Effect of Xiaochuanping powder on the inflammatory response and airway remodeling in asthmatic rats

Zhou Tao, Zhang Nianzhi, Hu Die, Wang Wendong, Xu Shunfu, Chen Xu

OBJECTIVE: To observe the effect of Xiaochuanping powder (XP), a traditional Chinese prescription for the treatment of cough and asthma, on serum concentrations of eosinophil cationic protein (ECP), tumor necrosis factor (TNF)-α, and interleukin (IL)-4, eosinophil counts, as well as expression of matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinase (TIMP)-1 in the lung tissues of asthmatic rats.

METHODS: Sixty clean-grade Sprague–Dawley rats were divided randomly into six groups: normal control (NC), asthma model, Guilong Kechuanning (GK) group, as well as high-, intermediate-, and low-dose XP groups. Rats were sensitized with ovalbumin (OVA) to trigger asthma. Serum concentrations of ECP, TNF-α and IL-4, eosinophil counts, as well as expression of MMP-9 and TIMP-1 in lung tissues were evaluated using an immunofluorescence method. mRNA expression of MMP-9 and TIMP-1 was determined using real-time quantitative polymerase chain reaction.

RESULTS: Compared with the asthma-model group, serum concentrations of ECP, TNF-α, and IL-4, and eosinophil counts decreased significantly in the high- and intermediate-dose XP groups and GK group (all \( P < 0.01 \)). Protein expression of MMP-9 and TIMP-1 decreased significantly in the high- and intermediate-dose XP groups and GK group (all \( P < 0.01 \)). Transcription of MMP-9 and TIMP-1 mRNA and the ratio of expression of MMP-9: TIMP-1 in lung tissue were significantly lower (\( P < 0.01 \)).

CONCLUSION: XP can reduce TNF-α secretion, suppress the infiltration / activation of eosinophils, reduce serum concentrations of ECP and IL-4, reduce the protein and mRNA expression of MMP-9 and TIMP-1 in lung tissue, and regulate the balance between expression of MMP-9 and TIMP-1. In these ways, XP alleviated the inflammation and remodeling of the airways.

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Keywords: Asthma; Airway remodeling; Eosinophils; Eosinophil cationic protein; Matrix metalloproteinase 9; Tumor necrosis factor-alpha; Tissue inhibitor of metalloproteinase 1; Xiaochuanping powder
INTRODUCTION

Bronchial asthma is a common, recurrent, protracted, and persistent respiratory disease. Inflammation and remodeling of the airways are important pathologic features of asthma. They can promote each other, jointly inducing the development and exacerbation of asthma. About 1%-18% of the populations of different countries are affected by asthma. Worldwide, > 300 million people are plagued by asthma, and the incidence and severity of asthma are increasing annually. Western Medicines such as glucocorticoids and β2-agonists have shown good efficacy in the early management of acute severe asthma by controlling symptoms, reducing damage to delicate lung tissue, and improving quality of life. However, Western Medicines cannot cure asthma. Furthermore, they often cannot be used for long periods or at high doses, especially in older adult people and young children. In comparison, Traditional Chinese Medicine (TCM) have several advantages in the prevention and treatment of asthma due to their documented efficacy, few side-effects, applicability to different populations, and low price. In recent years, their role in asthma control has been recognized increasingly.

Xiaochuanping powder (XP) is a TCM composed of medicinal materials such as Shanyao (Rhizoma Dioscoreae Opposite), Bihu (Gekko Swinhonii), Baizhu (Rhizoma Atractylodis Macrocephalae), Muhudic (Semen Qroxyli) and prepared Mahuang (Herba Ephedrae Sinica). XP has been used in the Department of Respiratory Medicine of the First Affiliated Hospital of Anhui University of Chinese Medicine (Hefei, China) for many years, with confirmed efficacy.

In the present study, we evaluated changes in serum levels of eosinophil cationic protein (ECP), tumor necrosis factor (TNF-α) and interleukin (IL)-4, ECP expression of MMP-9 and TIMP-1, and to obtain the eosinophil count, were purchased from Shanghai Yuanye Biotechnological (Shanghai, China). TRizol Reagent was obtained from Invitrogen (14105; Carlsbad, CA, USA). A RevertAid™ First Strand cDNA Synthesis kit was from Thermo Scientific (00145205; Waltham, MA, USA). An automatic embedding and slicing system was purchased from Leica (Wetzlar, Germany). A microplate reader was obtained from Bio-Rad Laboratories (Hercules, CA, USA). A microscope image-analysis system was obtained from Olympus (Tokyo, Japan). A PikoReal™ 96 RealTime Polymerase Chain Reaction (PCR) system was purchased from Thermo Scientific. A desktop high-speed refrigerated centrifuge (JW-3021HR) was obtained from Jiaven (Anhui, China).

MATERIALS AND METHODS

Animal groupings
Sixty clean-grade Sprague-Dawley rats [30 males and 30 females (200 ± 20 g)] were purchased from the Laboratory Animal Center of Anhui Medical University. The study was approved by the experi-mental animal ethics committee of Anhui University of Chinese Medicine. They were divided randomly into six groups of 10: normal control (NC); asthma model; Guilong Kechuanping (GK; a TCM used to treat cough and asthma); high-dose XP; intermediate-dose XP; low-dose XP. The animals were bred in a conventional manner at (25 ± 1) °C for 7 d before being experimented upon.

Drugs
XP was composed of the following medicinal materials: Shanyao (Rhizoma Dioscoreae Opposite, 20 g), Bihu (Gekko Swinhonii, 8 g), Baizhu (Rhizoma Atractylodis Macrocephalae, 10 g), Muhudic (Semen Qroxyli, 10 g) and prepared Mahuang (Herba Ephedrae, 6 g). These medicinal materials were purchased from the TCM Pharmacy in the First Affiliated Hospital of Anhui University of Chinese Medicine. Using a conventional method, a decoction with a crude drug concentration of 0.55 g/mL was prepared. GK granules were manufactured by Golong Medicine (No. Z20103119; Shanxi, China).

Reagents and equipment
Ovalbumin (OVA) was purchased from Sigma-Aldrich (A5253; Saint Louis, MO, USA). Aluminum hydroxide was obtained from Shanghai Dong Shan Chemical Plant (Shanghai, China). Kits to measure levels of TNF-α, IL-4, ECP expression of MMP-9 and TIMP-1, and to obtain the eosinophil count, were purchased from Bio-Rad Laboratories (Hercules, CA, USA). A microscope image-analysis system was obtained from Olympus (Tokyo, Japan). A PikoReal™ 96 RealTime Polymerase Chain Reaction (PCR) system was purchased from Thermo Scientific. A desktop high-speed refrigerated centrifuge (JW-3021HR) was obtained from Jiaven (Anhui, China).

Modeling and drug administration
In the NC group, sensitization and asthma induction were undertaken using physiologic (0.9%) saline. In the other groups, sensitization was triggered by 10% OVA (1 mL; i.p.); after day-15, asthma was induced by aerosol inhalation with 1% OVA (5 mL) for 20-30 min on a daily basis for 2 weeks consecutively. The OVA challenge was regarded as being successful if the rats developed symptoms of irritability, dyspnea, sneezing, wheezing and cyanosis, as well as bending their back and scratching their ears.

After 6 consecutive days of asthma induction, physiologic saline was administered (i.g.) to rats in NC and asthma-model groups. In the high-dose XP, intermediate-dose XP, low-dose XP, and GK groups, the corresponding drugs were administered (i.g.) at a daily dose (in g/kg body weight) of 0.5 (10-fold higher than the dose for human adults), 0.375 (7.5-fold), 0.25 (5-fold), and 0.375 (7.5-folds) respectively, for 8 consecutive days.
**Specimen collection**

In each group, animals were anesthetized using 10% chloral hydrate (0.35 mL/100 g, i.p.) within 24 h after the final challenge. Blood samples were obtained from the abdominal aorta. After leaving in a water bath for 20 min at 37 °C, specimens were centrifuged at 1509 (×g) for 10 min at room temperature. The supernatants were harvested and stored at −20 °C for further use.

Thoracotomy was carried out to harvest the two lobes of the left lung, which were placed in cryogenic tubes immediately for real-time quantitative PCR (qPCR). Right lung tissue was harvested, fixed in 100 g/L formaldehyde, embedded in paraffin, and sliced to sections of thickness 5 μm.

**Measurements**

Hematoxylin and eosin (HE) staining of lung sections was done to observe the pathologic/morphologic changes of the airways. In each group, serum levels of ECP, TNF-α, and IL-4, and eosinophil counts, were measured using an immunofluorescence method according to manufacturer instructions. Lung-tissue sections were processed according to the instructions provided with the MMP-9 and TIMP-1 assay kits. The bronchus with a perimeter of 2.0 mm was selected. The optical density was measured at four randomly selected sites at locations with positive expression, and the mean value used as the measurement value of that specimen. Changes in levels of MMP-9 and TIMP-1 at the same airway level were compared among these groups.

mRNA transcription of MMP-9 and TIMP-1 was determined using real-time qPCR. In brief, 1 mL of TRIzol was added into 100-ng lung tissue to extract total RNA. Reverse transcription (cDNA synthesis) and qPCR were undertaken following manufacturer instructions and using the following primer (forward and reverse, respectively) pairs: 5′-CCCATCTAT-GAGGGTTACGC-3′ and 5′-TTTAATGTCAAGCAAGATTTC-3′ (150 bp in length) for rat β-actin; 5′-CCCCCTCACTTGGGCCCCACAGGTCC-3′ and 5′-TTGGCTTCTCCTCCTGATT-3′ (185 bp) for rat MMP-9; 5′-CTCTGGCATCTGTGTTTGGT-3′ and 5′-CGCTGTATAAGTTGGTCT-3′ (156 bp) for rat TIMP-1.

The validity of the PCR was verified using solution curves. The relative quantification method was applied for analyses, and relative expression of mRNA was 2−ΔΔCt×100%. Statistical analyses

Experimental data were analyzed using SPSS v19.0 (IBM, Armonk, NY, USA). Measurement data are the mean ± standard deviation (x ± s). The Student’s t-test was used to compare differences between two groups. Univariate analysis of variance was applied to compare multiple groups. P < 0.05 was considered significant.

**RESULTS**

**Pathologic morphologies of the airways**

In the NC group, bronchial ciliated columnar epithelial cells were arranged in an orderly manner, and only a small number of Goblet cells were visible after HE staining. No infiltration of inflammatory cells was seen at the lumina or walls of the airways, or surrounding tissues. Mucosal epithelia at all bronchiolar levels were intact, and virtually no cells were within the bronchiolar lumen. No obvious thickening of the smooth-muscle layer or tracheal stenosis was found. The alveolar walls were intact, and alveolar sizes were normal.

In the asthma-model group, bronchial ciliated epithelial cells were arranged in a disorderly manner and some of them were exfoliated, and massive proliferation of Goblet cells was observed. Infiltration of inflammatory cells was seen at all layers of the walls, along with massive accumulation of inflammatory cells in the airway lumen. The terminal bronchiolar mucosal epithelium was arranged in a disorderly manner, together with edema and degradation. The alveolar structure was disordered, and the alveolar walls had become thin and broken. The alveoli were enlarged and merged, and some of them fused into bullae; as a result, emphysema occurred.

In the high-dose XP group, infiltration of inflammatory cells in bronchial and lung tissues was decreased remarkably. The walls were smooth, and the volume of intraluminal exudates decreased. Alveolar structures were slightly disordered.

In the intermediate-dose XP, low-dose XP, and GK groups, the morphologic changes of lung tissues were between those observed for the high-dose XP group and asthma-model group (Figure 1).

**Serum levels of ECP, TNF-α and IL-4, and eosinophil counts**

Serum levels of ECP, TNF-α and IL-4, and eosinophil counts, were significantly higher in the asthma-model group than in the NC group (all P < 0.01). Compared with the asthma-model group, the serum concentrations of ECP, TNF-α and IL-4, and eosinophil counts were decreased significantly in the high-, intermediate-, and low-dose XP groups and GK group (P < 0.01 or P < 0.05) (Table 1).

**Protein expression of MMP-9 and TIMP-1**

Compared with the NC group, the protein expression of MMP-9 and TIMP-1 in lung tissues increased significantly in the asthma-model group (both P < 0.01). Compared with the asthma-model group, the protein expression of MMP-9 and TIMP-1 in lung tissues decreased significantly in the GK group as well as in the high- and intermediate-dose XP groups (all P < 0.01) (Table 2).

**Transcription of MMP-9 and TIMP-1**

Compared with the NC group, the mRNA transcrip-
Table 1 Comparison of serum concentrations of ECP and eosinophil counts among six groups (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>EOS (×10³/L)</th>
<th>ECP (ng/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-4 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>23.87 ± 2.76</td>
<td>3.23 ± 0.12</td>
<td>65.32 ± 9.16</td>
<td>32.36 ± 2.87</td>
</tr>
<tr>
<td>Asthma model</td>
<td>10</td>
<td>184.76 ± 6.40</td>
<td>8.06 ± 0.29</td>
<td>93.73 ± 10.72</td>
<td>46.77 ± 4.14</td>
</tr>
<tr>
<td>GK</td>
<td>10</td>
<td>93.92 ± 3.40</td>
<td>4.38 ± 0.51</td>
<td>72.18 ± 9.04</td>
<td>39.92 ± 2.08</td>
</tr>
<tr>
<td>High-dose XP</td>
<td>10</td>
<td>90.74 ± 4.11</td>
<td>4.36 ± 0.33</td>
<td>71.27 ± 10.34</td>
<td>36.58 ± 3.66</td>
</tr>
<tr>
<td>Intermediate-dose XP</td>
<td>10</td>
<td>97.90 ± 3.14</td>
<td>4.57 ± 0.25</td>
<td>75.91 ± 9.24</td>
<td>37.68 ± 2.71</td>
</tr>
<tr>
<td>Low-dose XP</td>
<td>10</td>
<td>121.26 ± 3.83</td>
<td>5.21 ± 0.20</td>
<td>82.65 ± 11.72</td>
<td>44.51 ± 7.43</td>
</tr>
</tbody>
</table>

Notes: NC and Asthma model group: treated with physiologic (0.9%) saline; GK group: treated with 0.375 g GK kg⁻¹·d⁻¹; high-dose XP group: treated with 0.5 g XP kg⁻¹·d⁻¹; intermediate-dose XP group: treated with 0.375 g XP kg⁻¹·d⁻¹; low-dose XP group: treated with 0.25 g XP kg⁻¹·d⁻¹; EOS: eosinophils; ECP: eosinophil cationic protein; TNF-α: tumor necrosis factor-α; IL-4: interleukin-4; NC: normal control; GK: Guilong Kechuanning; XP: Xiaochuanping powder. Compared with NC group, P < 0.01; compared with asthma model group, 0.01 < P < 0.05; compared with GK group, P < 0.01, P > 0.05.

Table 2 Comparison of protein expression of MMP-9 and TIMP-1 in lung tissues among the six groups (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MMP-9</th>
<th>TIMP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>0.35 ± 0.09</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>Asthma model</td>
<td>10</td>
<td>0.82 ± 0.09</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>GK</td>
<td>10</td>
<td>0.49 ± 0.05</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>High-dose XP</td>
<td>10</td>
<td>0.47 ± 0.08</td>
<td>0.43 ± 0.11</td>
</tr>
<tr>
<td>Intermediate-dose XP</td>
<td>10</td>
<td>0.63 ± 0.06</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>Low-dose XP</td>
<td>10</td>
<td>0.75 ± 0.13</td>
<td>0.60 ± 0.07</td>
</tr>
</tbody>
</table>

Notes: NC and Asthma model group: treated with physiologic (0.9%) saline; GK group: treated with 0.375 g GK kg⁻¹·d⁻¹; high-dose XP group: treated with 0.5 g XP kg⁻¹·d⁻¹; intermediate-dose XP group: treated with 0.375 g XP kg⁻¹·d⁻¹; low-dose XP group: treated with 0.25 g XP kg⁻¹·d⁻¹; MMP-9: matrix metalloproteinase-9; TIMP-1: tissue inhibitor of metalloproteinase-1; NC: normal control; GK: Guilong Kechuanning; XP: Xiaochuanping powder. Compared with NC group, P < 0.01; compared with asthma model group, P < 0.01; compared with GK group, P > 0.05.

**DISCUSSION**

Asthma is a chronic inflammatory airway disease, and different cell types (e.g., eosinophils, mast cells, T lymphocytes) and cellular components are involved. It has a series of pathologic features: airway inflammation, airway hyper-responsiveness, and airway remodeling. TNF-α, a pro-inflammatory cytokine with broad biologic activities, can trigger eosinophils, mast cells, and T lymphocytes to release inflammatory mediators (e.g., IL-4) and, finally, induce or exacerbate asthma. Eosinophils, participate directly in chronic structural changes in the airways, and are closely associated with the severity of airway hyper-responsiveness and injury/exfoliation of airway epithelial cells. After activation, eosinophils release the toxic glycoprotein ECP that can cause direct injury to the respiratory epithelium. During the inflammatory process, ECP can induce mast
cells to release histamine and mediate injuries to the airway epithelium and horn cells. Thus, it has extremely strong cytotoxicity and is an important substance that can cause airway inflammation and airway hyper-responsiveness during asthma.15

In the present study, the asthma-model group had significantly higher serum concentrations of ECP, TNF-α and IL-4, and eosinophil counts, than the NC group (all \( P < 0.01 \)), suggesting the success of model creation. In the high- and intermediate-dose XP groups, serum concentrations of ECP, TNF-α and IL-4, and eosinophil counts decreased significantly compared with the asthma-model group (all \( P < 0.01 \)), and their effectiveness was superior (\( P < 0.01 \)) or equal (\( P > 0.05 \)) to GK (a licensed TCM). Thus, the high- and intermediate-dose XP groups could effectively inhibit the secretion of TNF-α in serum, reduce the accumulation and activation of inflammatory cells (e.g., eosinophils), and thus decrease the serum contents of inflammatory mediators such as ECP and IL-4, and alleviate the inflammatory response and asthma symptoms.

Recurrent attacks of asthma and persistent chronic inflammation can lead to the exfoliation and proliferation of epithelial cells, hyperplasia and hypertrophy of smooth muscle cells, deposition of the extracellular matrix (ECM), increased permeability of blood vessels, and regeneration of capillaries. These structural changes of the airways are termed “airway remodeling.” Research has revealed that excessive deposition of the ECM in airway walls is a major cause of airway fibrosis and airflow obstruction, and that ECM production is closely related to expression of MMP-9 and TIMP-1.1,17,15 MMP-9 can degrade type IV collagen, fibronectin and laminin, causing damage to the ECM.1,10,16 TIMP-1 is a specific inhibitor of MMP-9 expression. TIMP-1 can inhibit MMP-9 activity to protect ECM from being degraded. Thus, TIMP-1, along with MMP-9, maintains the stability of the normal ECM and internal environment. In an asthma attack, the expression of MMP-9 and TIMP-1 increases, and their proportions become imbalanced.1,15 High- and intermediate-dose XP and GK could decrease the expression of MMP-9 and TIMP-1 effectively (\( P < 0.01 \)) and alleviate the imbalanced ratio of expression of MMP-9:TIMP-1. Thus, XP and GK could reduce ECM deposition and affected airway remodeling.

In conclusion, XP can directly or indirectly reduce TNF-α secretion in the serum of asthmatic rats and inhibit the infiltration and activation of eosinophils. By doing so, it can reduce the serum concentrations of inflammatory mediators (e.g., ECP and IL-4) and expression of MMP-9 and TIMP-1 in lung tissues and, thus, effectively alleviate airway inflammation and airway remodeling. These may be the key mechanisms through which XP can be used to treat asthma, and warrant further investigation.

REFERENCES


Table 3 Comparison of mRNA expression of MMP-9 and TIMP-1 in the lung tissues among the six groups (x ± s)
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