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Research article

Majie cataplasm provides a shield against asthmatic punch from the neuroimmune system

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ABSTRACT

Asthma poses a threat to human health, and its pathogenesis is closely related to the neuroimmune system. *Majie* cataplasm can not only regulate the immune system but also the nervous system in asthma patients for its components. We speculate that *Majie* cataplasm may relieve asthmatic patients with sensitivity to hormone or not by regulating the body's neuroimmune system.

Methods: In this experiment, a mouse model of asthma was well established by ovalbumin. The lung function of animals was examined and pathological changes in the lung tissue were assessed by hematoxylin-eosin staining. Serum immunoglobulin E (IgE), calcitonin gene-related peptide (CGRP) and neurokinin A (NKA) were measured by ELISA. The location of CGRP, CD3 and neutrophil in lung tissue and their expressions were detected by immunofluorescence staining. In addition, contents of CGRP mRNA, Substance P (SP) mRNA, interleukin (IL)-17 mRNA and interleukin(IL)-13 mRNA were detected by quantitative polymerase chain reaction.

Results: Compared with the asthma model group, *Majie* cataplasm and dexamethasone can not only equivalently relieve airway hyperresponsiveness, but also make the content of serum IgE reduced. In addition, they can lower the content of serum CGRP and NKA after OVA stimulation, and this effect was more obvious for *Majie* cataplasm. Our results also showed that Majie Cataplasm and dexamethasone could inhibit the secretion of CGRP and the infiltration of T lymphocytes together with neutrophils in lung tissue and reduce expressions of CGRP mRNA, SP mRNA, IL-17 mRNA and IL-13 mRNA in lung tissue.

Conclusion: *Majie* cataplasm effectively relieves expressions of neuropeptides such as CGRP, reduces the infiltration of immune cells in lung tissue, regulates the body's neuroimmune system, and has a therapeutic potential for both Th2 asthma and neutrophilic asthma.

1. Introduction

Globally, the prevalence of asthma is in a sharp rising [1]. As one of the most common respiratory diseases, asthma seriously endangers the human health and poses great economic burden on patients. Research suggests that the essence of asthma is chronic airway inflammation. Therefore, most of the previous experiments focus on the body's immune system, deeming that the body's immune disorder and the release of inflammatory factors eventually lead to asthma. In clinical practice, asthma immunotherapy has limited effects [2]. This seems to suggest that there are other pathogenesis involved in the development of asthmatic respiratory inflammation. Increasing studies have verified that the nervous system is closely related to the immune system [3], and the interaction of neural and immune system monitors and regulates the inflammatory response. Thus, the pathogenesis of asthma is also bound up with neuro-immune disorders. Typical symptoms of asthma include bronchial hyperresponsiveness, cough, spasms and inflammation, which are all associated with impaired respiratory neurons and/or dysregulated immune responses [4], and asthmatic patients have a dense network of sensory fibers around the airways [5]. Researchers have gradually recognized the importance of neuroimmune interaction in maintaining stable homeostasis and defense in lung tissue.

Majie cataplasm achieves a therapeutic effect through skins, which is safe and non-toxic [6]. It consists of five traditional Chinese medicines: *Ephedra Herba* (Mahuang), *Semen Sinapis* (Baijiezi), *Semen Armeniacae Amarum* (Kuxingren), *Rhizoma Corydalis* (Yanhusuo), and *Rhizoma*

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Zingiberis Recens (ginger). *Ephedra Herba*, *Semen Sinapis*, and *Rhizoma Corydalis* all own the potential to regulate neuropeptides. *Ephedra Herba* prevents the development of pulmonary fibrosis by reducing the level of endothelin (ET)-1 in the blood [7] and suppresses the expression of Neuropeptide Y in hypothalamus of Food-Deprived Rat [8].“Eliminating phlegm and strengthening intelligence” prescription containing *Semen Sinapis* can improve neurite growth and elevate the expression of growth associated protein-43 [9]. Tetrahydropalmatine, the main compound of *Rhizoma Corydalis*, regulates D2 dopamine receptors in the central nervous system, which is the main analgesic mechanism [10]. In addition, both *Ephedra Herba* [11] and *Semen Sinapis* [12] modulate transient receptor potential vanilloid 1 (TRPV1) of sensory neurons. And they alleviate the capsaicin-induced pain and asthma respectively.

During the previous research of *Majie* cataplasm, we found that it can regulate the immune system in asthmatic patients, but this regulation mechanism is not a single one. It may exert an effect through other pathways [13]. Therefore, we speculate that *Majie* cataplasm probably exerts role in asthma by regulating the body's nervous system.

As a typical neuropeptide, CGRP links up with asthma promoting inflammation through the corresponding receptors on immune cells [14]. And no matter how asthma is classified, its pathogenesis is closely related to neuropeptides and immune cells [15]. In this paper, we explored the regulation of *Majie* cataplasm about the secretion of CGRP and other neuropeptides in ovalbumin (OVA) asthmatic mice and its influence on the interaction between CGRP and related immune cells. In addition, asthma is roughly divided into hormone-sensitive asthma and non-hormone-sensitive asthma. The former is mainly characterized by the infiltration of eosinophils with a Th2 inflammatory response [16]. For non-hormone-sensitive asthma, neutrophilic asthma is an important type with the overproduction of IL-17 and a infiltration of neutrophils [17, 18, 19, 20]. Therefore, we intend to evaluate the effects of *Majie* cataplasm and dexamethasone on eosinophilic and neutrophilic asthma and analyzed the reasons as well.

2. Methods

2.1. The making process of Majie cataplasm

Except for the Rhizoma Zingiberis Recens purchased from the local Walmart, other ingredients of *Majie* cataplasm were bought from Beijing Tongrentang Pharmaceutical Co. Ltd., China. According our previous experiments, the technique of *Majie* cataplasm is reliable. It has good reproducibility and can be mass-produced, which lays a good foundation for the subsequent medicinal mechanism experiments. The fixed dose of these five medicines for each piece of *Majie* Cataplasm is 4, 4, 4, 4, and 4 g respectively. One piece of *Majie* cataplasm is taken once a day for humans. The load of each piece of *Majie* cataplasm is uniform, and the contact area is 63cm^2 (length 9cm x width 7cm), which is converted to a mouse application area of about 0.2cm^2. We cut it into small square patches of 0.45 cm $\times$ 0.45 cm.

Refer to the supplementary material called “making process of Majie Cataplasm” to see more details.

2.2. Mice

In this experiment, WT C57/BL6 mice (aged 6–10 weeks) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China; No. SCXK 2019-0010). All mice were fed in house and kept under specific pathogen-free conditions. This study was approved by the Ethics Committee on Animal Experiments of Beijing University of Chinese Medicine (Approval number: BUCM-4-2019031001-1077).

2.3. OVA-induced asthma model [21] and drug intervention

Forty C57 mice except control group were induced by OVA and divided into the control group (n = 10), the asthmatic model group (n = 10), the *Majie* cataplasm group (n = 10) and the dexamethasone group (n = 10).

On Days 0, 7, and 14, C57 mouse (excepting which in the control group) received an intraperitoneal injection (i.p.) of a solution about 0.2 mL containing 0.05 mg of OVA and 1 mg of Alum gel. On Days 15–25, the animals were challenged with an intranasal injection of OVA (2.5 mg/mL diluted in PBS, 40 μL each mouse). In the control group, 0.2 mL phosphate buffer solution (PBS) was injected on days 0, 7, and 14 and during the days of 15–25, each mouse was challenged with 40 μL PBS.

On Day 15 of the experiment, the intervention measures of drugs for each group were as follows: the control group (without intervention); the asthma model group (without intervention); the dexamethasone group treated with intraperitoneal injection of dexamethasone sodium phosphate injection 0.1 mL each mouse (2 mg/kg); the *Majie* cataplasm group (put cataplasm on mouse's back, each cataplasm for 24 h). After 10 days, all mice were examined for airway resistance (AR), and then euthanized.

2.4. Quantitative analysis of serum IgE, CGRP, and NKA using enzyme-linked immuno sorbent assay (ELISA)

Levels of IgE and neuropeptides including CGRP and NKA in serum were detected according to the manufacturer's protocols of Raybiotech ELISA kits (Biovision, USA).

2.5. Hematoxylin and eosin staining (H&E)

After fetching left lung tissue, the tissue was fixed with 4% paraformaldehyde dehydrated, embedded in paraffin, and cut into 6 μm sections. After that, the sections were performed with H&E staining.

2.6. Immunofluorescent staining

After dissection, the mouse lungs were immediately placed in 4% paraformaldehyde. After 48h fixation, specimens were rinsed with 20% and 30% sucrose in 0.1 M PBS at 4 °C. The lungs were fetched and embedded in optimum cutting temperature compound (OCT), and immediately frozen in liquid nitrogen. Serial 7μm sections were prepared using a cryostat (Leica CM 1950, Wetzlar, Germany), and then stored in a freezer at -80 °C.

The primary antibodies used: mouse monoclonal anti-CGRP antibody (ab81887, 1:200) and Rat Monoclonal anti-Neutrophil antibody (ab53457, 1:80) were from Abcam (Cambridge, UK). Rabbit polyclonal anti-CD3 (17617-1-AP, 1:170) was from Proteintech (Chicago, USA). All secondary antibodies were diluted at 1:200 and purchased from ZSGB-BIO (Beijing, China) including goat anti-rabbit IgG-Alexa Fluor-488 (ZF-0511), goat anti-mouse IgG-Alexa Fluor-594 (ZF-0513), goat anti-RAT IgG-FITC (ZF-0315). Images were taken using a Leica DM4B fluorescence microscope (Wetzlar, Germany). The Fluorescence colocalization analysis was done through Image J.

2.7. Quantitative polymerase chain reaction (qPCR)

Total RNA was collected from lung tissue using a HiPure Unviersal RNA Mini Kit (Magen, Shanghai, China) reagent protocol in accordance with the manufacturer. RNA was diluted in RNase-free water prepared for cDNA using reverse transcription. 20 μL of cDNA per reaction was prepared using random primer, Oligio (dT), 5x reaction buffer, dNTPs (10 mM), Reverse Transcriptase, and RNase inhibitor (all from Thermo Fisher Scientific, Massachusetts, USA). Quantitative polymerase chain reaction (qPCR) was performed in triplicate using SYBR Green reagents (Thermo Fisher Scientific, Massachusetts, USA). Fluorescence intensities were monitored 50 cycles on a Step One real-time PCR system (Bio-Rad CFX). The relative expression of the target genes was calculated by the $2^{-\triangle\triangle}$CT with β-actin for normalization. All primers in the experiment are shown in Table 1.

Table 1. Primers used in this study.

β-acting	Forward: CGTAAAGACCTCTATGCCAA
	Reverse: TTGATCTTCATGGTGCTAGG
IL-13	Forward: AAAGCAACTGTTTCGCCACG
	Reverse: CCTCTCCCCAGCAAAGTCTG
IL-17	Forward: CTCCACCGCAATGAAGAC
	Reverse: CTTTCCCTCCGCATTGAC
SP	Forward: GGTCCGACAGTGACCAGATCAAG
	Reverse: AAAGAACTGCTGAGGCTTGGGTC
CGRP	Forward: CCTTTCCTGGTTGTCAGCATCTTG
	Reverse: CTGGGCTGCTTTCCAAGATTGAC

2.8. Statistical analysis

Data were presented as mean ± standard deviation (SD). Differences between groups were analyzed by GraphPad Prism 8 software with one-way ANOVA with Bonferroni multiple comparison post-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Evaluation on the improvement of Majie cataplasm on AR, serum IgE, and lung inflammation

Airway hyperresponsiveness reaction (AHR) and high level of serum IgE are important features in most asthma patients. The AR (Figure 1A) and content of serum IgE (Figure 1B) of the mice in each group were tested. The experimental results showed that the AR and the level of IgE in the asthma model group increased compared with those in the control group (both $P < 0.01$). The results of dexamethasone and *Majie* Cataplasm were consistent, both of them had a reduction in AR (both $P < 0.01$), and a decrease in IgE (both $P < 0.01$). We next evaluated the lung histopathology and immunohistochemistry to further clarify the effect of *Majie* cataplasm on lung tissue inflammation in asthma. From the result of H&E staining (Figure 1C) in the asthma model group, there was a large infiltration of inflammatory cells and alveolar septal thickening in the lungs and hyperplasia appeared around the alveolar septum and local trachea. After intervention with dexamethasone and *Majie* cataplasm, the infiltration of inflammatory cells in the lung tissue was significantly reduced. Among them, the effect of dexamethasone was similar to that of *Majie* cataplasm.

3.2. Majie cataplasm reduced serum secretion of CGRP and NKA

We then observed the regulation of asthma-related neuropeptides after the treatment of dexamethasone and *Majie* cataplasm based on the content of serum CGRP and NKA in each group. Results in Figure 2 showed that contents of CGRP and NKA were significantly increased in the asthma model group (both $P < 0.01$). After the intervention of dexamethasone and *Majie* cataplasm, contents of neuropeptides decreased, and the downregulation of *Majie* cataplasm was more obvious.

3.3. Majie cataplasm inhibited CGRP expression in lung tissue and reduced infiltration of T lymphocytes and neutrophils around CGRP

To clarify the effect of *Majie* cataplasm on CGRP and its related immune T cells and neutrophils, we labeled CGRP, T cells (stained with CD3) and neutrophils (stained with neutrophil) by immunofluorescence, not just for their precise localization, but also observing the infiltration of these two immune cells around CGRP.

Figure 3 shows the double staining of CGRP and $CD3^+$T cells in lung tissue of each group. A small amount of CGRP in the lung tissue expressed in the alveolar septa space around the trachea in the control group along with little $CD3^+$T cells infiltration. In the asthma model group, the expression of CGRP increased and a large number of $CD3^+$T cells aggregated around the trachea. In some of these $CD3^+$T cells, the colors of CD3 (green) and CGRP (red) overlapped shown in Figure 5A. According to the analysis of colocalization of CGRP and CD3 in Figure 5C, the Pearson's correlation coefficient (PCC) which is used for evaluating the degree of colocalization is between 0.6 and 0.8, indicating that the degree of colocalization of CGRP and CD3 is strong [22, 23]. Therefore, CGRP was expressed in some $CD3^+$T cells. Additionally, the expression of CGRP was obvious in non-$CD3^+$inflammatory cells surrounding the trachea. There was no apparent infiltration of $CD3^+$T cells in the dexamethasone group and the *Majie* cataplasm group, and the secretion of CGRP around the trachea reduced compared with the asthma model group.

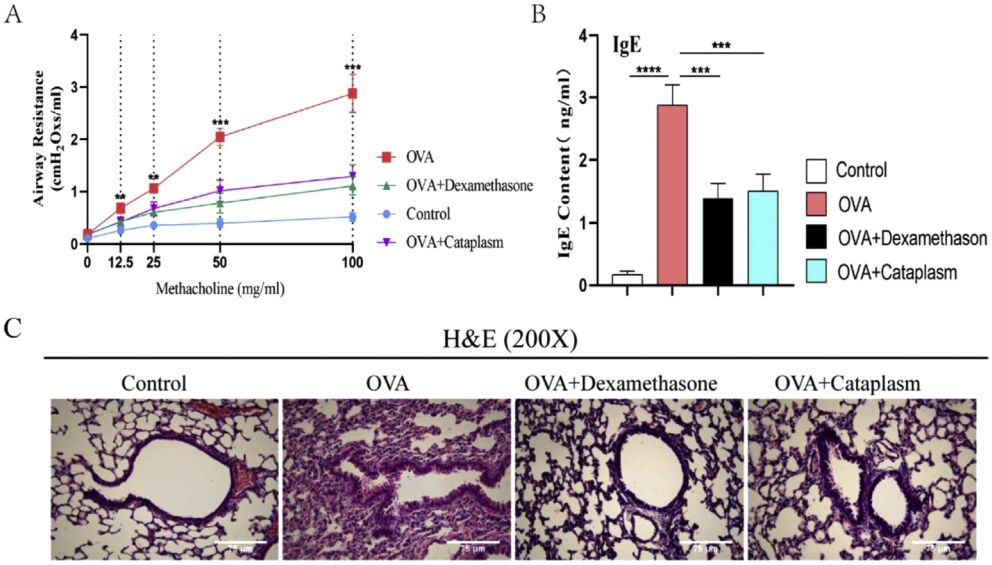

Figure 1. (A) Airway resistance was measured in response to increasing doses of aerosolized methacholine in mice of each group. (B) The level of serum IgE was measured by ELISA. (C) H&E staining of lung sections from mice of each group, the pictures are 200X (scale bars = 75 μm). NS, not significant. $*P < .05$, $**P < .01$, $***P < .001$ and $****P < .0001$.

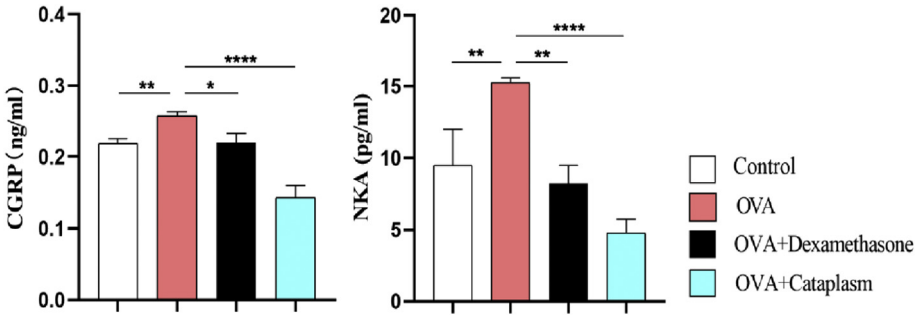

Figure 2. Levels of CGRP and NKA in the serum were assessed by ELISA. NS, not significant. *$P < .05$, **$P < .01$, ***$P < .001$ and ****$P < .0001$.

Figure 4 shows the double staining of CGRP and neutrophils in lung tissue of each group. The CGRP barely expressed in the lung tissue of the control group, and there was no evident neutrophils infiltration in the visual field. In the asthma model group, CGRP and neutrophil densely expressed around the trachea and they co-expressed in some cells as the colors of neutrophil (green) and CGRP (red) overlapped shown in Figure 5B. Combined with the result of colocalization of CGRP and neutrophil in Figure 5C, the PCC is about 0.8, indicating that the degree of colocalization of CGRP and neutrophil is also strong. Thus, CGRP expressed in some neutrophils. Also, CGRP is present in the remaining inflammatory cells. In the dexamethasone group, the expression of CGRP secretion reduced as well as inflammatory cells infiltration, but neutrophils related CGRP were still visible in the visual field. In the *Majie* cataplasm group, the expression of CGRP reduced with scattered inflammatory cells secreting CGRP, but the neutrophils related CGRP significantly reduced, which was more obvious than that in the dexamethasone group.

3.4. Majie cataplasm reduced the expression of CGRP mRNA, SP mRNA, IL-13 mRNA and IL-17 mRNA in lung tissue

We focused on genes related to the neuropeptides together with Th2 inflammation-related cytokine IL-13 and Th17 inflammation-related cytokine IL-17 to further clarify the regulation of neuropeptides in the lung tissue and asthma-related immune inflammation. According to Figure 6, contents of CGRP mRNA and SP mRNA in the asthma model group increased ($P < 0.01$). After interventions of dexamethasone and *Majie* cataplasm, expressions of these neuropeptides were inhibited ($P < 0.01$, $P < 0.05$). Levels of IL-17 mRNA and IL-13 mRNA increased after OVA stimulation, while dexamethasone and *Majie* cataplasm had a regulatory effect on them. However, dexamethasone had a strong inhibitory effect on IL-13 mRNA ($P < 0.01$), while *Majie* cataplasm presented with a great control of IL-17 mRNA ($P < 0.01$).

4. Discussion

As a chronic airway inflammation with AHR, asthma poses a heavy burden on the health and life of the body [24]. In asthma treatment strategy, alleviation of asthmatic symptoms and prevention of asthma deterioration are the therapeutic goal for asthma [25]. In this experiment, by assessing the severity of inflammation in the lung tissue of each group, the level of AR and the content of serum IgE, we could find that dexamethasone and *Majie* cataplasm exert a marked relief on asthma. As a traditional therapeutic drug, although the efficacy of dexamethasone is a bit superior to that of *Majie* cataplasm and widely used in clinical practice, there are still some adverse reactions such as poor patient compliance [26], side effects [25]. And some patients have fear of hormone therapy, hence they prefer to Chinese herbal medicine therapy [27]. The *Majie* cataplasm is composed of *Ephedra Herba, Semen Sinapis*, *Semen Armeniacae Amarum*, *Rhizoma Corydalis*, and *Rhizoma Zingiberis Recens*. As a topical Chinese herbal medicine, it has advantages of good

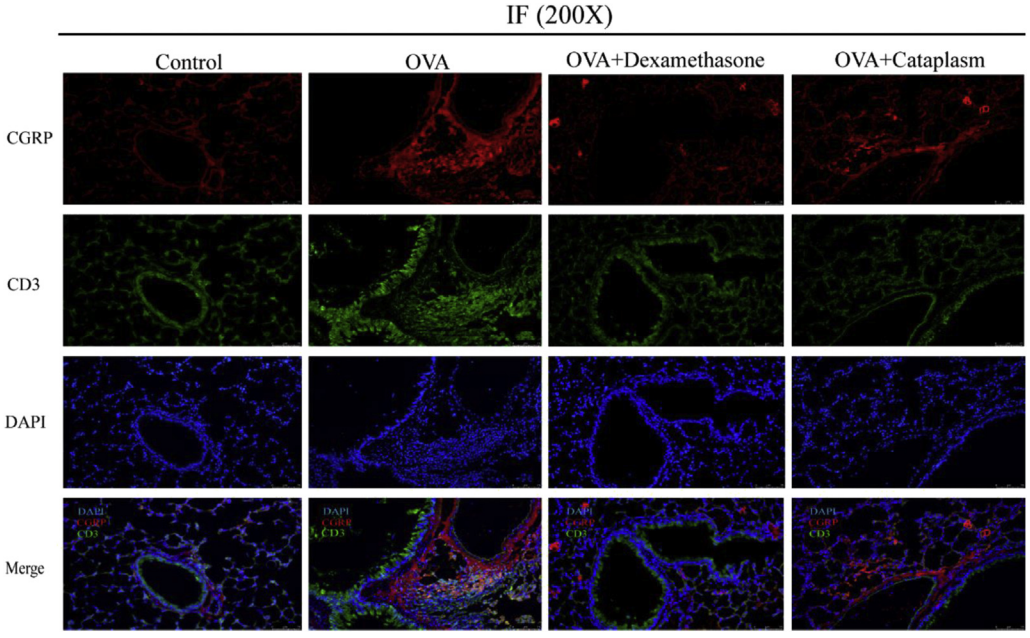

Figure 3. CD3 labeling of T cells with CGRP among the four groups. Red represents CGRP marker; green represents CD3 marker; blue represents DAPI (4′, 6-diamidino-2-phenylindole). Magnification × 200.

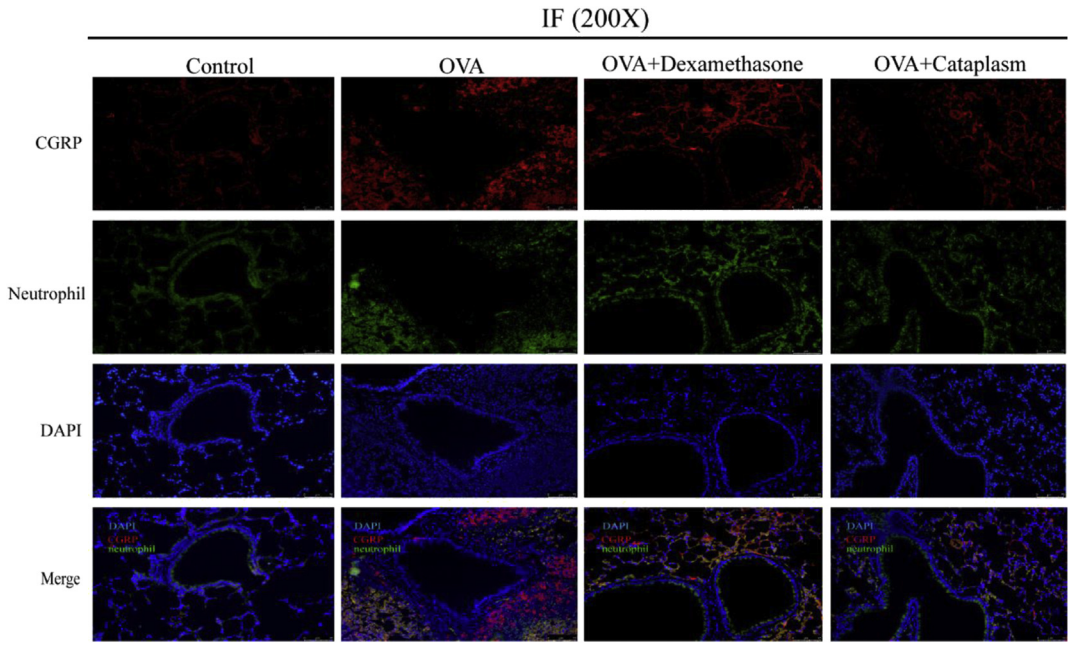

Figure 4. Neutrophil labeling of Neutrophils with CGRP among the four groups. Red represents CGRP marker; green represents neutrophil marker; blue represents DAPI. Magnification × 200.

biocompatibility with skin, breathability and sweat-resistant without obvious side effects. It seems to be an optimal herbal medicine to control asthma, especially chronic asthma.

The pathogenesis of asthma is closely relative to neuropeptides, and the development of new drugs also focuses on the blockade of neuropeptides [28]. In general, asthma is regulated by both afferent and efferent nerves. Afferent vagus neurons account for 80% of the vagus nerve [29], the nodular ganglion neurons dominate the visceral, while the jugular ganglion neurons control the respiratory and esophagus. The majority of afferent sensory neurons belong to nociceptors and are sensitive to thermal, mechanical, and chemical stimuli. When noxious stimuli activate the axonal terminals of nociceptors, nerve cells immediately release a variety of neuropeptides [30], inducing and aggravating asthma. Lungs also undergo efferent innervation of ganglionic cholinergic neurons from the parasympathetic nervous system. These cholinergic neurons mediate bronchoconstriction [31]. Postganglionic sympathetic neurons derived from the paravertebral ganglia innervate lymphoid tissue, bone marrow, joints, spleen, lung and airways, gastrointestinal tract, liver, and kidney, mainly releasing norepinephrine (NE), epinephrine (E) and other catecholamines, while NE and E can relieve bronchospasm to alleviate asthma.

In respiratory diseases such as asthma, the interaction between nerve and immune cells is deleterious. These changes are included in neurogenic inflammation [32].

CGRP, SP and NKA are important asthmatic neuropeptides. They are all released in response to TRPV1 activation in the nervous and immune systems. TRPV1 is expressed in c-sensory fiber neurons [33]. These neurons govern every organ and tissue of the body [34]. TRPV1 is activated by mechanical and chemical signals in and out of the body, resulting in the release of neuropeptides, which in turn leads to

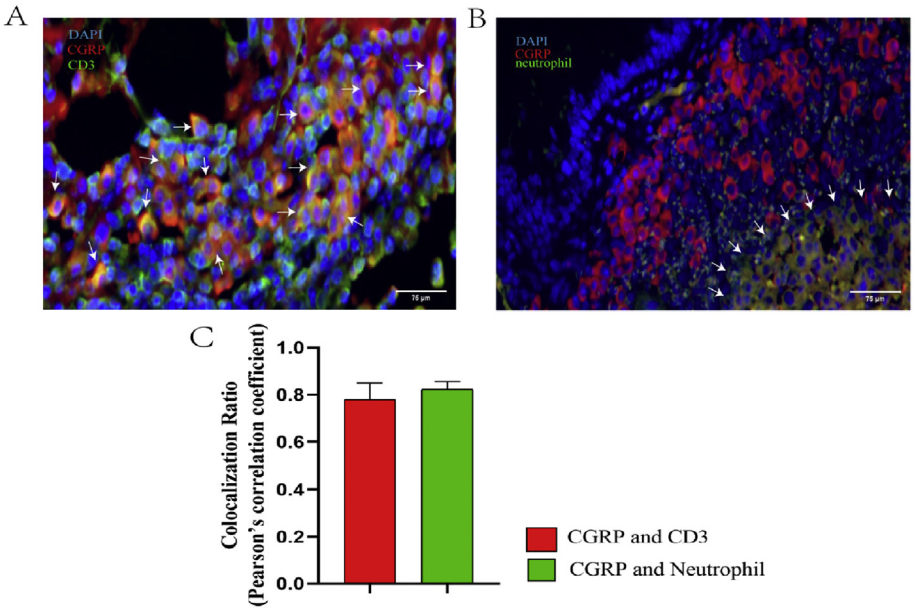

Figure 5. (A) Red represents CGRP marker; green represents CD3 marker; blue represents DAPI, Magnification × 200. As indicated by the arrows, cells in lung tissue were stained both CGRP and CD3. (B) Red represents CGRP marker; green represents neutrophil marker; blue represents DAPI, Magnification × 200. As indicated by the arrows, cells with overlap of colors in lung tissue were stained both CGRP and Neutrophil. (C) Colocalization analysis of immunofluorescence. The colocalization ratios of CGRP and CD3, CGRP and Neutrophil were evaluated by Pearson's correlation coefficient (PCC).

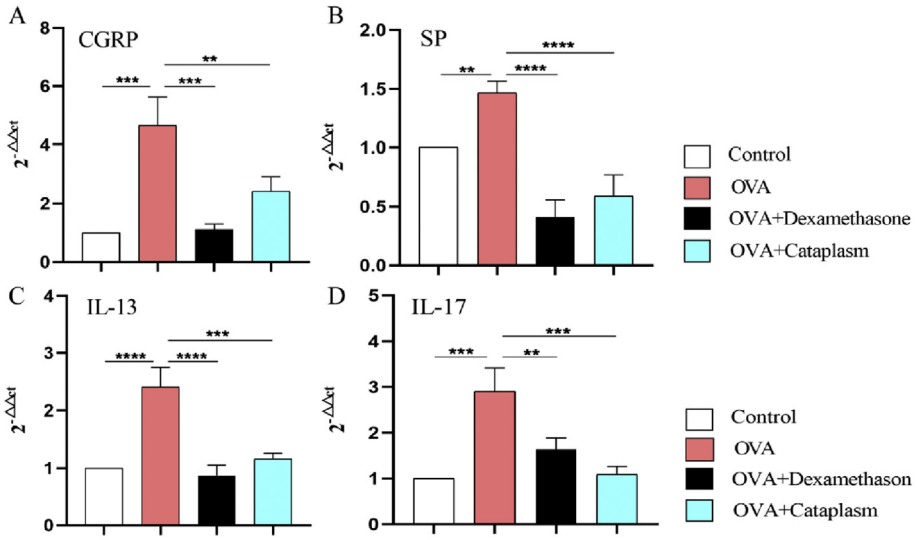

Figure 6. The expressions of CGRP, SP, IL-13 and IL-17 mRNA were adjusted by *Majie* cataplasm. Data are expressed as means of gene expression fold changes relative to β-actin. NS, not significant. $*P < .05$, $**P < .01$, and $***P < .001$.

inflammatory reactions [35]. Still, neuropeptides such as SP and CGRP can also act on C-fibers in the airways, as a result of increased expression of TRPV1 causing cough hypersensitivity [36].

CGRP is secreted by nerve cells because it is released at the site of stimulation, affecting immediate response and mediating information flow to other parts of the nervous system. Therefore, CGRP, as a neuro-immune connector, can guide a variety of immune cells to produce pro-inflammatory responses [37]. Regulations of macrophages [38], DC cells [39], ILC2 cells [40], and mastocytes [41] contribute to Th2 inflammation and lead to pathological reactions of asthma. In addition, sufficient studies demonstrated that some key immune cells, namely lymphocytes [42], monocytes [43] and macrophages [44] also synthesize CGRP.

SP is also a neuropeptide secreted mainly by neurons, encoded by the TAC1 gene (located on human chromosome 7) and is one of the members in the tachykinin family [45]. It can also be secreted by some immune cells, including T cells, macrophages, dendritic cells, and eosinophils. SP participates in multiple biological processes and plays an indispensable role in the immune response. SP can mobilize immune cells, promote their activation and proliferation, and exert a remarkable pro-inflammatory effect [46]. NKA is also a member of the tachykinin family encoded by TAC1 [47]. Similar to SP, it aggravates asthma symptoms together with SP. Emerging asthma therapeutic agents have been developed for NKR antagonists and have achieved some success [48].

The nerve cells in the lung tissue surround immune cells such as macrophages, DCs, and T cells, and the cytokine receptors, lipid receptors, growth factor receptors, and other immune signaling molecules are expressed on their envelope. The proximity of neurons to immune cells in location and their interaction help their cooperation [14]. For lung tissue, once smog, dust mites, and pollen enter the respiratory tract, nerve cells are stimulated and immediately released a variety of neuropeptides. These neuropeptides combine with the corresponding neuropeptide receptors of immune cells such as macrophages, DCs, MCs, and ILC2s, then activate the surrounding immune cells [49]. The activated immune cells secrete inflammatory cytokines and promote Th2 to rapidly expand the body's immune response. It can be seen that the interaction of neuropeptides with immune cells leads to asthma.

Our results showed that the neuropeptide represented by CGRP in the asthma model was not only secreted by lung tissue cells but also widely expressed in aggregated inflammatory cells. In this experiment, we selected two typical inflammatory cells, $CD3^+$T cells and neutrophils, which not increased in the asthma model alone but also secreted CGRP itself. Therefore, some immune cells are characterized by "neuralization", which not only interacts with neuropeptides but always secretes neuropeptides to further activate other immune cells and aggravate asthma. In addition, these inflammatory cells with neuralization may rapidly secrete neuropeptides and inflammatory cytokines after antigen stimulation, which is the culprit leading to the rapid onset of asthma. Neuropeptides interact with immune cells to regulate the occurrence and deterioration of asthma.

Both dexamethasone and *Majie* cataplasm can reduce the expression of CGRP in lung tissue and alleviate the aggregation of inflammatory cells, which has an obvious inhibitory effect on neuralization inflammation. This indicates that dexamethasone and *Majie* cataplasm can regulate inflammation caused by neuropeptides and immune cells. In addition, the reduction of serum CGRP and NKA in mice and expressions of asthma-associated neuropeptide CGRP mRNA and SP mRNA in lung tissue can demonstrate that dexamethasone and *Majie* cataplasm regulate the neuroimmune-associated inflammation.

However, we still have no idea whether the regulation of dexamethasone and *Majie* cataplasm on inflammation can be achieved by reducing nerve cells to directly hinder neuropeptides. It may also affect the secretion of neuropeptides indirectly through the regulation of immune cells, which is the main content of our subsequent experiments. Dexamethasone seems to get some answers. Experimental results have shown that the relief of asthma inflammation by dexamethasone does not prevent the increase of CGRP-related neurons [50]. However, the specific regulatory mechanism of *Majie* cataplasm on the neuropeptide and immune cells is still suspected, and we are expecting a result in our subsequent experiments.

With the deepening research on asthma, its classification is becoming more and more accurate. The most common asthma in clinical practice is hormone-sensitive asthma, of which eosinophilic asthma takes the majority. The main pathology is high Th2 inflammation which brings increasing of cytokines such as IL-4, IL-5, IL-13 and IgE caused by B cells proliferation and activation. This type of asthma is sensitive to hormonal therapy. It can quickly relieve asthmatic airway inflammation and symptoms [51]. In this experiment, both dexamethasone and *Majie* cataplasm can effectively inhibit $CD3^+$T cells infiltration in lung tissue, and the inhibition of Th2 typical cytokine IL-13 can also prove the alleviation of dexamethasone and *Majie* cataplasm on Th2 inflammation. Non-hormone-dependent asthma has also aroused great attention in asthma types. The clinical manifestations of this type of asthma are insensitive to hormonal therapy, which even exacerbates asthma symptoms. The most important one is neutrophilic asthma [52] with a

infiltration of neutrophils in the lung tissue. Still, the IL-17 content increases [53], and the use of hormones is ineffective, or even aggravates the secretion of IL-17 [54]. The OVA-induced severe asthma model, although mainly presented with Th2 inflammation, widespread neutrophils infiltration was still observed in the lung tissue. After the intervention of dexamethasone and *Majie* cataplasm, the total number of neutrophils decreased, and the expression of IL-17 mRNA in lung tissue was also decreased. On the one hand, this effect may be directly exerted by inhibiting neutrophils and IL-17-related targets; on the other hand, it may be indirectly regulated by inhibition of Th2 inflammatory cells. However, the effect of *Majie* cataplasm is superior to that of dexamethasone with a remarkable inhibitory effect on neutrophils and IL-17 mRNA. We deduce that *Majie* cataplasm probably exerts a direct regulatory effect on neutrophils and IL-17, so it has the potential to regulate non-hormone-dependent asthma.

To sum up, *Majie* cataplasm can regulate the interaction between neuropeptides and immune cells, and the effect on alleviating Th2 inflammation is similar to dexamethasone. In addition, *Majie* cataplasm also has a potential to regulate non-hormone dependence asthma. However, whether *Majie* cataplasm has a direct effect on the neuropeptides or not, and its specific regulation on neutrophils and IL-17 still needs further in-depth exploration.

Declarations

Author contribution statement

W. Ji and X. Yu: Performed the experiments; Wrote the paper.

Y. Gao, B. Ren and S. Zhang: Analyzed and interpreted the data; Wrote the paper.

Q. Wang: Conceived and designed the experiments; Wrote the paper.

X. Wang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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