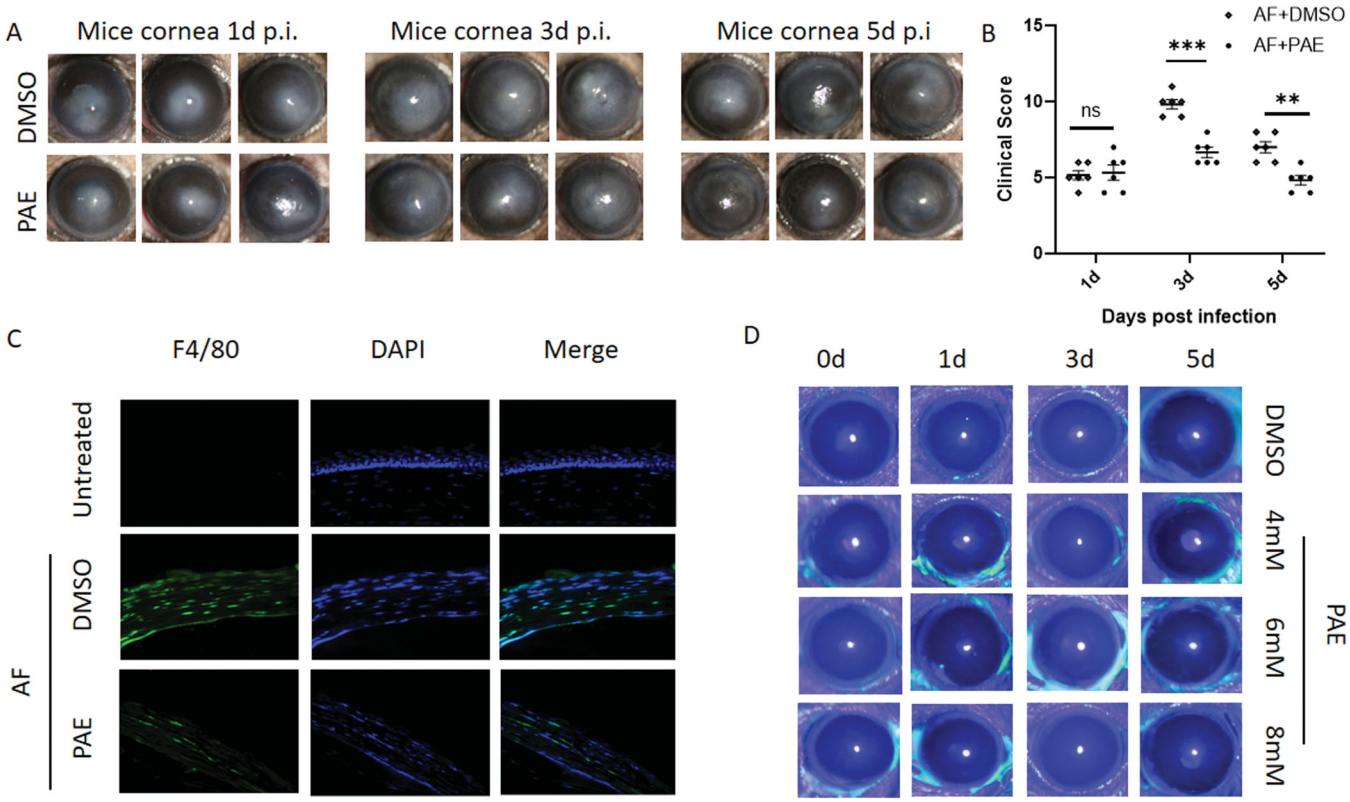

Figure 2. Therapeutic effect of PAE on fungal keratitis in mice. Photographs of mice corneas taken with a slit lamp at 1, 3, and 5 d p.i. after PAE and DMSO treatment (A). The corneal clinical scores were significantly reduced after PAE (6 mM) treatment at 3, and 5 d p.i. compared with DMSO-treated (B). F4/80 labeled macrophages in normal group, DMSO-treated group, PAE-treated group, indicating PAE significantly reduced the number of macrophages in corneas at 3 d p.i. (C). Green: F4/80-FITC staining; blue: nuclear staining (DAPI); merge: macrophages localization (magnification ×400). The corneas treated with PAE at 4, 6, and 8 mM or 1%DMSO for 1, 3, and 5 d to detect the corneal toxicity of PAE. Images of corneal fluorescein staining were presented (D). (ns: no significance; $^{*}p<.05$, $^{**}p<.01$, $^{***}p<.001$).

we used the goat anti-rat secondary antibody with FITC-conjugated (1:200; Elabscience) for staining in the dark for 1 h. The nuclei were stained by DAPI (1:100; Solarbio) for 10 min. Finally, slices could be photographed by fluorescence microscope (Zeiss Axio Vert; magnification ×400).

Statistical analysis

The analysis of the data was obtained by GraphPad Prism (GraphPad Software, La Jolla, CA). Differences in clinical scores between two groups at each time point were analyzed by Mann–Whitney U test. Statistical significance between two groups was evaluated by two-tailed Student's t test, and One-way ANOVA test was used to make comparison among three or more groups. All the data were expressed in the form of mean ± SEM, which $p<.05$ ($^{*}p<.05$, $^{**}p<.01$, $^{***}p<.001$) could indicate the difference between two groups is representative. All experiments were repeated at least three times to ensure the reproducibility.

Results

PAE inhibited the biofilm formation, adhesion ability, and membrane integrity of A. fumigatus

In order to assess the biofilm forming capacity, DMSO and different concentrations of PAE were added to the medium with conidia and incubated together for 48 h. The results showed that 0.2 mM PAE had a remarkably inhibitory effect on the formation of *A. fumigatus* biofilm, and presented a concentration-dependent manner (Figure 1(A)). HE staining showed that the number of conidia attached on the surface of HCECs treated with PAE (0.6 mM) was significantly reduced compared with the DMSO group (Figure 1(B–D)), indicating that PAE could inhibit the adhesion ability of *A. fumigatus* to HCECs. The integrity of the membranes could be detected by PI staining (Figure 1(E–H)), in which PI solution (a red-fluorescent counterstain) was used to stain the nucleic acids exposed from the damaged membranes. Compared with DMSO treated group, the fluorescence intensity of hyphae treated with PAE was significantly enhanced.

PAE alleviated the severity of A. fumigatus keratitis in mice and the infiltration of macrophages

To evaluate the functional role of PAE in FK, 6 mM PAE and 1% DMSO treatment were used for topical treatment in infected mice corneas. Corneal inflammation in mice was recorded by photographs taken with slit lamp. Clinical scores were used to assess disease severity. Compared with DMSO group, the infected corneas treated with PAE exhibited decreased ulcer area and corneal opacity (Figure 2(A)). The clinical scores were markedly reduced at both 3 and 5 d

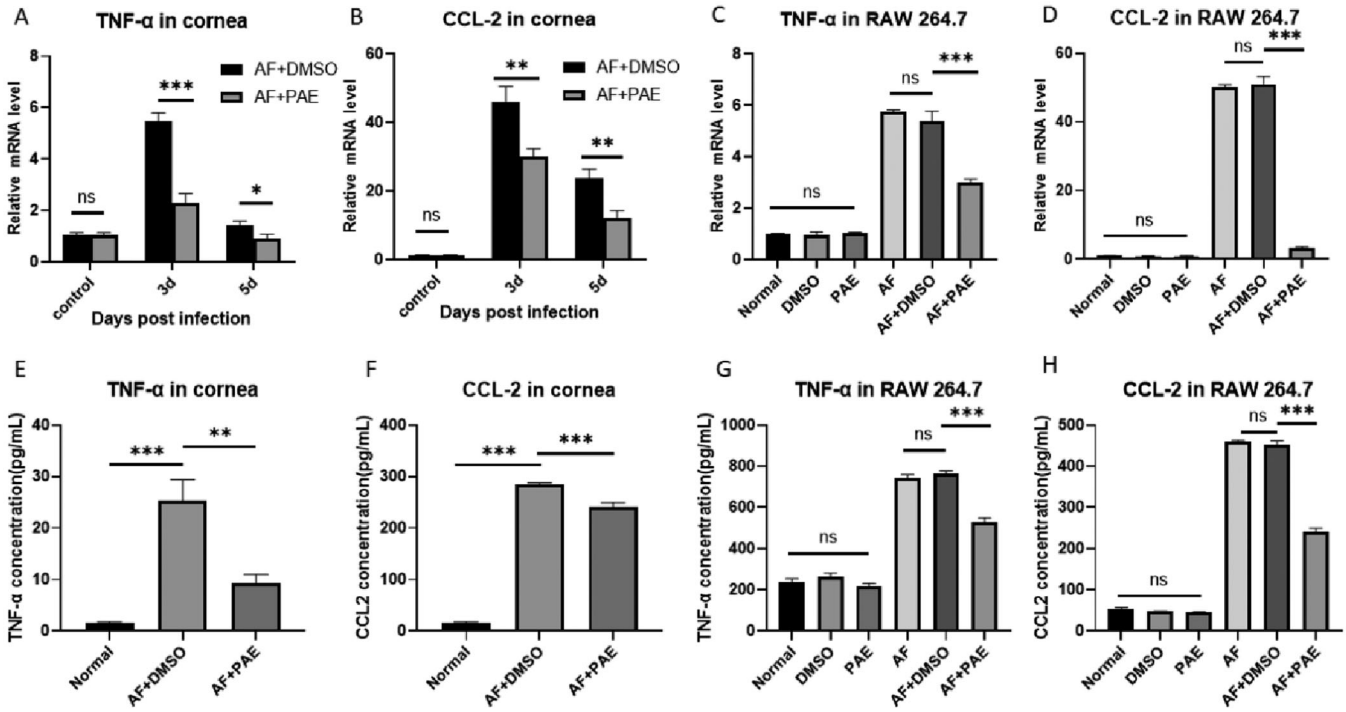

Figure 3. PAE inhibited the secretion of cytokines induced by *A. fumigatus* in infected corneas, PAE decreased mRNA levels (A, B) of TNF-α and CCL-2 at 3 and 5 d p.i. and protein levels (E, F) at 3 d p.i. compared with DMSO-treated. PAE also reduced the mRNA(C, D) and protein (G, H) levels of TNF-α and CCL-2 in RAW264.7 cells. All the data were analyzed in the form of mean ± SEM by an unpaired, two-tailed Student's t-test (ns: no significance;*$p<.05$, **$p<.01$, ***$p<.001$).

p.i. in PAE-treated corneas, which were consistent with the images taken by slit lamp (Figure 2(B)). To investigate whether PAE could affect the infiltration of macrophages, we stained for F4/80. IFS showed that the number of macrophages accumulated in the stroma of infected corneas treated with PAE was less than that in DMSO group (Figure 2(C)). Furthermore, we evaluated the toxicity of PAE to the corneal epithelium. Draize eye test revealed that after exposed to different concentrations of PAE for 1, 3, and 5 d, there were no sodium fluorescein staining in treated corneas (Figure 2(D)), indicating that PAE which concentration below 8 mM was no-toxic to mice corneas.

PAE inhibited A. fumigatus-induced inflammatory cytokines production in vivo and in vitro

We further examined whether PAE could affect the expression of inflammatory cytokines in infected mice corneas and RAW264.7 cells. RT-PCR and ELISA were used to examine the mRNA and protein expression levels of inflammatory cytokines. PAE remarkably reduced the mRNA levels of TNF-α and CCL-2 at 3 and 5 d p.i. compared with DMSO-treated group (Figure 3(A,B)) in infected corneas. Consistently, protein levels of TNF-α and CCL-2 at 3 d p.i. were also decreased in PAE-treated corneas (Figure 3(E,F)). Similarly, PAE pretreatment notably inhibited mRNA expressions of TNF-α and CCL-2 in RAW264.7 cells stimulated by *A. fumigatus* 8 h later (Figure 3(C,D)), and suppressed protein expression within 24 hours following infection with the comparison of DMSO (Figure 3(G,H)).

PAE inhibited the expression of LOX-1 in RAW264.7 cells and A. fumigatus keratitis

In order to explore the anti-inflammatory mechanism of PAE, we detected the expression of LOX-1 in RAW264.7 cells and infected corneas by RT-PCR and Western blot. Results showed that PAE pretreatment markedly inhibited mRNA level (Figure 4(A)) and protein level (Figure 4(B,C)) of LOX-1 in *A. fumigatus* infected RAW264.7 cells. In addition, the experiments *in vivo* showed that LOX-1 mRNA level was significantly suppressed after PAE treatment at 3 and 5 d p.i. compared with DMSO-treated in infected mice corneas (Figure 4(D)), while the protein level was inhibited at 3 d p.i. (Figure 4(E,F)).

Discussion

FK is a serious infectious disease, which usually leads to ulceration and even perforation of infected corneas.[27] Fungal virulence factors, the excessive inflammatory responses can accelerate the progression of FK and increase the difficulties in clinical treatments.[28] PAE is a natural monocyclic terpenoid compound extracted from the *Perilla*, and the safety has been judged by several international expert panels.[29] The remarkable antifungal activities and anti-inflammatory effects of PAE have been confirmed in a variety of diseases. In our study, we investigated the antifungal mechanism of PAE and its inhibition of inflammatory responses in FK.

Recent researches showed that PAE had prominent antifungal activities. Our previous experiments suggested that PAE could efficiently inhibit conidial germination and

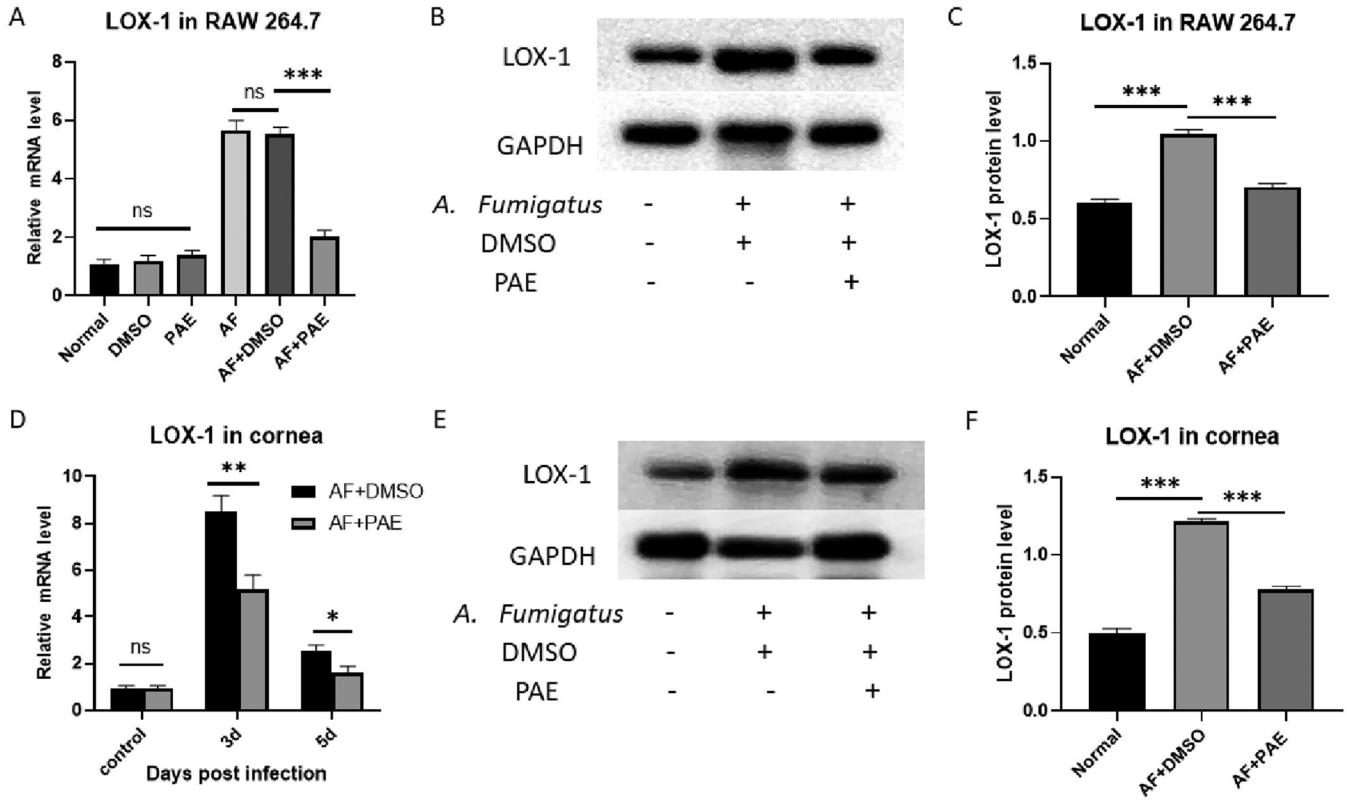

Figure 4. PAE inhibited the expression of LOX-1 induced by A. *fumigatus* In RAW 264.7cells, after stimulated by *A. fumigatus*, PAE (600 μM) pretreatment could significantly reduce the expression of LOX-1 in the mRNA (A) and protein levels (B, C). In mice models of *A. fumigatus* keratitis, the mRNA level of LOX-1 was decreased after the treatment of PAE (6 mM) compared with DMSO-treated (1%) (D). The protein level was showed the similar trend. Grayscale value and quantitative analysis were represented (E, F). All the data were analyzed in the form of mean ± SEM by an unpaired, two-tailed Student's t-test (ns: no significance; $^{*}p<.05$, $^{**}p<.01$, $^{***}p<.001$).

hyphae growth of *A. fumigatus*, and it could also significantly reduce the fungal load of the corneas infected by *A. fumigatus in vivo*. In this study, we further explored the antifungal mechanism of PAE. The adhesion ability of spores and formation of biofilm are critical steps for fungi to invade and resist the host immune system.[30] After the fungal spores are implanted into the host, they produce extracellular matrix to encapsulate themselves to form the biofilm. Our results indicated that PAE was able to inhibit the formation of biofilm and presented a concentration correlation. Studies antecedently showed that PAE inhibited biofilm formation of *C. albicans*' which is related to the reduction of genes *ALS3, HWP1,* and *ECE1* related to biofilm synthesis.[12,13] PAE could also reduce the release of virulence factors like rhamnolipids, cell surface hydrophobicity(CSH), and extracellular polysaccharides (EPS) of *Pseudomonas aeruginosa*, which are important factors in biofilm matrix formation and microbial adhesion.[31]We also confirmed that PAE could inhibit the adhesion of *A. fumigatus* spores to HCECs. Cell membranes play a significant role in maintaining the homeostasis of the intracellular environment, exchanging matter, and conveying energy and information to maintain the health and survival of cells.[6] The lipophilicity of PAE as an essential oil facilitates the interaction with fungal membranes.[32] Our data indicated that PAE decreased the membrane integrity of *A. fumigatus*, which could cause the leakage of ions from the inside of the cell, resulting in intracellular and extracellular osmotic pressure imbalance and irreversible damage to the fungal cell plasma membrane.[6,11,33] Results presented demonstrated that PAE inhibited the formation of fungal biofilm, reduced conidial adhesion, and increased the permeability of fungal membrane to exert antifungal activity.

In addition, we also illustrated that PAE could ameliorate the inflammatory response after infected by *A. fumigatus*. PAE was used for topical treatment in mice with FK, resulting in the alleviated inflammation. The changes of corneal infection could be manifested as alleviated degree of corneal edema and opacity, the reduced ulcer area compared with the control group treated by DMSO. The findings were consistent with others that PAE could alleviate the inflammation of vaginitis.[17] Macrophages are critical in regulating host immune resistance to corneal fungal infection.[34] Recruitment of macrophages in corneal stroma induced by fungal infection may further trigger the production of pro-inflammatory cytokines and chemokines, increase the infiltration of polymorphonuclear neutrophil (PMN), and induce a stronger and more lasting immune response.[35–37] Excessive macrophages recruitment could provoke an excessive immune response and lead to an exacerbation of FK.[38–40] Therefore, controlling the excessive recruitment of macrophages is of great importance in FK. IFS illustrated that the number of macrophages in infected corneas was significantly decreased in PAE group compared with the

DMSO-treated. The results were consistent with the previous researches that PAE played a protective role in the regulation of macrophages in colitis.[41] In addition, our research tested the corneal toxicity of PAE *in vivo*. PAE has been widely used as additives of food, ingredients of perfume, and traditional medicine concoctions.[6] Also, PAE is certified "generally recognized as safe" (GRAS) and not mutagenic.[6] In our study, according to the Draize eye test, PAE is basically non-toxic to the healthy corneas of mice in the concentration of 8 mM, which further confirmed the low toxicity of PAE.

Research showed that PAE reduced secretion of inflammatory factors in oropharyngeal candidiasis.[13] We demonstrated the anti-inflammatory effects of PAE by detecting the expression of inflammatory cytokines. Our results also indicated that PAE treatment notably inhibited the expression of TNF-α and CCL-2 in mice corneas infected by *A. fumigatus*. CCL-2 is one of the potent chemokines correlated with the regulation of macrophages migration and infiltration,[42] and its reduction was consistent with our finding showing that PAE regulated the number of macrophages in corneal tissues of FK. The RAW264.7 cells were used for experiments *in vitro* to explore how PAE affects the function of macrophages. Results suggested that PAE could remarkably reduce the expression of TNF-α and CCL-2 after stimulated by *A. fumigatus*. All the results reflected that the anti-inflammatory effects of PAE were important in relieving corneal inflammation and repairing tissue.

LOX-1 is a kind of PRRs, which has the ability to recognize pathogens and promote the fungus-induced inflammation.[43]Our previous studies demonstrated that LOX-1 was expressed in corneal epithelium and could be up-regulated by fungal stimulation. The neutralization of LOX-1 could attenuate the expression of inflammatory cytokines in *A. fumigatus* keratitis, suggesting that LOX-1 is a crucial pro-inflammatory target in FK. We used RAW264.7 cells and fungal-infected mice corneas to verify whether the inhibition of inflammatory response by PAE is related to LOX-1. In our study, we revealed that being pretreated by PAE could reverse the up-regulation of LOX-1 stimulated by *A. fumigatus* in RAW264.7 cells. We also observed that after PAE treatment in *A. fumigatus* keratitis of mice model, LOX-1 was decreased in mRNA and protein levels.

In conclusion, our study illustrated that PAE reduced fungal load by inhibiting the formation of biofilm and adhesion ability, and disrupting the membrane integrity of fungi. In addition, PAE alleviated inflammation by reducing the recruitment of macrophages and decreasing the secretion of inflammatory cytokines, which may be attributed to the down-regulation of LOX-1. Collectively, our research indicated that PAE had the potential to be a promising therapeutic agent toward *A.fumigatus* keratitis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data for this study are available upon request to the corresponding author.

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