Protective Effects of Recombined Mussel Adhesive Protein against AD Skin Inflammation in Mice

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Abstract: (1) Background: Atopic dermatitis (AD) is characterized as a chronic inflammatory skin disease with a significant incidence rate. The pathophysiological mechanisms underlying AD remain incompletely understood. However, extensive research demonstrates that a complex interplay among genetic, immune, and environmental factors contributes to the disruption of skin barrier function. Inflammation is identified as one of the pathological mechanisms in AD. Recombined mussel adhesive protein exhibits anti-inflammatory properties. However, recombinant mussel adhesive protein has been used less frequently for AD, so we explored the therapeutic effect of recombinant mussel adhesive protein for AD and the potential mechanism. (2) Methods: We established a mice model of AD in vivo and an LPS-induced inflammation model in HaCaT cells in vitro. Through assessment of skin lesion scores, itch frequency, transepidermal water loss, skin microcirculation, HE staining, Elisa assays for IL-6, IL-12, IL-13, IL-4, IL-5, IFN-γ, IgE, and TNF-α, immunohistochemical staining for filaggrin and CK14, Masson staining, and Western blot analysis of NF-κB p65, P-P65, Keap1, and Nrf2, the effects of recombined mussel adhesive protein on AD symptoms, pathology, inflammation, and its mechanisms are investigated. (3) Results: The recombined mussel adhesive protein significantly improved the compromised skin barrier, reduced scratching frequency in mice, decreased transepidermal water loss, and lowered the expression of inflammatory factors, thus ameliorating skin inflammation damage. Mechanistically, recombined mussel adhesive protein downregulated the expression of P-p65/p65 and Keap1 while upregulating the level of Nrf2. (4) Conclusions: Overall, our results demonstrate the effectiveness of recombined mussel adhesive protein in attenuating DNFB-induced AD by inhibiting NF-κB and activating the Keap1/Nrf2 signaling pathway. Thus, recombined mussel adhesive protein is a promising therapeutic candidate for the treatment of AD.

Keywords: atopic dermatitis; recombinated mussel adhesive protein; inflammation; NF-κB; Keap1/Nrf2

1. Introduction

Atopic dermatitis (AD) is a prevalent chronic inflammatory skin disorder characterized by pruritus and impaired barrier function [1]. Globally, it affects millions, presenting symptoms that include swelling, dryness, erythema, erosion, vesiculation, and crust. These symptoms, which vary among individuals, are manifested on the skin surface and are known to adversely affect patient quality of life. The pathogenesis of AD is affected by multiple factors, including environmental factors such as climate, temperature, and humidity, as well as lifestyle and genetic factors [2–4]. Current foundational treatments for AD include the application of topical moisturizers and anti-inflammatory drugs, along with the avoidance of irritants and allergens. The current diagnostic and treatment guidelines for AD recommended baseline treatments for all patients include educational programs regarding the disease course, proper utilization of treatments, avoidance of triggering factors and
allergens, and consistent and appropriate application of emollients to restore impaired skin barrier and address xerosis [5,6]. For patients with mild AD, treatment usually consists of topical corticosteroids and topical calcineurin inhibitors. For moderately severe AD cases, a proactive approach should be taken, including continued use of topical corticosteroids and topical calcineurin inhibitors in combination with various phototherapy approaches. According to the most recent treatment guidelines, advanced systemic therapies should be considered for severe AD, which include drugs such as dupilumab, baricitinib, abrocitinib, upadacitinib, and tralokinumab, as well as traditional immunosuppressants including cyclosporine A, methotrexate, azathioprine, mycophenolate mofetil, and systemic corticosteroids [7–9]. Despite recent advancements in AD treatment, challenges and unmet needs persist in achieving the safe and effective control of moderate-to-severe AD. The primary challenge is the unresponsiveness to advanced treatment options of a subset of patients. The second challenge is the adverse effects that restrict the utilization of recent advanced treatments, particularly in patients with comorbidities. Finally, existing treatments have not been shown to alter the course of the disease. Therefore, there is an urgent need to seek alternative and effective treatment approaches to alleviate inflammation, relieve symptoms, and improve skin barrier function [10].

The pathophysiological mechanisms of AD are complex, involving impaired skin barrier function, alterations in skin microbiota, and immune dysregulation. Recent research indicates that inflammatory responses play a crucial role in the development of AD [11]. In the chronic phase of AD, cytokines such as TNF-α, IFN-γ, and IL-6 are produced by Th1 cells, contributing to the inflammatory response. During the acute phase of AD mediated by Th2 cells, IL-4, IL-5, and IL-31 are produced and stimulate B cells to secrete IgE. Excessive IgE activates mast cells, leading to the further production of inflammatory cytokines, thus exacerbating AD [12,13]. NF-κB is a central regulator of inflammatory responses. It collaborates with other cytokines and signaling pathways to control the expression of inflammatory mediators and enzymes, thereby mediating the initiation and progression of inflammation. Activation of NF-κB results in the production of multiple inflammatory cytokines and chemokines, which are specifically associated with microbial infections and compromised immune responses in AD [14,15]. In summary, selectively inhibiting NF-κB activity may serve as an effective therapeutic approach for treating AD.

Nrf2 (nuclear factor erythroid 2-related factor 2), an essential endogenous antioxidant transcription factor, interacts with Keap1 (Kelch-like ECH-associated protein 1) within the cytoplasm. Normally, Keap1 functions as an inhibitor by binding with Nrf2 to form a complex, thus preventing the transcriptional activation of Nrf2. During oxidative stress, the active site of Keap1 is oxidized, leading to the dissociation of Nrf2 from Keap1 and its subsequent translocation into the nucleus [16–18]. After translocating into the cell nucleus, Nrf2 binds with antioxidant response elements (AREs). This interaction activates downstream antioxidant genes, including HO-1 and NQO1, thereby mitigating oxidative stress responses [19,20]. It has been reported that activation of the Nrf2 signaling pathway can inhibit NF-κB activity and reduce inflammatory responses. Conversely, when Keap1 and Nrf2 expression are inhibited, NF-κB expression increases, indicating the involvement of the Keap1/Nrf2 signaling pathway in NF-κB-mediated inflammation [21].

Mussel adhesion protein (MAP), also known as mussel pedunculated filament protein, is a unique protein secreted by marine mollusks and is a mucin belonging to the glycoprotein family. It is produced and stored in the foot glands of mussels. This foot filament protein secreted by mussels has adhesion and detachment properties on different surfaces and responds to changes in salinity, humidity, and pH in the marine environment. It has three functions: bioadhesion, formation of protective membranes, and promotion of cell adhesion and crawling [22]. Mussel adhesive protein, also known as mussel foot silk protein, is a unique protein secretion from marine mollusks. This type of foot silk protein, secreted by mussels, demonstrates adhesive and detachment properties on diverse surfaces, responding to changes in salinity, humidity, and pH levels in the marine environment [23]. Recombined mussel adhesive protein are proteins obtained by genetic
engineering technology and produce clear sources without any animal origin and no risk of virus contamination. Recombined mussel adhesive protein is recognized for its significant potential in applications, especially within the fields of biomedicine and materials science [24,25]. Furthermore, due to the inherent antimicrobial and anti-inflammatory properties of recombined mussel adhesive protein, along with its potential to promote wound healing, it is speculated to offer assistance in skin inflammation [24,26,27]. With advancements in technology and deeper research, recombined mussel adhesive protein may become one of the significant tools for treating skin diseases and promoting skin health. Therefore, this study aims to investigate the protective effects of recombined mussel adhesive protein on mice with AD, by establishing an in vivo model of AD in mice and an in vitro inflammation model induced by LPS in HaCaT cells, and explore the potential mechanisms involved.

**2. Materials and Methods**

**Drugs:** Recombined mussel adhesive protein, supplied by JUYOU (Xian, China), 0.5 mg/g × 15 g/bottle; Positive drug: Mometasone furoate gel, products of China Resources Sanjiu (Nanchang, China) Pharmaceutical, 2209004j; hair removal cream; 2,4-dinitrofluorobenzene (DNFB, 70-34-8, Sigma, Louis, MO, USA).

**Animals:** Male BALB/c mice, weighing 18–22 g, were purchased from Dashuo, Chengdu, China Certificate number: SCXK (Chuan) 2020-030. The mice were housed in the animal facility of the Department of Pharmacology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University. They underwent an acclimation period of three days with a 12 h light/dark cycle, relative humidity of about 50%, and a temperature of approximately 23 °C. The mice were accommodated in the animal facility for three days to adapt to the environment.

**Animal grouping and modeling:** Before the start of the experiment, the mice were depilated on their backs using a razor and depilatory cream, and the depilated area was cleaned with a medical swab. The mice were then allowed to acclimatize for another three days, during which time their skin condition was observed. The mice were randomly assigned to groups, seven of which were designated as the control group and the rest were assigned to the experimental group. DNFB was dissolved in a 3:1 mixture of acetone and olive oil to make 100 µL of 1.0% DNFB solution and 50 µL of 0.5% DNFB solution. The experimental groups were sensitized using 100 µL of 1.0% DNFB applied to the skin of the backs of the mice, and dermatitis was induced by applying 50 µL of 0.5% DNFB solution to the skin of the backs of the mice for two consecutive weeks on days 5, 8, and 14 [28]. The recombinant mussel adhesive protein group was administered 5 µL twice a day on the skin of the backs of the mice from day 5 for 21 days. The positive control group was administered in the same manner, with mometasone furoate gel applied to the back.

**Skin lesion scores and scratching frequency:** According to specific dermatitis scoring criteria, on the 7th, 14th, and 21st days of the experiment, the presence of erythema, epidermal peeling, scaling, and edema on the backs of the mice was assessed and categorized into four levels: none, mild, moderate, and severe, assigned scores of 0, 1, 2, and 3, respectively. The aggregate of these scores represented the total symptom score, with higher values indicating more severe skin damage. At the conclusion of the experiment, the frequency of scratching at the ears and back within a 10 min interval was recorded for each mouse, with continuous scratching being counted as a single occurrence.

**Transcutaneous water loss rate:** A skin-testing device was used to randomly test three locations on the backs of the mice, and the transepidermal water loss (TEWL) values were recorded and averaged. The Tewameter (model TM300, Courage and Khazaka, Köln, Germany) employed an open chamber continuous measurement method. Once the readings stabilized, three measurements were taken and averaged; the final results were obtained by averaging the values from both the left and right sides.

**Detection of skin microcirculation:** Before conducting the skin microcirculation assessments, the mice were anesthetized using a small animal anesthesia machine, during which
the machine’s parameters were appropriately adjusted. The mice were then restrained, exposing their dorsal affected areas. The probe was firmly applied to these exposed areas, following which data collection and processing were carried out.

HE staining: Fixed skin tissues were embedded in paraffin, and sections were then dewaxed and rehydrated. Initially, nuclei were stained blue or purple using hematoxylin. Following this, the cytoplasm was stained red with eosin. After staining, the sections underwent a progressive dehydration process using increasing concentrations of alcohol, followed by clearing and mounting. Finally, the prepared sections were examined under a microscope.

Masson staining: Paraffin-embedded sections were dewaxed to water, either chromized or treated to remove mercury salt precipitates. Initially, collagen was stained blue using Weigert’s iron hematoxylin solution. Following this, myoglobin and cytoplasm were stained red with Biebrich Scarlet-Acid Fuchsin solution. The cell nuclei were then stained using an ammonium molybdate-orange G solution. Post-staining, the sections underwent differentiation with an acidic solution and bluing with a diluted lithium hydroxide solution. Once the staining process was complete, the sections were rehydrated and mounted for subsequent microscopic examination to assess the tissue’s morphological structure.

Immunohistochemical staining: Paraffin-embedded tissue blocks were cut into sections with a thickness of 4 µm. Specimens were deparaffinized with xylene, debenesinated with gradient ethanol, and hydrated before immunohistochemical staining. The main antibodies were filaggrin (bs-6327R, Bioss, Beijing, China) and CK14 (AB181595, Abcam, Cambridge, Britain).

Cell culture: HaCaT cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% double antibody in a constant temperature incubator at 37 °C with 5% CO2 saturation and humidity. Passaging was performed when the cells reached 80–90% confluence. Cells in the logarithmic growth phase and exhibiting good growth status were selected for the experiments.

Cell viability analysis: HaCaT cells (5 × 10^3 cells per well) were seeded in a 96-well culture plate (100 µL per well). After 12 h of adherence, recombined mussel adhesive protein (0, 1, 3, 10, 30, 90, 270 µg/mL) was added to the culture for 24 h, with each well set up in quadruplicate. Cell viability was assessed using the CCK-8 assay (CA1210-100, Solarbio, Beijing, China). Ten microliters of CCK-8 solution was added to each well and incubated in the dark for 2 h before measuring absorbance at 450 nm.

Establishment of the HaCaT inflammatory injury model: HaCaT cells in the logarithmic growth phase were seeded into 6-well plates with 5 × 10^3 cells per well. The cells were then divided into the control group, the LPS (L2880, Sigma, Bandai, Japan) group, and the recombined mussel adhesive protein (3, 10, 30 µg/mL) + LPS group. When the adherent growth reached 70%, LPS was added at a final concentration of 1 µg·L⁻¹, and the cells were incubated for 6 h. After incubation, the corresponding concentration of LPS was added to the drug group, and the cells were further incubated for 18 h. The control group received no treatment.

Western blot assay: Cells from each group were seeded in 6-well plates. Once the cells reached a density of 70–80%, they were collected to extract total protein using the BCA method (P0012, Beyotime, Shanghai, China). The protein concentration was determined, and then the samples were heated at 100 °C for 10 min in a constant temperature metal bath. Twenty micrograms of proteins per sample was separated by 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5% nonfat milk in Tris-buffered saline and Tween 20 buffer for 1 h. After blocking, the membranes were incubated overnight at 4 °C with primary antibodies, followed by incubation with HRP-conjugated secondary antibodies (Beyotime Institute of Biotechnology) for 1 h at room temperature. Subsequently, the membranes were washed three times with TBST at room temperature, and immunoreactivities were detected using an ECL detection kit. The following antibodies were used: NF-κB p65 (#ET1603-12, HUABIO, Hangzhou, China),
Phospho-NF-κB p65 (#ET1604-27, HUABIO), Keap1 (R26935, Zen-bio, Chengdu, China), and Nrf2 (ab137550, Abcam, Cambridge, UK).

ELISA detection: Serum samples collected from mice at each time point were thawed from −80 °C. The cell supernatant was collected and centrifuged at 1000× g for 10 min to remove debris. Experiments were conducted following the protocols provided in each ELISA kit and were detected using a full-wavelength microplate reader. ELISA assays for IL-6 (EK206/96, liankebio, Beijing, China), IL-12p70 (EK212/3-96, liankebio, Beijing, China), IL-13 (EK213/2/96, liankebio, Beijing, China), IL-4 (EK204/2-96, liankebio, Beijing, China), IL-5 (EK205-96, liankebio, Beijing, China), IFN-γ (EK280/3-96, liankebio, Beijing, China), IgE (1218202, dakewe, Shenzhen, China), and TNF-α (EK282/4-96, liankebio) were conducted according to the manufacturer’s instructions.

Statistical analysis: The experimental results were presented as “mean ± SD”. Histological measurements were processed using ImageJ software, and statistical analysis was conducted using SPSS 19.0 software, with one-way analysis of variance (ANOVA) performed among groups. A significance level of p < 0.05 is considered statistically significant.

3. Results
3.1. Recombined Mussel Adhesive Protein Can Improve Skin Damage in Mice with AD Subsection

The skin lesion scores results shown in Figure 1A indicated that, on the 7th day, the model group exhibited severe skin damage compared to the control group, confirming successful model creation. On the 14th day, the skin of mice in the experimental group had shown substantial recovery when compared to the model group. By the 21st day, the skin in the positive control group was nearly healed and that in the recombined mussel adhesive protein group had essentially recovered. The lesion scores corresponded closely with the visual outcomes. As shown in Figure 1B, the HE staining results revealed significant damage to the skin structure in the model group, characterized by a discontinuous stratum corneum. Even after 7 days, the skin structure had not completely recovered, and it remained impaired on the 21st day. However, recombined mussel adhesive protein facilitated effective repair of the damaged skin by the 14th and 21st days, showing no significant difference when compared to the positive control group. Additionally, Masson staining highlighted that collagen fibers, stained blue, demonstrated uneven distribution in the model group. As shown in Figure 1C, it was demonstrated that the administration of both a positive control drug and recombined mussel adhesive protein facilitates the gradual repair of skin collagen on days 14 and 21.
Figure 1. The effect of recombined mussel adhesive protein on the skin lesions in mice with AD. (A) Skin lesion scores: #: compared with the control group, $p < 0.05$; *: compared with the experimental group, $p < 0.05$. (B) H&E staining of the skin lesion: scale bar = 200 μm. (C) Masson staining of the skin lesion: scale bar = 200 μm.
3.2. Recombined Mussel Adhesive Protein Improves the Skin Barrier Function of Mice with AD

Following successful induction of the model, the mice exhibited severe symptoms of AD, characterized by pronounced itching. As shown in Figure 2A, itching incidents were significantly higher in the model group on the 7th day, but markedly reduced by the 21st day. Administration of the positive control medication resulted in a decrease in itching incidents, and the recombined mussel adhesive protein effectively reduced the frequency of itching. TEWL reflects the amount of water evaporating from the skin surface and is an important parameter for assessing skin barrier function. Due to severe skin lesions on the 7th day, TEWL was measured on the 14th and 21st days. As shown in Figure 2B, compared to the control group, the modeling group exhibited a significant increase in TEWL. However, in the recombined mussel adhesive protein group, TEWL decreased on the 14th day and notably decreased on the 21st day, indicating a good recovery of skin barrier function.

![Figure 2](image_url)

**Figure 2.** The effect of recombined mussel adhesive protein on the skin barrier function in mice with AD. (A) Scratching times. (B) Transcutaneous water loss rates. (C) Microcirculation index. (D) Statistical results of microcirculation index. Values represent the mean ± SD; a t-test was used for significance analysis. #: compared with the control group, \( p < 0.05 \); *: compared with the model group, \( p < 0.05 \).

The skin superficial blood vessels in each part of the skin were directly and clearly observed from the microcirculation meter. The number and rate of moving red blood cells in the skin can also be measured based on the changes in frequency and amplitude signals generated by the scattering of low-energy laser light by moving red blood cells.
in blood vessels. It has a high application value for the study of superficial vascular-related diseases and inflammatory diseases. The microcirculation of skin in mice with AD was detected by a microcirculation analyzer. Figure 2C,D shows the results of the dorsal skin microcirculation in mice. The results showed that there were significant differences between the model group and the control group, indicating that the model was successfully established. In mice, the level of microcirculation was poor on the 3rd day of administration and gradually recovered on the 14th and 21st days. After positive drug administration, microcirculatory indicators improved. There was a significant difference between the recombined mussel adhesive protein group and the model group \( (p < 0.05) \), showing a better effect on improving microcirculation.

### 3.3. Recombined Mussel Adhesive Protein Can Improve Skin Keratin in Mice with AD

Figure 3A–C represents the results of the immunohistochemistry maps of CK14 in the dorsal skin of mice. CK14, associated with the stratum corneum, was observed to increase gradually on the 14th and 21st days following treatment with recombined mussel adhesive protein, suggesting its efficacy in repairing the stratum corneum. The immunohistochemical staining results for filaggrin in the dorsal skin of mice can also be obtained from Figure 3D–F. In the mice with AD model, filaggrin, crucial for skin barrier function, exhibited a decrease but was seen to increase as skin recovery ensued, indicating enhancements in both the skin barrier and the stratum corneum due to the recombined mussel adhesive protein.

**Figure 3.** The effect of recombined mussel adhesive protein on the skin keratin in mice with AD. (A–C) Immunohistochemical results of CK14 in the skin of mice with specific dermatitis after drug intervention, ((A)—4×), ((B)—10×); (D–F) Immunohistochemical results of filaggrin in the skin of mice with specific dermatitis after drug intervention, ((D)—4×), ((F)—10×). Values represent the mean ± SD; a t-test was used for significance analysis. *: compared with the model group, \( p < 0.05 \).

### 3.4. Recombined Mussel Adhesive Protein Can Ameliorate the Inflammatory Response in Mice with AD

The ELISA results shown in Figure 4 demonstrated that recombined mussel adhesive protein significantly suppressed the expression of IL-4, IL-12, and IL-5 and reduced the levels of immunoglobulin IgE, TNF-α, and IL-13. These findings suggested that recombined mussel adhesive protein effectively alleviated skin damage and decreased inflammatory cell infiltration in the dorsal skin of mice with AD. The probable mechanism involves the inhibition of the immune responses of IL-4 and IL-13 cytokines in Th2 cells, alongside a reduction in IgE levels.
**Figure 4.** The effect of recombined mussel adhesive protein on inflammation in mice with AD. (A–G) Results of Elisa assay for inflammatory factor: TNF-α, INF-γ, IL-12, IL-13, IL-5, IL-4, and IgE. Values represent the mean ± SD; a t-test was used for significance analysis. #: compared with the control group, \( p < 0.05 \); *: compared with the model group, \( p < 0.05 \).

3.5. In Vitro Anti-Inflammatory Effects of Recombined Mussel Adhesive Protein

To further investigate the anti-inflammatory effect of recombined mussel adhesive protein, HaCaT cells (human immortalized keratinocytes) were cultured, and an inflammatory model was induced using LPS. A CCK-8 assay was performed to ascertain the optimal dosage of the recombined mussel adhesive protein. As shown in Figure 5A, within the range of 1–90 µg/mL, no significant drug toxicity was observed. At a concentration of 270 µg/mL, a notable reduction in cell viability occurred. Furthermore, a significant promotion of cell growth was evident in the 30 µg/mL group. Therefore, 30 µg/mL was
chosen as the dosage for further treatment. Following the induction of an inflammatory response with 1 µg/mL of LPS, cell supernatants were collected to assess the expression levels of the inflammatory cytokines TNF-α, INF-γ, IL-6, and IL-13. The ELISA results shown in Figure 5B–E demonstrated that recombined mussel adhesive protein significantly suppressed the expression of TNF-α, IL-6, and IL-13, while enhancing the expression of INF-γ. These findings are consistent with the in vivo results, indicating that recombined mussel adhesive protein effectively reduces inflammatory cytokine expression.

![Image](https://via.placeholder.com/150)

**Figure 5.** The effect of recombined mussel adhesive protein on inflammation in HaCaT cells. (A) Effect of recombined mussel adhesive protein on the cell viability of HaCaT cells. (B–E) Effect of recombined mussel adhesive protein on the LPS-induced expression of inflammatory factors in HaCaT cells: TNF-α, INF-γ, IL-6, IL-13. Values represent the mean ± SD; a t-test was used for significance analysis. #: compared with the control group, p < 0.05; *: compared with the model group, p < 0.05.

### 3.6. Recombined Mussel Adhesive Protein Exerts Its Anti-Inflammatory Effects by Inhibiting NF-κB and Activating the Keap1/Nrf2 Pathway

The NF-κB signaling pathway plays a crucial role in inflammatory responses, and the transcriptional activity of NF-κB is regulated by the complete activation of the p65-NF-κB subunit through phosphorylation and acetylation. Nrf2 is a key transcription factor that initiates the antioxidant stress response pathway in the body. Under normal circumstances, it binds with Keap1 and is present in the cytoplasm. Upon activation of the Nrf2 pathway, downstream antioxidant enzymes’ expression is promoted. To investigate the anti-inflammatory mechanism of recombined mussel adhesive protein, we examined whether it affects the expression of NF-κB, Keap1, and Nrf2. According to the Western blot results shown in Figure 6A,B, compared to the control group, LPS stimulation significantly increased the expression of P-p65/p65. However, treatment with recombined mussel adhesive protein reduced the expression of P-p65/p65 compared to the LPS group. As shown in Figure 6C,D, to further explore the relationship between recombined mussel adhesive protein and Keap1/Nrf2, we utilized the Nrf2 inhibitor ML385 to reduce the expression of Nrf2. LPS stimulation increased the expression of Keap1, while treatment with recombined mussel adhesive protein decreased Keap1 expression. Keap1 negatively regulates the expression of Nrf2. Compared to the LPS group, treatment with recombined mussel adhesive protein increased Nrf2 expression. Administration of ML385 reversed this effect, thereby reducing Nrf2 expression. These results indicate that the anti-inflammatory effect of recombined mussel adhesive protein is mediated through the inhibition of NF-κB and activation of the Keap1/Nrf2 pathway.
Although AD is not associated with mortality, making it underestimated relative to other fatal diseases, it imposes a significant burden of morbidity due to its profound humanistic and psychosocial effects on patients and their families [30]. Although AD is not associated with mortality, making it underestimated relative to other fatal diseases, it imposes a significant burden of morbidity due to its profound humanistic and psychosocial effects on patients and their families [30].

However, due to the complex etiology and diverse pathogenicity of AD, finding effective treatment remains a significant challenge. The main pathogenic mechanisms of AD include an abnormal skin barrier, T-cell-induced skin inflammation, and increased IgE response [30]. Recombined mussel adhesive protein possesses various effects such as anti-inflammatory, antiallergic, antioxidant, antimicrobial, inhibition of pigment deposition, wound-healing promotion, and the formation of protective films. In this study, contrary to the severe skin inflammation observed in AD mice, we found that the severity of skin damage in AD mice treated with recombinant mussel adhesive protein was reduced. This reduction included manifestations such as skin tissue swelling, peeling, and discontinuity of the stratum corneum. AD causes local itching of the skin, which was significantly reduced after treatment with recombinant mussel adhesive protein, as evidenced by a decrease in scratching behavior in mice. AD leads to damage to the skin barrier, and both transepidermal water loss rate and superficial vascular manifestations can reflect the status of the skin barrier. Treatment with recombinant mussel adhesive protein improved the water loss conditions, reduced transepidermal water loss rate, and improved microcirculation indicators.

Figure 6. The effect of recombinant mussel adhesive protein on the NF-κB and Keap1/Nrf2 pathways. (A,B) Protein levels of NF-κB p65 and P-p65. (C,D) Protein levels of Keap1 and Nrf2. Values represent the mean ± SD; a t-test was used for significance analysis. #: compared with the control group, p < 0.05; *: compared with the model group, p < 0.05; @: compared with the recombinant mussel adhesive protein group, p < 0.05.

4. Discussion

The pathogenesis of AD is a complex process, influenced by a variety of factors such as external environmental conditions, genetic predispositions, impaired skin barrier function, and immune responses [13]. AD is typically characterized by dry skin, itching, and eczematous lesions. These clinical manifestations can significantly impair quality of life, resulting in sleep disturbances, emotional distress, and secondary infections. Treatment strategies generally prioritize restoring the skin barrier, managing itching and inflammation, and preventing exacerbations and infections [2]. Currently, the treatment of AD mainly consists of topical corticosteroids and topical calcineurin inhibitors, or oral antihistamines; systemic therapies include drugs such as dupilumab, baricitinib, abrocitinib, upadacitinib, and tralokinumab, as well as conventional immunosuppressants and systemic corticosteroids. However, these medications are limited in efficacy and associated with side effects [29].

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However, due to the complex etiology and diverse pathogenicity of AD, finding effective treatment remains a significant challenge. The main pathogenic mechanisms of AD include an abnormal skin barrier, T-cell-induced skin inflammation, and increased IgE response [30]. Recombined mussel adhesive protein possesses various effects such as anti-inflammatory, antiallergic, antioxidant, antimicrobial, inhibition of pigment deposition, wound-healing promotion, and the formation of protective films. In this study, contrary to the severe skin inflammation observed in AD mice, we found that the severity of skin damage in AD mice treated with recombinant mussel adhesive protein was reduced. This reduction included manifestations such as skin tissue swelling, peeling, and discontinuity of the stratum corneum. AD causes local itching of the skin, which was significantly reduced after treatment with recombinant mussel adhesive protein, as evidenced by a decrease in scratching behavior in mice. AD leads to damage to the skin barrier, and both transepidermal water loss rate and superficial vascular manifestations can reflect the status of the skin barrier. Treatment with recombinant mussel adhesive protein improved the water loss conditions, reduced transepidermal water loss rate, and improved microcirculation indicators.
Keratin proteins exhibit highly resistant physicochemical properties and have special biological functions, playing a crucial role in maintaining the normal physiological function of the epidermis. The high expression of CK14 can repair the stratum corneum of the skin. Filaggrin is an important molecule that links keratin fibers in the stratum corneum of human skin [31,32]. We used immunohistochemistry to observe the expression of CK14 and filaggrin. In AD mice, the expression of these three proteins was reduced, while treatment with recombinant mussel adhesive protein significantly increased their expression, indicating that recombinant mussel adhesive protein had a certain repairing effect on the stratum corneum of the skin. Collagen within the skin, produced by cells, is located predominantly in the extracellular matrix of the dermis. This protein, characterized by a complex triple-helix macromolecular structure, is essential for maintaining skin integrity, particularly type I collagen. Masson staining can reflect collagen expression. In this study, Masson staining revealed that collagen fibers appeared blue. In the model group, collagen staining was uneven. The group treated with recombinant mussel adhesive protein demonstrated the repair of these collagen fibers.

AD is a chronic inflammatory skin disease caused by the complex interactions of immune responses, particularly increased skin inflammation and IgE reactions [33]. In our study, we observed upregulation of various pro-inflammatory cytokines in the skin of AD-like lesion mice, such as IL-4, IL-12, IL-5, TNF-α, and IL-13. Treatment with recombinant mussel adhesive protein significantly downregulated the expression of pro-inflammatory cytokines. IgE is a type of antibody found exclusively in mammals, playing a crucial role in type I hypersensitivity reactions such as allergic rhinitis, chronic urticaria, and AD. Elevated levels of IgE in serum can indicate a genetic predisposition to allergies or the presence of type I hypersensitivity reactions [33]. By measuring the levels of IgE in mouse serum, we observed a significant increase in IgE expression in AD mice, whereas treatment with recombinant mussel adhesive protein significantly reduced IgE expression. These experimental results indicated that recombinant mussel adhesive protein could alleviate the inflammatory response in AD.

To further elucidate the anti-inflammatory mechanisms of recombinant mussel adhesive protein, we established an in vitro inflammation model by inducing HaCaT cells with LPS. The expression levels of inflammatory cytokines TNF-α, IL-6, and IL-13 and the anti-inflammatory cytokine INF-γ were assessed using ELISA. Consistent with the in vivo findings, the expression of TNF-α, IL-6, and IL-13 was significantly suppressed, while INF-γ expression was promoted by recombinant mussel adhesive protein. These findings reinforce the anti-inflammatory properties of recombinant mussel adhesive protein. Consequently, we speculate that its therapeutic effect on AD primarily arises from its anti-inflammatory activity.

NF-κB is a class of transcription factors, which includes RelA (p65), RelB, c-Rel, p50, and p52. Normally, NF-κB is bound to the inhibitory protein IκB and remains inactive outside the nucleus. When cells are exposed to inflammatory stimuli, such as infection, stress, or injury, IκB is degraded. This degradation releases NF-κB, which then enters the nucleus and activates various genes associated with inflammation, including cytokines, chemokines, and adhesion molecules, thus inducing an inflammatory response. Prolonged activation of NF-κB is linked to the development and progression of various chronic inflammatory diseases, including arthritis, asthma, and inflammatory bowel disease [34]. Through these mechanisms, NF-κB is directly involved in the production of inflammatory mediators and the regulation of inflammatory responses, serving as an indispensable key factor in the inflammatory process. In addition, NF-κB can also regulate immune response genes, induce various pro-inflammatory genes in innate immune cells, and modulate the activation, differentiation, and effector functions of inflammatory T cells. Therefore, dysregulated NF-κB activation serves as a hallmark of chronic inflammatory diseases [35,36]. Due to its association with various inflammatory conditions, targeting the NF-κB signaling pathway can serve as an effective anti-inflammatory therapy. P65-RelA, a member of the mammalian NF-κB family, regulates the transcriptional activity of NF-κB
through post-translational modifications such as phosphorylation and acetylation of the p65-NF-κB subunit. Several studies have shown that inhibition of the NF-κB pathway can improve AD. For example, inhibition of the MAPK/NF-κB pathway and Th2 axis in macrophages by mangiferin can improve Mc903-induced AD [37]; the TPS240 peptide is a new type of treatment, inhibiting the nf-kappa B and STAT3 activation resistance of atopic dermatitis [38]. In this study, the expression levels of NF-κB p65 and P-p65 were assessed through Western blot analysis. In the model group, P-p65 expression was found to be upregulated. Conversely, treatment with recombinant mussel adhesive protein significantly reduced the expression of P-p65. Thus, recombinant mussel adhesive protein was demonstrated to exert anti-inflammatory effects by inhibiting the protein expression of NF-κB.

Nrf2 serves as a transcription factor primarily involved in cellular defense mechanisms, playing a critical role in antioxidative and anti-inflammatory responses. Nrf2 is recognized as a crucial regulator of antioxidative defenses across different cell types. Under normal physiological conditions, Nrf2 is bound to Keap1 in the cytoplasm, where it is inhibited by Keap1, thus preventing the transcription of genes associated with antioxidants. Under stress conditions, including ROS stimulation, genetic mutations, interference with autophagy, and metabolic changes, the Keap1–Nrf2 complex is disrupted, resulting in the activation of Nrf2. Upon activation, Nrf2 accumulates in the nucleus and induces the expression of a range of antioxidant response genes [18,20,39]. By reducing oxidative stress, Nrf2 helps contributes to the modulation of signaling pathways associated with inflammation, thereby exerting an anti-inflammatory effect [40]. The mutual regulation of the Nrf2 and NF-κB pathways is observed. Firstly, the activity level of NF-κB is decreased by the Nrf2 pathway, which increases the levels of antioxidants and cellular protective enzymes. Additionally, the degradation of NF-κB inhibitor IκB-α is prevented by the Nrf2 pathway, thereby inhibiting NF-κB-mediated transcription. Furthermore, the activation of Nrf2 is inhibited by NF-κB, which reduces ARE transcription [41,42]. Therefore, Nrf2 is a critical factor in cellular antioxidative and anti-inflammatory defenses; the regulation of its activity plays a vital role in maintaining cellular health and preventing associated diseases. Several researchers have demonstrated that the activation of the Nrf2 pathway can exert an antidermatitis effect, for example, ursolic acid ameliorates DNCB-induced atopic dermatitis-like symptoms in mice by regulating TLR4/NF-κB and Nrf2/HO-1 signaling pathways, and a related review of the role of KEAP1–NRF2 system in atopic dermatitis has been elucidated [43,44]. Based on the existing studies reported, we speculated whether the ameliorative effect of recombinant mussel mucin on AD was related to the Keap1/Nrf2 pathway. In this study, the expression levels of Keap1 and Nrf2 were assessed through Western blot analysis. In the model group, Keap1 expression was upregulated. Following treatment with recombinant mussel adhesive protein, a noticeable decrease in Keap1 expression was observed. To examine the impact on Nrf2, the Nrf2 inhibitor ML385 was administered. Compared to the model group, a significant increase in Nrf2 expression was exhibited by the group treated with recombinant mussel adhesive protein. However, the activating effect of recombinant mussel adhesive protein on Nrf2 was reversed upon the addition of ML385. These results indicated that recombinant mussel adhesive protein may alleviate DNFB-induced AD-like symptoms by inhibiting the NF-κB pathway and promoting activation of the Keap1/Nrf2 pathway. In addition, we need further studies to elucidate the direct site of action of recombinant mussel adhesive protein.

5. Conclusions

In conclusion, our research indicates that recombinant mussel adhesive protein exhibits effective anti-inflammatory activity in both DNFB-induced AD mice and LPS-stimulated HaCaT cells by inhibiting the expression of NF-κB P65 and activating the Keap1/Nrf2 pathway, thereby exerting anti-inflammatory effects. In summary, these findings provide a better understanding of the anti-inflammatory mechanism of recombinant mussel adhesive protein in AD and offer new insights for further development of its application in skincare.
Nevertheless, more AD models and clinical trials are needed to demonstrate the effects of recombinant mussel adhesive protein on AD. We believe that recombinant mussel adhesive protein is important as a promising drug in future AD-related studies.

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