The “time-response” effect of Wenyang Pingchuan Formula on miR-19a in asthmatic mice experiment

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Received 29 October 2021; Accepted 16 February 2022
Available online 1 September 2022

Abstract

Background: Wenyang Pingchuan Formula (WPCF) is an empirical formula for the treatment of acute childhood asthma. However, the “time-effect” relationship of this prescription is not clear. This paper explores the relationship between Janus activated kinase signal transducer and activator of transcriptions (JAK/STAT), nuclear factor-κB (NF-κB), and microRNA (miR-19a), and also preliminarily determines the best time-effect relationship of WPCF in reducing the airway inflammation in asthmatic mice.

Method: 80 BALB/c mice were randomly divided into four groups: control (CON) group, model (MDL) group, dexamethasone (DEX) group, and WPCF group. MDL group was established through intraperitoneal injection of 10% ovalbumin (OVA) and Al(OH)3 solution and the inhalation of aerosolized 5% OVA solution. Enzyme-linked immunosorbent assay (ELISA), real-time PCR and Western blot were conducted to determine the levels of interleukin (IL)-4, IL-13, interferon-γ (IFN-γ) in bronchoalveolar lavage fluid (BALF), contents of miR-19a mRNA and STAT6, phosphorylated signal transducers and activators of transcription 6 (p-STAT6), p65, phosphorylated p65 (p-p65), suppressors of cytokine signaling 1 (SOCS1), and tumor necrosis factor α-induced protein-3 (Tnfaip3) proteins after 7 and 28 days of intervention respectively.

Results: Significant down-regulation of IL-4 and IL-13 expression (P<0.05) and up-regulation of IFN-γ expression (P<0.05) in BALF have been observed for WPCF group compared with the MDL group. The significant down-regulation of miR-19a mRNA and STAT6, p-STAT6, p-p65 proteins (P<0.05) and up-regulation of SOCS1 and Tnfaip3 proteins (P<0.05) in BALF was also observed for WPCF group compared to the MDL group. During the experiment, the weight of the mice in DEX group significantly decreased (P<0.05) compared with the other groups.

Conclusions: WPCF could restore Th1/Th2 balance. The longer the intervention time, the more effective the treatment. The down-regulation of miR-19a mRNA by activating JAK/STAT and NF-κB signal pathways may be a possible mechanism by which WPCF alleviates airway inflammation.

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https://doi.org/10.15586/aei.v50i5.531
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Background

Bronchial asthma is a complex inflammatory airway disease involving a variety of inflammatory factors and structural cell activation, clinically presenting as airway obstruction, airway inflammation, mucus secretion and airway hyperresponsiveness.1 Allergic asthma refers to a subset of asthma triggered by allergens, which is mainly driven by immune response involving T-helper 2 (Th2) cells. The National Health and Nutrition Survey (NHANES) from 2005-2006 reports an asthma prevalence of 8.8%, of which 62.1% is caused by allergic asthma.2 According to the American severe asthma research program, allergic asthma accounts for 78.8% of severe asthma onsets.3 Epidemiological surveys carried out in 1990, 2000, and 2010 on children aged 0-14 years in urban areas reported prevalence rates of 0.91%, 1.97%, and 3.02% in the respective years, with significantly increasing trend. The epidemiological survey in 2010 reports that more than 70% of the children had allergic asthma, which is often associated with allergic diseases such as allergic rhinitis, eczema, and atopic dermatitis.4,5 At present, the conventional western medicine (bronchodilators and corticosteroids) can provide symptomatic relief, but may cause adverse side effects such as skin rash and affecting growth. Drug resistance may also occur with long term usage. Traditional Chinese Medicine is proven to have better clinical efficacy and safety, and is an increasingly popular form of treatment for asthma. The advantages of traditional Chinese medicine (TCM) should be explored for establishing better treatment and prevention options for asthma, as well as developing TCM proprietary medicine.

MicroRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in the post-transcriptional regulation of gene expression in multicellular organisms by affecting the stability and translation of mRNAs. miR-19a is an miRNA encoding gene which is reported to promote the secretion of interleukin (IL)-4, IL-5, and IL-13 by inhibiting the expression of A20 and suppressors of cytokine signaling1 (SOCS1) protein and gene in type 2 innate lymphoid cells (ILC2).4 miR-19a targets phosphatase and tensin homology deleted on chromosome ten (PTEN), SOCS1 and tumor necrosis factor α-induced protein-3 (Tnfaip3) directly to activate phosphatidylinositol-3 kinase (PI3K), Janus activated kinase signal transducer and activator of transcription (JAK/STAT) and nuclear factor-kB (NF-kB) signaling pathways, thus promoting Th2 cytokine production.5 In this study, we observed the effects of Wenyang Pingchuan Formula (WPCF) on inflammatory cytokines (IL-4, IL-13, interferon-γ (IFN-γ)), and related signal pathway indicators (miR-19a, signal transducers and activators of transcription 6 (STAT6)), phosphorylated signal transducers and activators of transcription 6 (p-STAT6), p65, phosphorylated p65 (p-p65), SOCS1, Tnfaip3) in asthmatic mice to explore the possible intervention mechanisms and determine the best intervention time of WPCF.

Materials and methods

Laboratory animals and grouping

Around 80 male BALB/c mice (5-6 weeks old, SPF) were purchased from Shanghai Super-B&K Laboratory Animal Corp. Ltd (Certificate No: SCXK2013-0016). The animals were accommodated in macrolon cages at a controlled environment of temperature 20-24°C and humidity 50-70%, food and water available freely. All experimental procedures were approved by the Animal Care and Use Sub-committee of the Shanghai Traditional Chinese Medicine Hospital.

The 80 mice were randomly divided into 4 groups (20 mice/group): control (CON) group, model (MDL) group (asthma induced, no treatment), dexamethasone (DEX) group (asthma induced, DEX treatment for 7d or 28d), and WPCF group (asthma induced, WPCF treatment for 7d or 28d).

Allergen-induced asthma model and standard of evaluation for successful modelling

The establishment of murine model of allergic asthma involves the ovalbumin (OVA) sensitization and challenge stage.4 OVA sensitization stage: the mice were sensitized by an intraperitoneal injection of 10% OVA solution on days 1 and 15, the dosage calculated by weight (50 ml/kg). OVA solution was prepared by dissolving 10g OVA (Sigma, No. A5378) and 1g Al(OH)3 (Thermofisher) in 100ml of 0.9% NaCl solution.

OVA challenge stage: The mice were challenged with 5% OVA solution for 40 min, once a day for a total of 1 week. At the same time, the control group was sensitized and challenged in the same way with equivalent amounts of 0.9% sodium chloride solution.

According to the results of hematoxylin and eosin (HE) staining, the presence of a large amount of inflammatory cell infiltration around the trachea serves as the criteria for successful establishment of asthma model (Figure 1)

Dosage and method of administration

WPCF (provided by Shanghai Hospital of TCM) consists of 12 kinds of TCM herbs, including MaHuang (Ephedra Herba) 5g, KuXingRen (Amygdalus Communis Vas) 10g, LaiFuZi (Raphani Semen) 10g, ZisSuZi (Perillae Fructus) 10g, HuangQin (Scutellariae Radix) 10g, DiLong (Pheretima) 10g, and the dosage of WPCF is 5 g/kg per day. The control group received intraperitoneal injection of 10% saline with 5 ml/kg. The dosage of WPCF was determined by calculating body weight. The WPCF group was intraperitoneal injection with 5 ml/kg/day.

Figure 1  Animal modeling and administration flow chart.
10g, JiaoMu (Semen Zanthoxyl) 10g, XianLingPi (Epimedi Foliuim) 10g, XianMao (Curculiginis Rhizome) 10g, TaoRen (Persicae Semen) 10g, DanShen (Salviae Mittiorrhizae Radix et Rhizoma) 10g, and GanCao (Licorice) 10g. The extract was prepared at a concentration of 5.33mg/ml according to the referenced method of preparation.10 1260g of WPCF herbs were weighed, and the extraction was done by first simmering with 7.56 L of water for 30 min to obtain the first round of filtrate, then simmering for the same amount of time after adding 6.30 L of water to obtain the second round of filtrate. The filtrates from both rounds of extractions were mixed and concentrated with a rotary evaporator. The preparation of the extract was completed in the experimental center of the affiliated Municipal Hospital of TCM, Shanghai University of TCM. It was stored at 4°C and warmed before gastric infusion.

DEX (Shanghai Xinyi Co., Ltd., No. H31020793) solution was prepared at a concentration of 0.075mg/ml and administered routinely. Administration for both treatment groups began the first day after the sensitization inhalation, at the fixed dosage of 20 ml/kg·d (10 times of the clinical dosage of 30 kg for children). D23 to D50 was the time of intragastric administration. The mice were sensitized in the morning and intragastric administration was done in the afternoon. CON group and MDL group were administered the reference method of preparation. 10 1260g of WPCF (Persicae Semen) 10g, DanShen (Salviae Miltiorrhizae Radix et Rhizoma) 10g, XianMao (Curculiginis Rhizome) 10g, TaoRen (Semen Zanthoxyli) 10g, JiaoMu (Semen Zanthoxyli) 10g, and GanCao (Licorice) 10g. The extract was prepared at a concentration of 5.33mg/ml according to the referenced method of preparation. 10 1260g of WPCF was dispersed in 5.33mg/ml of 0.9% NaCl solution of the same dosage. After 7 and 28 days, the mice in every group were sacrificed and sampled.

Analysis of lung tissue morphology by HE

The lung tissue of the mice was cut into sizes of 1.5 cm × 1.5 cm × 0.3 cm, immersed with formalin, dehydrated with ethanol (Shanghai Zhenxing chemical first plant), and embedded in wax (Shanghai Guoyao group, No.69018961). The waxed sample was subsequently sectioned into thicknesses of 4-7 μM after solidifying. The samples were dyed using hematoxylin solution (Solarbio, No. R1010) for 5 min followed by alcohol eosin staining solution (GIBCO, No.21985023) according to the manufacturer’s protocol and separated via SDS-PAGE. Subsequently, the separated proteins were transferred onto a PVDF membrane, (Thermofisher, No. PICPI23223) according to the manufacturer’s protocol, and ran using the RT-PCR instrument (ABI, 7500).

The expression of JAK/STAT and NF-κB signal transduction factors in lung tissues

SOCS1, Tnfaip, STAT6, p-STAT6, p65, p-p65 levels were measured using Western blot. Total proteins were extracted, centrifuged at 2000 r/20 min/4°C, and stored at -80°C. Total protein was measured using the BCA Protein Assay Kit (Sinopharm Chemical Reagent Co., Ltd. No. CAS67-66-3), mixed for 5 s, left to stand for 3 min, and centrifuged at 12000 r/15 min/4°C. Total protein was measured using the BCA Protein Assay Kit (Shanghai Xinyi Co., Ltd., No. H31020793) solution was prepared at a concentration of 0.075mg/ml and administered routinely. Administration for both treatment groups began the first day after the sensitization inhalation, at the fixed dosage of 20 ml/kg·d (10 times of the clinical dosage of 30 kg for children). D23 to D50 was the time of intragastric administration. The mice were sensitized in the morning and intragastric administration was done in the afternoon. CON group and MDL group were administered the reference method of preparation. 10 1260g of WPCF was dispersed in 5.33mg/ml of 0.9% NaCl solution of the same dosage. After 7 and 28 days, the mice in every group were sacrificed and sampled.

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The expression of miR-19a in mouse lung tissues

miR-19a mRNA expression in lung tissues was detected using RT-PCR (Table 1). The tissue was added with 200 μl of chloroform (Shanghai Suyi Chemical Reagent Co., Ltd, CAS67-66-3), mixed for 15 s, left to stand for 3 min, and centrifuged at 12000 r/15 min/4°C. Subsequently, the supernatant was removed and added with isopropanol (Sinopharm Chemical Reagent Co., Ltd. No. CAS67-63-0), left to stand for 10 min before centrifuging at 12000 r/15 min/4°C, then discarding the supernatant. The precipitate was washed with 1 ml anhydrous ethanol, dissolved in 20 ul DEPC (Beyotime biotechnology company, No. R0022) water, and stored at -80°C. Nanodrop 2000 software was used to record the sample concentration and RNA260/280 ratio. Reverse transcription was carried out according to manufacturer’s protocol, and ran using the RT-PCR instrument (ABI, 7500).

Table 1 Primers for RT-PCR

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Primer sequence (5’-3’)</th>
<th>Product (bps)</th>
</tr>
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<tbody>
<tr>
<td>miR-19a</td>
<td>sense 5’-CAG CCC TCT GTT AGT TTT GCA T-3’</td>
<td>80</td>
</tr>
<tr>
<td>Antisense 5’-CAG GCC ACC ATC AGT TTT G-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense 5’-CAC AGG GGA AAA ATT CCT GA-3’</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>B-actin</td>
<td>Antisense 5’-AGC TTG ACG GTG TCT TTT GC-3’</td>
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</tr>
</tbody>
</table>

miR-19a expression level and inflammatory cytokine levels in mouse bronchoalveolar lavage fluid (BALF)

IL-4, IL-13, IFN-γ levels in BALF were analyzed using enzyme-linked immunosorbent assay (ELISA). Diluting and sampling was proceeded according to the manufacturer’s protocol on the ELISA kits (Shanghai Fanke Industry Co. Ltd, No. 201906), and the reaction was carried out at 37°C for 30 min. Subsequently, 50ul of enzyme was added, followed by a color developing agent. Enzyme labeling instrument (Rabo, MK3) was run at 450 nm wavelength to read OD value, and ELISA calc1 software was used to calculate sample concentration.

Statistical methods

Statistical analyses were performed using SPSS18.0 (SPSS, Chicago, IL). Data were analyzed using Student’s T Test, one-way ANOVA, and least significant difference (LSD). P<0.05 was considered to indicate a statistically significant difference.

Results

The effect of WPCF on the weight of asthmatic mice

The weight of mice was measured. On the one hand, the weight of mice can determine the drug dosage, on the
other hand, we can observe the other effects besides therapeutic effects of drug on mice. The experimental results are as follows:

After 7 days of intervention, the weights of mice in MDL, DEX, and WPCF groups were lower (P<0.001, P<0.01) compared with the CON group; while the weight loss of mice in both treatment groups is less than that of MDL group (P<0.01). After 28 days of intervention, the weight of mice in DEX group was significantly lower than that of the MDL group (P<0.05). However, the WPCF group recorded significantly higher weight compared with MDL (P<0.05) and DEX (P<0.001) groups, and was comparable with CON group (Table 2). The weight of mice in WPCF group continued to increase, while the weight of mice in DEX group decreased after 28 days. It suggested that long-term use of hormones may have adverse effects on growth.

The effect of WPCF on histological changes in lung tissue of asthmatic mice

HE staining is helpful to observe the lung tissue structure and cell morphology of asthmatic mice. After 7 days of intervention, the histological changes in lung tissue were as follows: the structure of bronchioles and alveoli of CON group was normal with no infiltration of inflammatory cells in the lumen; the bronchial epithelium in MDL group was damaged and thickened, with large amounts of inflammatory cell infiltration and edema; the airway walls in DEX group and WPCF group were partially damaged and thickened, with moderate inflammatory cell infiltration and edema, indicating attenuated airway inflammation and remodeling with treatment (Figure 2). After 28 days of intervention, there were no remarkable changes for CON group and MDL group; however, reduced extent of inflammatory cells infiltration was observed for both treatment groups, indicating effective reduction of airway inflammation by WPCF (Figure 2).

The inflammatory reaction of the two drug groups relieved after 7 days of intervention and 28 days of intervention. The intervention effect of 28 days was better than that of 7 days.

Table 2  Body weight comparison of mice among groups in different intervention time (mean± SD, g)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Treated for 7d</th>
<th>Treated for 28d</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>10</td>
<td>23.62±0.88</td>
<td>24.58±1.038</td>
</tr>
<tr>
<td>MDL</td>
<td>10</td>
<td>21.33±1.421***</td>
<td>23.29±1.47*</td>
</tr>
<tr>
<td>DEX</td>
<td>10</td>
<td>22.62±1.156**</td>
<td>22.03±1.122***</td>
</tr>
<tr>
<td>WPCF</td>
<td>10</td>
<td>22.22±0.943**</td>
<td>24.63±1.228aaa</td>
</tr>
</tbody>
</table>

Compared with the CON group, *P<0.05, **P<0.01, ***P<0.001, compared with the MDL group, #P<0.05, ##P<0.01, ###P<0.001, compared with the DEX group, ▲P<0.05, ▲▲P<0.01, ▲▲▲p<0.001.

Effect of WPCF on inflammatory factors IL-4, IFN-γ and IL-13 in asthmatic mice

There are many inflammatory cells in the pathogenesis of asthma. At the same time, these inflammatory cells synthesize and release cytokines such as Th2 cytokines IL-4 and IL-13 and Th1 cytokines IFN-γ. These cytokines can be observed for the inflammatory response. The results are as follows:

After 7 days of intervention, compared with the MDL group, the level of IL-13 and IL-4 were significantly lower (P<0.001, P<0.001), and the level of IFN-γ was significantly higher (P<0.001) in DEX group. While in WPCF group, the level of IL-13 was significantly lower (P<0.001) and the level of IFN-γ was significantly higher (P<0.01) compared with MDL group (P<0.01). Compared with DEX group, the changes in the levels of IL-4, IL-13, and IFN-γ was less remarkable in WPCF group (P<0.01, P<0.001, P<0.001) (Figure 3).

After 28 days of intervention, no significant difference in the levels of IL-4 and IL-13 in both treatment groups was observed, while the level of IFN-γ in WPCF group was moderately lower (P<0.01) compared with DEX (Figure 4).

Compared with 7 days of intervention, the levels of IL-4 and IL-13 in MDL group significantly increased after 28 days...
of intervention (P<0.001, P<0.05). The levels of IL-4 and IL-13 in both the treatment groups significantly decreased after 28 days of intervention (P<0.01, P<0.05). Moreover, a significant increase in the level of IFN-γ (P<0.05) was recorded in WPCF group (Figure 5).

Under different intervention time, the levels of IL-4 and IL-13 decreased, and the level of IFN-γ increased in the two drug groups. The airway inflammatory environment of asthma was improved. In addition, under the long-term intervention, the levels of IL-4 and IL-13 in MDL group increased and the level of IFN-γ had not changed. It showed that the inflammatory response in the later stage of asthma may be more closely related to Th2 cells. The effect on IL-4 and IL-13 of WPCF group for 28 days was better than that for 7 days. With the continuous use of WPCF, the effect of inhibiting Th2 is more remarkable.

Effect of WPCF on miR-19a mRNA content in asthmatic mice

miR-19a can enhance Th2 differentiation and induce asthma. Observing the change of miR-19a mRNA content is conducive to understand the inflammatory reaction process of asthma. The results are as follows:
After 7 days of intervention, compared with MDL group, DEX group and WPCF group induced significant lower in the miR-19a mRNA content of mice (P<0.001) (Figure 6A).

After 28 days of intervention, compared with MDL group, the miR-19a mRNA content of mice in both treatment groups was significantly lower (P<0.001), with more remarkable lower observed in DEX group (P<0.05) (Figure 6B).

Under the short-term and long-term intervention, the miR-19a mRNA content became lower. It was obvious that miR-19a maybe involved in the pathogenesis of asthma, which was related to the regulation of asthma inflammatory response.

Effect of WPCF on JAK/STAT signal network

JAK/STAT is a classical signaling pathway of inflammatory response, and it participates in the inflammatory response of asthma. STAT6 is one of the signal factors of JAK/STAT signaling pathway, which triggers Th2 inflammatory response. SOCS1 is a cytokine signal inhibitor, which is related to JAK/STAT signaling pathway.

After 7 days of intervention, compared with MDL group, DEX and WPCF groups recorded a smaller increase in the protein content of STAT6 and p-STAT6 (P<0.001), and smaller decrease in the protein content of SOCS1 (P<0.001). Compared with DEX group, the protein content of STAT6 and p-STAT6 in WPCF group decreased to a smaller extent (P<0.05) (Figure 7).

After 28 days of intervention, compared with MDL group, the protein content of STAT6 and p-STAT6 for both treatment groups was significantly lower (P<0.001), and the protein content of SOCS1 was significantly higher (P<0.001). Compared with DEX group, WPCF group recorded similar levels of STAT6 and SOCS1, but smaller decrease in p-STAT6 (P<0.01) (Figure 8).

Under different intervention time, the protein content of STAT6 and p-STAT6 was lower, and that of SOCS1 was higher in the two treatment groups. The mechanism may be related to JAK/STAT signaling pathway.

![Figure 6](image1.png) miR-19a mRNA content among each group in different intervention time. (A) miR-19a mRNA content in each group after 7 days of intervention, (B) miR-19a mRNA content in each group after 28 days of intervention, compared with CON group, *P<0.05, **P<0.01, ***P<0.001, compared with MDL group, #P<0.05, ##P<0.01, ###P<0.001, compared with DEX group, ▲P<0.05, ▲▲P<0.01, ▲▲▲P<0.001.

![Figure 7](image2.png) STAT6, p-STAT6, SOCS1 protein in each group after 7 days of intervention. (A) STAT6, p-STAT6, SOCS1 protein bands, (B) comparison of STAT6 protein gray values, (C) comparison of p-STAT6 protein gray values, (D) comparison of SOCS1 protein gray values, compared with CON group, *P<0.05, **P<0.01, ***P<0.001, compared with MDL group, #P<0.05, ##P<0.01, ###P<0.001, compared with DEX group, ▲P<0.05, ▲▲P<0.01, ▲▲▲P<0.001.
Effect of WPCF on NF-κB signal network

NF-κB activation is associated with asthma attack. p65 is a member of NF-κB signaling pathway, whose expression change will affect the related cytokines. As the negative regulatory factor of NF-κB, Tnfaip3 can inhibit NF-κB activation.

After 7 days of intervention, compared with MDL group, the protein content of p65 and p-p65 in DEX and WPCF groups was significantly lower (P<0.001), and the protein content of Tnfaip3 was significantly higher (P<0.001, P<0.01). Compared with DEX group, the protein content of p65 and p-p65 in WPCF group was significantly higher (P<0.001), and the protein content of Tnfaip3 in WPCF group was significantly lower (P<0.01) (Figure 9).

After 28 days of intervention, compared with MDL group, the protein content of p65 and p-p65 in the two WPCF groups was significantly lower (P<0.001), and the protein content of Tnfaip3 was significantly higher (P<0.001, P<0.01). Compared with DEX group, the protein content of p65 and p-p65 in WPCF group was significantly higher (P<0.001), and the protein content of Tnfaip3 in WPCF group was significantly lower (P<0.01) (Figure 9).
The “time-response” effect of Wenyang Pingchuan Formula on miR-19a in asthmatic mice experiment

The treatment groups was significantly lower (P<0.001), and the protein content of Tnfaip3 was significantly higher (P<0.001). Compared with DEX group, the changes in protein content of p65, p-p65, and Tnfaip3 in WPCF group was to a smaller extent (P>0.05) (Figure 10).

Under different intervention time, the protein content of p65 and p-p65 was lower, and that of Tnfaip3 was higher in the two treatment groups. The mechanism may be related to NF-κB signaling pathway.

Discussion

Allergic asthma involves the activation of both adaptive and innate immune response in response to environmental triggers. The adaptive immune response includes two stages: the first stage being allergen sensitization which is established through intraperitoneal OVA injection in this study and the second stage is the immune response stage, referring to the release of a variety of inflammatory mediators upon recontact with the allergen, causing contraction of bronchial smooth muscle, mucosal edema and mucus secretion, resulting in an acute asthma response, which in this study, is triggered through inhalation of atomized OVA. Acute asthmatic response is triggered when CD4⁺ T lymphocytes and eosinophils release type 2 cytokines (IL-4, IL-5, and IL-13), which are the key mediators of airway inflammation.¹¹

In this study, markedly different levels of expression of IL-4, IL-13, and IFN-γ of mice in each group were observed, which also varied in different points in time. Higher expression of IL-4 and IL-13, and lower expression of IFN-γ in MDL group were recorded than CON group. The decrease in the expression of IL-4 and IL-13, and increase in the expression of IFN-γ were exhibited in both the treatment groups. The effect of WPCF was comparable with that of DEX, indicating a similar effectiveness in the reduction of airway inflammation. The efficacy of DEX and WPCF after 28 days of intervention was significantly greater than that of intervention for 7 days, which suggests that the effectiveness of reducing the airway inflammation in asthmatic mice is time dependent. With long-term intervention, the expression of IL-4 and IL-13 in asthmatic mice increased, but the expression of IFN-γ remained the same. The results above suggest that asthma onset is mediated predominantly by Th2 inflammatory response, which is consistent with results of Dong’s study.¹² It can also be concluded that the efficacy of WPCF was similar to that of DEX.

The possible intervention mechanism of WPCF was explored in this study. Changes in IL-4, IL-13 and IFN-γ expression may be caused by regulated expression of miR-19a and balanced JAK/STAT and NF-κB network. miR-19a could promote expression of IL-4, IL-5 and IL-13 by inhibiting the expression of SOCS1 and ILC2s. It also regulates target networks in Th2 cells, such as PTEN, SOCS1, and Tnfaip3, to activate PI3K, JAK/STAT, and NF-κB signaling networks, which further promotes Th2 cytokine generation. In this study, the effects of WPCF on inflammatory cytokines (IL-4, IL-13, IFN-γ) and respective signal pathway indicators (miR-19a, STAT6, p-STAT6, p65, p-p65, SOCS1, Tnfaip3) in asthmatic mice were observed to explore its possible intervention mechanism and determine the best intervention time. For this purpose, corresponding markers of the JAK/STAT and NF-κB network were quantified. miR-19a mRNA, STAT6, p-STAT6, p65, and p-p65 protein content of mice in MDL group were higher than that of CON group, while the SOCS1 and Tnfaip3 protein content were lower. miR-19a mRNA content, STAT6, p-STAT6, p65, and p-p65 protein content of mice in both treatment groups at different intervention times decreased, and SOCS1 and Tnfaip3 protein content increased. Finally, we concluded that a possible mechanism of WPCF may be through regulating the JAK/STAT and
NF-κB signaling pathways to alleviate airway inflammation. In addition, after 28 days of intervention, the effect of WPCF was close to that of DEX.

In Chinese medicine, the pathogenesis of asthma is phlegm and blood stasis. In clinic, WPCF can significantly improve the clinical symptoms of children with asthma. WPCF controls the recurrence of asthma by removing yin-natured “phlegm” and “blood stasis” to restore yin-yang balance. In the clinical study, WPCF was composed of MaHuang (Ephedra Herba), KuXingRen (Amygdalus Communis Vas), LaiFuZi (Raphani Semen), ZiSuZi (Perillae Fructus), HuangQin (Scutellariae Radix), DiLong (Pereretima), JiaoMu (Semen Zanthoxyli), XianMao (Curcurliginis Rhizoide), etc. Modern pharmacology study showed that Ephedra total alkaloids has apparent anti-asthmatic and antitussive effects. Icariin can effectively inhibit the generation and infiltration of eosinophil, thus improving OVA induced airway inflammation. KuXingRen (Amygdalus Communis Vas) produces trace hydrocyanic acid under the action of the emulsin, which can relieve cough and wheezing by depressing respiratory function. Cryptotanshinone remarkably reduces the total number of inflammatory cells and expression of Th2 cytokines (IL-4, IL-5, and IL-13) in BALF of asthmatic mice. JiaoMu (Semen Zanthoxyli) oil down-regulates the expression of inhibitor of kappa B kinase α (IKK-α) and phosphorylated inhibitor of kappa B (p-IKB) thereby inhibiting the generation and release of inflammatory factors and chemokines, reducing infiltration of inflammatory cells. With the combined use of all above mentioned herbs, effective treatment of asthma is achieved through regulating pulmonary qi, reducing phlegm, removing blood stasis, and relieving wheezing.

In conclusion, WPCF could reduce miR-19a to affect the secretion of inflammatory factors such as IL4, IL13, IFN-γ, and regulate Th1/Th2 balance. A possible mechanism by which it can alleviate airway inflammation of asthmatic mice may be through regulating the JAK/STAT and NF-kB signaling pathways. The longer the intervention time, the greater the treatment efficacy. This provides novel experimental basis for the treatment of asthma using TCM.

Conflict of Interest

There were no conflicts of interest to declare.

Reference