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Original article

Mechanism of modified *Yupingfeng* naristillae in protecting the nasal mucosal epithelial barrier during allergic rhinitis

Mécanisme des naristilles Yupingfeng modifiées dans la protection de la barrière épithéliale de la muqueuse nasale pendant la rhinite allergique

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ABSTRACT

Objectives. – Modified *Yupingfeng* naristillae (MYN) is an intranasal traditional Chinese medicinal preparation used to treat allergic rhinitis (AR) by repairing the nasal mucosal epithelial barrier. In this study, the mechanism underlying the effect of MYN was explored to provide a scientific reference for the development and application of new Chinese medicines.

Methods. – Rat models of AR were randomly divided into the normal, AR and MYN groups. After 14 days of intervention, nasal mucosae were collected for histopathological examination. Quantitative real-time PCR was used to detect claudin-1 mRNA and zona occludens-1 (ZO-1) mRNA.

Results. – (1) MYN could improve the swelling, shedding and gap enlargement of the nasal mucosal epithelium and promote the expression of claudin-1 mRNA ($P < 0.05$). However, it did not show a significant regulatory effect on ZO-1 mRNA ($P > 0.05$). (2) MYN could significantly inhibit inflammatory cell infiltration and eosinophil numbers in the nasal mucosa.

Conclusions. – MYN repairs the nasal mucosal epithelial barrier by upregulating the transcription of claudin-1 and reduces nasal inflammatory infiltration.

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R É S U M É

Objectifs. – Le *Yupingfeng* naristillae modifié (MYN) est une préparation médicamenteuse traditionnelle chinoise intranasale utilisée pour traiter la rhinite allergique (RA) en réparant la barrière épithéliale de la muqueuse nasale. Dans cette étude, le mécanisme sous-jacent à l'effet de MYN a été exploré afin de fournir une référence scientifique pour le développement et l'application de nouveaux médicaments chinois.

Méthodes. – Les modèles de RA chez les rats ont été répartis au hasard en groupes normaux, RA et MYN. Après 14 jours d'intervention, les muqueuses nasales ont été prélevées pour un examen histopathologique. Une PCR quantitative en temps réel a été utilisée pour détecter l'ARNm de la claudine-1 et l'ARNm de la zone occludens-1 (ZO-1).

Résultats. – (1) MYN pourrait améliorer la tuméfaction, l'exfoliation et l'élargissement de l'espace épithéliale de l'épithélium de la muqueuse nasale et favoriser l'expression de l'ARNm de la claudine-1 ($p < 0,05$). Cependant, il n'a pas montré d'effet régulateur significatif sur l'ARNm de ZO-1 ($p > 0,05$). (2) MYN pourrait inhiber de manière significative l'infiltration des cellules inflammatoires et le nombre d'éosinophiles dans la muqueuse nasale.

Mots clés :

Yupingfeng

Naristilles

Rhinite allergique

Barrière épithéliale

Inflammation

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Conclusions. – MYN répare la barrière épithéliale de la muqueuse nasale en régulant à la hausse la transcription de la claudine-1 et réduit l'infiltration inflammatoire nasale.

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1. Abbreviations

MYN	modified Yupingfeng naristillae
AR	allergic rhinitis
ZO-1	Zona Occludens-1
TJs	tight junctions
BV	bed volume
OVA	ovalbumin
sIgE	specific IgE
qPCR	quantitative real-time PCR
LSD	Least significant difference
HE	Hematoxylin–Eosin
EM	Epithelial monolayer
EMT	Epithelial–mesenchymal transition

2. Introduction

Allergic rhinitis (AR) is an immune globulin E-mediated inflammation of the nasal mucosa; this condition is characterised with nasal congestion, a runny nose, sneezing and itching [1]. AR, one of the most common immune diseases globally, is a risk factor for asthma and affects 10–40% of the world's population [1,2], which affect the quality of life of patients and may pose a heavy economic burden [1]. The nasal mucosal epithelium, an important immunological barrier, resists the penetration of allergens and microbes. Epithelial barrier dysfunction is central to the development of AR [3]. Tight junctions (TJs) are a key factor in maintaining epithelial barrier function [4]. TJ proteins, which mainly include claudin, zona occludens-1 (ZO-1) and occludin, maintain the connection between epithelial cells and ensure the integrity of the epithelial barrier [5]. In allergic diseases, allergens can directly or indirectly reduce the expression of TJ proteins [6], thereby damaging epithelial barrier leading to persistent allergen exposure and local chronic inflammation [7]. The constant inflammation inhibits the nasal mucosal epithelial repair. Therefore, the essence of protecting nasal mucosa epithelial barrier function involves the regulation of TJ proteins and anti-inflammation in AR.

Studies indicate that the epithelial barrier is a crucial target for the development of new therapies for AR [3]. Local therapy presents several advantages, including quick onset of action and reduced risk of systemic side effects [8], over oral therapy, because it can establish high concentrations of drugs in the nasal mucosa lesions of AR. Modified *Yupingfeng naristillae* (MYN) is developed by enhancing the classic recipe for *Yupingfeng San*, which is composed of astragalus, *Fangfeng*, *atractylodes*, tree peony bark and *Chuanxiong*. *Yupingfeng San* is a traditional Chinese medicine immunomodulator with a history of use of over 800 years [9]. It was first recorded in *Jiu yuan fang* (formulary of exploring cause) and is believed to enhance immunity and anti-allergic reactions. Network pharmacological analysis has shown that the target of action of the concoction is autoimmune diseases, such as AR and asthma [10]. Its efficacy has been clinically proven. Given that *Yupingfeng* is mainly used to treat AR via the oral route, however, its effects are limited by its easy decomposition by digestive enzymes due to its slow absorption in the digestive tract. Topical administration of MYN conforms to the focal characteristics of AR, thus avoiding the limitation of first-pass elimination by oral administration and

decomposition by the gastrointestinal tract. A clinical study confirmed that the effectiveness of MYN in the intranasal treatment group is higher than that in the internal treatment group [11]. AR easily causes local microcirculation stasis because of its prolonged and repeated course. Pathological studies have also found that the nasal mucosa exhibits increased vascular diameters [12] and vascular hyperplasia [13] in AR. MYN is prepared by enhancing *Yupingfeng* with *Chuanxiong* and tree peony bark, which promote *qi* flow, blood circulation and local microcirculatory improvement. In vivo experiments revealed that MYN can reduce the telangiectasia and congestion of the nasal mucosa of AR guinea pigs [14] and reduce the blood viscosity and platelet-activating factor of AR rats [15]. Comparative studies have found no significant differences between the effects of MYN and those of triamcinolone acetonide [14] and mometasone furoate nasal sprays in improving epithelial swelling and spaces and reducing epithelial inflammatory infiltration and nasal symptom scores [15,16]. Network pharmacology confirmed that *Yupingfeng San* has a positive effect on epithelial cell proliferation and steroid hormone responses [17]. The nasal effects of MYN have also been verified in clinical observations [11]. However, the nasal therapeutic mechanism of MYN remains unclear.

The occurrence of nasal mucosal epithelial barrier dysfunction is closely related to the low expression of epithelial TJ proteins and inflammation in AR. Therefore, this study observes the effects of drugs on TJ proteins and inflammation to investigate the mechanism of MYN on the nasal mucosal epithelial repair of the AR rat model.

3. Materials and methods

3.1. Ethics statement for animal studies

The female rats (7–8 weeks old; weight, 220–240 g) used in the experiment were obtained from the Experimental animal research centre of Chengdu University of Traditional Chinese Medicine and raised under SPF conditions. The animal licence number is SCXK (Sichuan) 2019-11 (Chengdu, China), and the animal research centre licence number is SYXK (Sichuan) 2019-049 (Chengdu, China). The experimental protocol was approved by the medical ethics committee of the Hospital of Chengdu University of Traditional Chinese Medicine.

3.2. Preparation of MYN

MYN consists of 5 crude herbs: *Astragalus membranaceus* (Fisch.) Bunge (30 g, D1906015), *Atractylodes macrocephala* Koidz (15 g, D1905086), *Saposhnikovia divaricata* (Trucz.) Schischk (10 g, D1910031), *Paeonia suffruticosa* Andr (15 g, 1909085) and *Ligusticum chuanxiong* Hort (10 g, 1908134), corresponding to the common dose for adult humans, which was prepared and provided by the Department of Pharmacy, Hospital of Chengdu University of Traditional Chinese Medicine.

The best anti-allergic effect on the rat model of AR was obtained with 1.05 g/mL MYN (Fig. 1). This concentration was selected through preliminary experiments. The medicinal herbs were decocted with water thrice, adding water 10 times the amount of the herbs, each time for 1 hour. And then, filter the decoction.

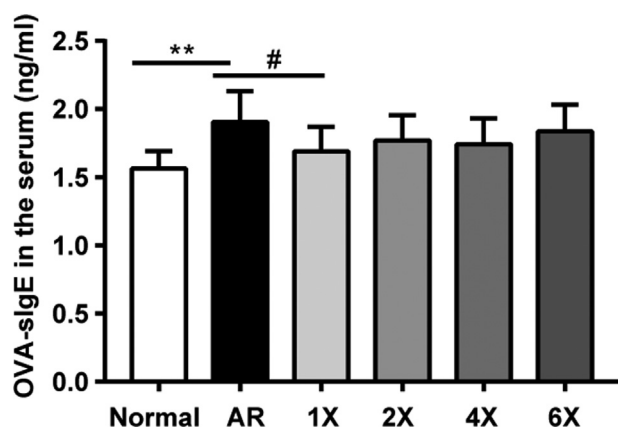


Fig. 1. Therapeutic effects of different concentrations of modified *Yupingfeng* naris-tillae (1.05, 2.10, 4.20 and 6.30 g/mL) on ovalbumin (OVA)-induced AR rats. Serum levels of OVA-specific IgE (sIgE) were determined via ELISA. OVA-sIgE protein expression in the 1× (1.05 g/mL) group remarkably decreased compared with that in the AR group. Data are expressed as mean ± SD ($n=6-8$, ** $P<0.01$ and * $P<0.05$ vs. the normal group; # $P<0.05$ vs. the AR group).

The filtrate was concentrated to a relative density of approximately 1.05. After centrifugation, the supernatant was diluted with water to a volume of 2000 mL and passed through an AB-8-type macroporous resin column with a diameter-to-height ratio of 1/6 and 1 BV of water for purification; the volume and flow rate of the feeding samples were maintained at 2 BV/h. Subsequently, elution was carried out with 4 BV of 70% ethanol solution at a flow rate of 4 BV/h. The alcohol eluate was collected, and ethanol was recovered under reduced pressure. The pH of the eluate was adjusted to 6.0–7.0 with 5% NaOH solution. The eluate was then refrigerated and allowed to stand for 24 h. After filtration, the liquid was added to 1000 mL of distilled water, and the osmotic pressure was adjusted to obtain an MYN preparation with a concentration of 1.05 g of crude drug per millilitre of solution.

3.3. Establishment and intervention of the AR model

The rats were randomly divided into the normal, AR and MYN groups with 7 rats per group. On days 1, 3, 5, 7, 9, 11 and 13, rats in the AR and MYN groups were intraperitoneally injected with 1 mL of normal saline + 0.3 mg of ovalbumin (OVA, BoMei, LA5253, Wuhan, China) + 30 mg of hydrogen alumina (Mayhui Biotech, MB0215, Dalian, China) for sensitisation. From days 15 to 21, sensitised rats were intranasally stimulated (50 μ L/nos-tril/day) with 0.5 g of OVA dissolved in 10 mL of PBS daily. After the final stimulation of the nasal cavity with OVA, the sum of the symptom scores for sneezing and scratching in each group was recorded. The specific scoring criteria were as follows:

- nasal itching: 1 point for mild nasal itching (the nose is rubbed several times), 2 points for moderate nasal itching (the nose and face are scratched occasionally) and 3 points for severe nasal itching (rubbing and scratching around points);
- sneezing: 1 point for sneezing 1–3 times, 2 points for sneezing 4–10 times and 3 points for sneezing 11 times or more.

The symptom scores of the AR (4.79 ± 0.76) and MYN (4.64 ± 0.75) groups were significantly higher than those of the normal group (1.64 ± 0.85). After 30 min, the tail was removed from each rat. Blood was collected from the tail vein, and changes in serum OVA-specific IgE (sIgE) were observed. Serum OVA-sIgE levels in the AR (1.64 ± 0.27) and MYN (1.65 ± 0.17) groups were significantly higher ($P<0.01$) compared with those in the normal

group (1.30 ± 0.20). Thus, the model was considered to have been successfully established.

From days 22 to 35, rats in the normal, AR and MYN groups received intervention respectively. The normal and AR groups received 0.9% normal saline via a nasal drip (10 μ L/nos-tril) once a day, whilst the MYN group received MYN via a nasal drip (10 μ L/nos-tril) once a day. The AR and MYN groups were stimulated intranasally with 5% OVA solution (50 μ L/nos-tril) after 30 min of intervention [15].

3.4. Histological examination of nasal mucosae

After treatment (day 36), the rats were anaesthetised through intraperitoneal injection of 3% sodium pentobarbital (30 mg/kg), and the nasal mucosae were separated and fixed with 4% neutral buffered formalin at room temperature. The tissues were embedded in paraffin, sectioned and stained with haematoxylin and eosin (HE, Art. Nos. G1005-1 and G1001; Wuhan, China). A BA200 digital microscope camera system (Motic China Group Co.) was used to obtain images of the samples. Gross pathological changes in each slice were observed under 40× magnification. Subsequently, the diseased area was evaluated, and photographs were acquired under 100× and 400× magnification to score lesion degrees. Three photographs obtained under 400× magnification were collected for eosinophil counting (number/400× field of view), and the average eosinophil count was calculated.

3.5. Quantitative real-time fluorescence polymerase chain reaction

Quantitative real-time PCR (qPCR) was applied to test changes in claudin-1 and ZO-1 expression in the nasal mucosa. An Animal Total RNA Isolation Kit (Art. No. RE-03014, Foregene, Chengdu, China) was used to extract total RNA from samples. Complementary DNA synthesis was performed by using a PrimeScript RT reagent kit (Art. No. RR047A, TAKARA, Japan) in accordance with the manufacturer's instructions. qPCR was performed with TB Green TM Premix Ex Taq TM II (Art. No. RR820A, TAKARA, Japan) on an RT-PCR instrument (Thermo Fisher, USA). cDNA was denatured through 45 PCR cycles (95°C, 30 s; 95°C, 5 s; 55°C, 30 s and 72°C, 30 s). β -Actin was applied as the invariant control, and the relative level of mRNA was calculated via the $2^{-\Delta\Delta C_t}$ method by using Thermo Scientific PikoReal software (Thermo, USA). The primer sequences are listed: β -actin, GAA GAT CAA GAT CAT TGC TCC (forward) and TAC TCC TGC TTG CTG ATC CA (reverse); claudin-1, GGT GCC CTA CTT TCC TGC TCC TGT C (forward) and GAT CTC TTC CTT CGC CGC TGT CAC A (reverse); ZO-1, GGA GTG TCA TTG CCG TCG CAT GTA GA (forward) and TGT ATG GTG GCT GCT CAA GGT CTC TG (reverse).

3.6. Statistical analysis

SPSS 25.0 software was used for statistical analysis. The Shapiro–Wilk method was used to check whether the data followed a normal distribution. The Levene method was used to test the uniformity of variance. One-way analysis of variance was used for multigroup comparisons of data conforming to a normal distribution and showing homogeneity of variance. Homogeneity of variance was tested via the least significant difference method, and the heterogeneity of variance was tested via Tamhane's T^2 test. The results are expressed as mean ± standard deviation. $P<0.05$ was considered to indicate statistically significant differences.

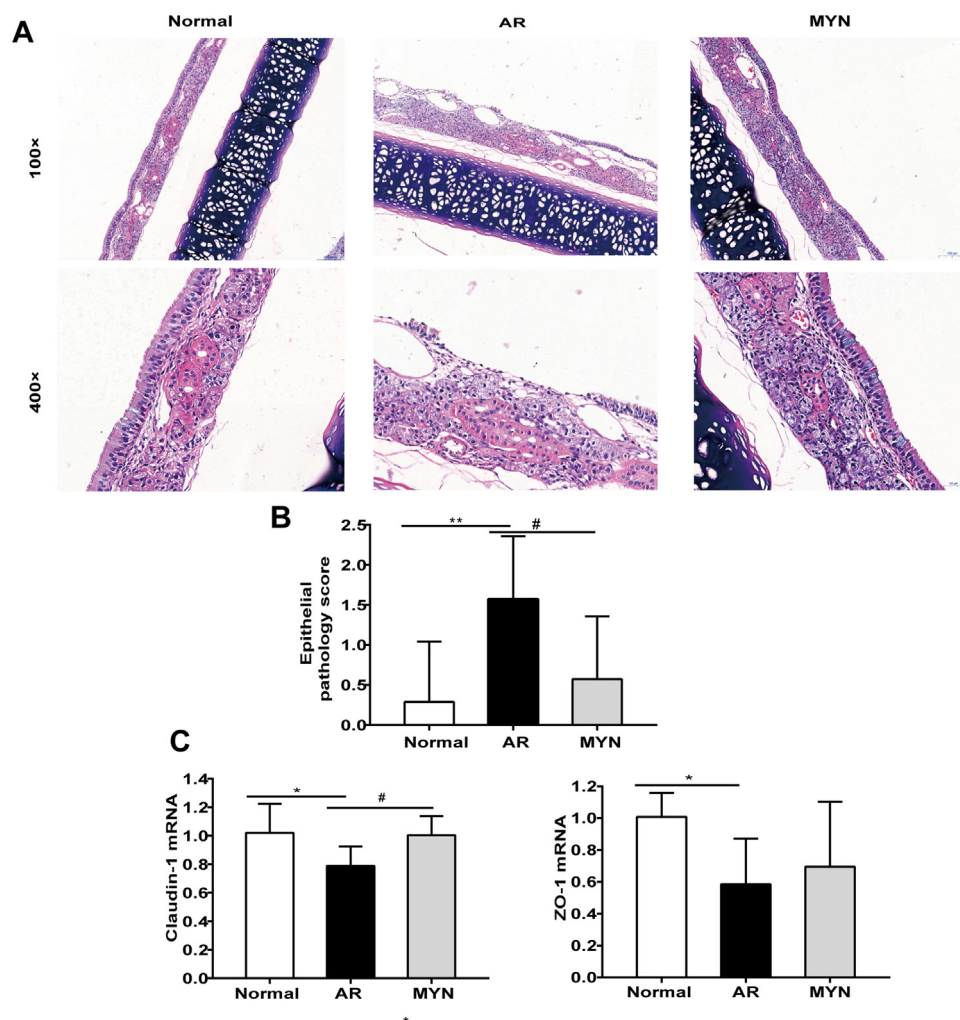


Fig. 2. Effect of modified *Yupingfeng* naristillae (MYN) on the nasal mucosal epithelial barrier. A. Photograph of the pathology of the nasal mucosal tissue (HE, 100 $\times$, 400 $\times$). The structure of the epithelial layer of the mucosal tissue in the MYN group improved compared with that in the AR group. B. Pathological score of the epithelial layer. Epithelial damage in rats in the MYN group was relieved relative to that in rats in the AR group. C. The expression levels of claudin-1 and ZO-1 mRNA in nasal mucosae were quantified by using qPCR. Compared with those in the AR group, the expression levels of claudin-1 mRNA, but not those of ZO-1 mRNA, increased in the MYN group. Data are expressed as mean $\pm$ SD ($n = 6-7$, * $P < 0.05$ vs. the normal group; # $P < 0.05$ vs. the AR group).

4. Results

4.1. MYN reduces damage to the epithelial barrier in the rat model of AR

HE staining was performed to observe the epithelial layer of rat nasal mucosae (Fig. 2A). In the normal group, the structure of the nasal mucosa was clear, continuous and complete, and the ciliated columnar epithelium of the respiratory area was normal and neatly arranged. In the AR group, the structure of the nasal mucosal tissue was damaged to varying degrees, the epithelial cilia were nearly completely shed and a small amount of residual cilia lodging was disordered. In addition, the epithelium was swollen, the epithelial gap was enlarged and part of the epithelium was necrotic and shed. Residual epithelial cell defects were clearly observed. The structure of the nasal mucosal tissue of the MYN group was clear with a small amount of disorderly arranged cilia. In this group, the swelling of epithelial cells was not obvious, the intercellular space was normal and goblet cells were occasionally slightly enlarged in some areas. Quantitative analysis showed (Fig. 2B) that the score of epithelial lesions in the AR group was significantly higher than that in the normal group ($P < 0.05$) and that the lesion score in the MYN group was significantly lower than that in the AR group ($P < 0.05$). The

score of the normal group has no differences with the MYN group ($P > 0.05$).

4.2. MYN upregulates the transcription of TJ proteins between epithelial cells

qPCR technology was used to detect the transcription of claudin-1 and ZO-1 mRNA in the nasal mucosa (Fig. 2C). Compared with those in the normal group, the expression levels of claudin-1 and ZO-1 mRNA in the AR group significantly decreased ($P < 0.05$). Compared with that in the AR group, the expression level of claudin-1 mRNA in the MYN group significantly increased ($P < 0.05$). However, the increase in ZO-1 mRNA expression in the latter was not statistically significantly different compared with that in the former ($P > 0.05$). These results suggest that MYN could promote the transcription of claudin-1 mRNA, but has no significant effect on the transcription of ZO-1 mRNA.

4.3. Reduction in the inflammatory infiltration of nasal mucosae by MYN

Rat nasal mucosae were subjected to HE staining to observe inflammatory infiltration (Fig. 3A). HE staining showed the absence

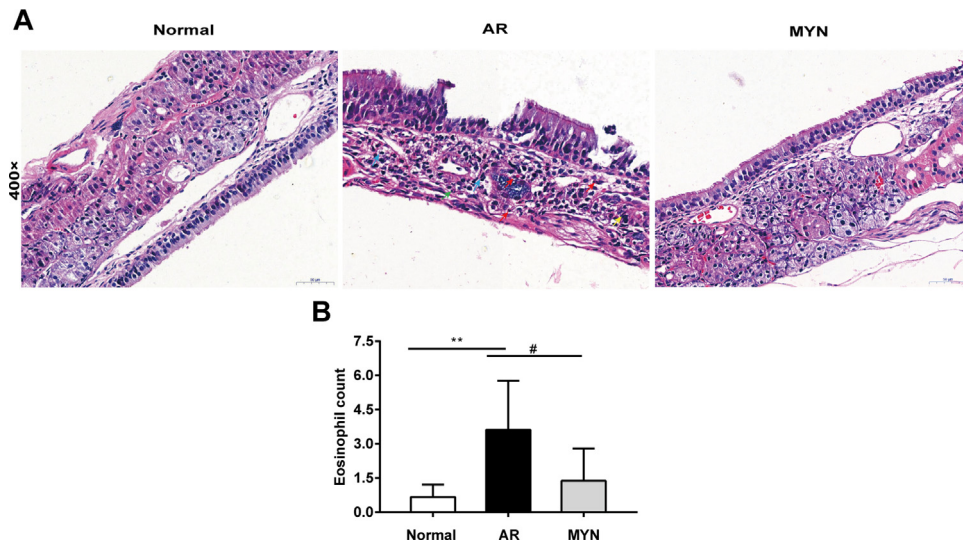


Fig. 3. Effect of modified *Yupingfeng* naristillae (MYN) on nasal mucosal inflammation. A. Photograph of nasal mucosa tissue pathology (HE, 400 $\times$). The inflammatory infiltration of mucosal tissue in the MYN group improved compared with that in the AR group (blue arrows, lymphocytes; red arrows, eosinophils; green arrows, neutrophils; yellow arrows, mast cells). B. Eosinophil counts in nasal mucosae. The eosinophil count in the MYN group decreased compared with that in the AR group.

of inflammatory cell infiltration in the epithelial layer of the normal group. In this group, abundant glands, blood vessels and nerves were seen in the connective tissue of the lamina propria, and a small number of inflammatory cells were occasionally scattered in some areas. No other obvious pathological changes were seen. By contrast, in the AR group, inflammatory cells were occasionally seen in the epithelial layer. Cell degeneration and necrosis were observed in the lamina propria, and the structure of the necrotic area was disordered. Neutrophils and lymphocytes were scattered in the necrotic area. Visible eosinophil infiltration was observed, and mast cells were occasionally seen. No other obvious pathological changes were noted. In the MYN group, no inflammatory cell infiltration was observed in the epithelial layer, very few lymphocytes were occasionally scattered in the lamina propria and no other obvious pathological changes were seen. The degree of inflammatory infiltration in the experimental groups followed the order normal group < MYN group < AR group. Compared with that in the normal group, the number of eosinophils in the AR group extremely significantly increased ($P < 0.01$; Fig. 3B). By contrast, the increase in eosinophil count in the MYN group was not statistically significant ($P > 0.05$). Compared with that in the AR group, the eosinophil count in the MYN group extremely significantly decreased ($P < 0.01$).

5. Discussion

The epithelial barrier is the first physiological barrier of the body's defence system [18]. Airway epithelial cells are connected by TJ proteins, which form the first barrier against external pathogens, including bacteria, viruses, chemicals and allergens [19,20]. Airway epithelial cells can coordinate the immune defence of the body, transmit external signals to immune cells to clear the external pathogens in the respiratory tract and avoid causing inflammation [21]. Allergens containing proteases and environmental contaminants promote the paracellular transport of epithelial cells by destroying the TJ proteins of the epithelial barrier, resulting in the transmission of allergens to the subcortical layer [22]. This process causes epithelial barrier damage and immune inflammation, which are important pathological links in the occurrence of AR [20]. The destruction of the airway epithelium increases the penetration of allergens and microorganisms, which drive mast cell degranulation and induce AR [18]. This process also causes the release of

epithelial-derived cytokines, which drive type 2 immune response [23]. Cytokines such as IL-4, IL-13, IL-31, TSLP and IL-33 in turn affect local tissue damage and repair in allergic diseases [24]. Therefore, protecting the epithelial barrier function is key to treat AR [22].

The destruction of the airway epithelium may be caused by defective or the loss of TJs or adherens junctions. TJs are crucial molecules that constitute the epithelial barrier. They can connect and fix adjacent nasal mucosal epithelial cells and close intercellular spaces, maintaining the epithelial barrier function of nasal mucosa epithelium [22] and preventing the infiltration of allergens. Defects and dysfunctions of epithelial TJs are one of the characteristics of AR. The disruption of the nasal mucosal epithelial barrier was observed in an animal model of AR, leading to more penetration of the inhaled allergen into the subepithelial space and resulting in increased antigen uptake and activation of epithelial signal transduction and immune response [25].

Claudin-1 and ZO-1 are essential transmembrane protein of TJs [6]. Claudin-1 is located at the top of the TJ of columnar cells and basal cells in the airway [26]. The increase of claudin-1 increases the tightness and trans-epithelial electrical resistance of the epithelial monolayer (EM) and reduces the permeability of the EM [27]. Claudin-1 is essential for the formation and maintenance of the EM barrier and regulating the tightness and selectivity of the TJ chain; it is also directly involved in the transport of substances through the paracellular pathway. By contrast, mice lacking claudin-1 showed severely defective permeability barrier of the epidermis and damaged epidermis barrier and died within 1 day after birth [28]. ZO-1 is located in the uppermost layer of the human nasal mucosal epithelium and usually assembles with claudins to form a TJ barrier between epithelial cells. The decomposition of ZO-1 destroys the paracellular barrier of epithelial cells. Thus, ZO-1 plays an important role in maintaining epithelial barriers by linking other TJ molecules to seal epithelial cells against infiltration by allergens [20]. In AR mouse models, low expression of ZO-1 causes increased epithelial barrier permeability, allergen permeability and aggravation of allergic reaction [29]. Impaired ZO protein is also observed in patients with AR, causing allergens to enter the subepithelial layer and leading to AR. Therefore, restoring ZO protein expression and improving the integrity of the nasal epithelial barrier of AR are critical for the future development of targeted therapies [20].

Allergens and inflammatory factors damage the epithelial barrier by destroying epithelial cells and their connexins [30]. In the present study, the nasal mucosal cilia of AR rats fell off in a disorderly manner, the epithelial swelling gap enlarged, the amounts of claudin-1 mRNA and ZO-1 mRNA decreased and part of the epithelium fell off. MYN treatment repaired cilia damage and arrangement, improved epithelial cell swelling, upregulated the expression of claudin-1 mRNA between epithelial cells, restored intercellular spaces, increased the fixation of epithelial cells and inhibited shedding. MYN upregulated the expression of ZO-1 mRNA ($0.70 \pm 0.41/0.59 \pm 0.29$), but no statistical significance was observed. Studies have found that the nasal mucosal epithelial-mesenchymal transition (EMT) is elevated in patients with AR [31]. When the normal balance of the epithelium is disrupted by allergens, the repair process is initiated to minimise further damage caused by microorganisms and the environment. In this process, mature differentiated epithelial cells undergo EMT, lose their attachment to the basement membrane, lose their polarity with each other and begin to divide and migrate to rapidly cover the injured area. During this process, epithelial cells lose the expression of TJ proteins ZO-1 and E-cadherin [32]. Studies have confirmed that the proliferation of retinal pigment epithelium and the increase of EMT-like phenotype were correlated with the downregulation of ZO-1 [33]. Claudin-1 can induce matrix metalloproteinase-2 activity, promote EMT and increase the invasion and migration of hepatocellular carcinoma cells [34]. Suh et al. also supported this view. The overexpression of claudin-1 induced the expression of EMT-related transcription factors, thereby inhibiting the expression of E-cadherin and β -catenin and resulting in the loss of cell adhesion and enhancement of cell movement [35]. In summary, MYN may increase the expression of claudin-1 by upregulating claudin-1 mRNA and promoting EMT. The EMT process reduces the expression of ZO-1 mRNA and downregulates ZO-1, which counteracts the upregulation effect of MYN on ZO-1 mRNA and makes the change insignificant. However, this hypothesis needs to be verified by subsequent experiments. The overall results indicate that MYN reduced damage to the epithelial barriers of the AR rat model by upregulating the expression of claudin-1 mRNA.

High-performance liquid chromatography analysis showed that MYN contains three flavonoids, namely, prim-O-glucosylcimifugin, calycosin-7-O- β -D-glucoside and 5-O-methylvisammioside. The contents of prim-O-glucosylcimifugin, calycosin-7-O- β -D-glucoside and 5-O-methylvisammioside in 1 mL of qualified MYN sample solution were 0.2995, 0.1335 and 0.1498 mg, respectively. Prim-O-glucosylcimifugin can regulate the expression levels of ZO-1 and occludin proteins in the intestinal barrier dysfunction model, thereby maintaining the permeability and integrity of the epithelium [36]. Formononetin, the main component of astragalus, exerts a protective effect on the epithelium [37] and may be a potential factor endowing MYN with epithelial repair effects. The main component of MYN, calycosin, can inhibit hypoxia-inducible factor-1 [38], upregulate the interepithelial TJ proteins (i.e. occludin and ZO-1) and the adherens junction protein (i.e. E-cadherin) and protect epithelial barrier function [39].

Considering that nasal inflammatory infiltration aggravates damage to the nasal mucosal epithelium, controlling nasal inflammation is vital to repair the epithelial barrier and treat AR. The epithelial cell-derived cytokines are warning [40] and initiating [41] factors of allergic inflammation. They can mediate the proliferation and differentiation of a variety of immune cells, such as type 2 innate lymphocytes, natural killer cells and mast cells [42], and promote in situ eosinophil production in a type-2 microenvironment [43]. They can also activate eosinophils to release granular protein [44] and mediate local inflammatory infiltration.

The current study demonstrated that flavonoids in traditional Chinese medicine can reduce the release of inflammatory factors in human airway epithelial cells induced by lipopolysaccharides [45]. Calycosin can reduce allergic inflammation in a mouse model of AD by downregulating the activation of NF- κ B [46] and inhibiting the release of epithelial cell-derived TSLP [39]. Prim-O-glucosylcimifugin and 4'-O- β -D-glucosyl-5-O-methylvisammioside can inhibit histamine-mediated allergic inflammation [47]. 4'-O- β -D-glucosyl-5-O-methylvisammioside can also block cell mitosis and the expression of pro-inflammatory genes, thus exerting strong anti-inflammatory activity [48]. Therefore, the nasal anti-inflammatory effect of MYN may be related to its flavonoids. Network pharmacological research has found that *Yupingfeng San* exerts a steroid hormone response [17]. Moreover, it can regulate the expression of the epithelial-derived proallergic cytokine TSLP, thus reducing allergic inflammation [49]. Given these findings, the pharmacological characteristics of individual MYN components have been suggested to be potential factors influencing the preparation's nasal anti-inflammatory effect. Studies of *Chuanxiong* and tree peony bark found that their chemical components of gallic acid and chuanxiongrolide can inhibit the maturation of dendritic cells in allergic inflammation [50] and reduce mast cell-mediated allergies [51], thereby exerting anti-inflammatory effects. This may be another mechanism through which MYN achieves epithelial barrier repair. Other components of MYN must be identified to establish a solid foundation for research on the efficacy of this treatment.

6. Conclusion

This study analysed the epithelial barrier protective mechanism of MYN in an AR rat model. MYN could promote the transcription of TJ proteins between epithelial cells to reduce the pathological damage of the epithelial layer of the nasal mucosa. It could also reduce nasal inflammatory infiltration. Thus, MYN exerts a local therapeutic effect on AR by repairing the epithelial barrier. This study provides experimental scientific evidence of the clinical application of MYN in the treatment of AR. Other experimental explorations on MYN are needed to confirm the findings because of the multi-component, multi-target and multi-channel characteristics of traditional Chinese medicine compound prescriptions.

Ethics approval and consent to participate

The experimental protocol was approved by the Medical Ethics Committee of the Hospital of Chengdu University of Traditional Chinese Medicine and under the guidelines of "Measures for the Quality Management of Laboratory Animals" (1997), "Regulations on the Management of Laboratory Animals" (2011), "Guiding Opinions on Treating Laboratory Animals Kindly" (2006), and "Quality Management Standards for Non-clinical Research of Drugs" (2003). The study conformed with European and French legislation.

Availability of data and materials

The data and materials used to support the findings of this study are available from the corresponding author upon request on reasonable request.

Disclosure of interest

The authors declare that they have no competing interest.

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