Clinical study on Mailuoning injection combined with conventional medical treatment of acute attack of bronchial asthma

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ABSTRACT

Objective: To study the effect of Mailuoning injection combined with conventional medical treatment for acute attack of bronchial asthma on inflammatory factor and protease levels as well as signal molecule expression. Methods: Patients with acute attack of bronchial asthma who were treated in Traditional Chinese Medicine Hospital of Shunyi District Beijing between January 2015 and January 2018 were selected as the research subjects and randomly divided into the Mailuoning group who accepted Mailuoning injection combined with conventional treatment and the control group who accepted conventional treatment. The levels of inflammatory factors and proteases in serum as well as the expression of signal molecules in peripheral blood were determined before treatment and 3 d after treatment. Results: Compared with those of same group before treatment, serum IL-18, IL-33, MIP-1 α, S1P, Eotaxin, MC-CP, ADAM33 and MMP9 levels as well as peripheral blood TIM3, Galectin-9, GATA-3 and ROR γ t expressions of both groups of patients were significantly lower whereas serum TIMP1 levels and peripheral blood Foxp3 expression were significantly higher 3 d after treatment. Moreover, serum IL-18, IL-33, MIP-1 α, S1P, Eotaxin, MC-CP, ADAM33 and MMP9 levels as well as peripheral blood TIM3, Galectin-9, GATA-3 and ROR γ t expression of Mailuoning group 3 d after treatment were lower than those of control group whereas serum TIMP1 level and peripheral blood Foxp3 expression were significantly higher than those of control group. Conclusion: Mailuoning injection combined with conventional medical treatment for acute attack of bronchial asthma can more effectively inhibit the inflammatory response, reduce the activity of proteases and regulate the expression of signal molecules.

1. Introduction

Bronchial asthma is the respiratory system disease characterized by airway hyperresponsiveness and reversible bronchial smooth muscle airway limitation; paroxysmal wheeze, dyspnea and chest distress are the common symptoms in patients with asthma. The lymphocyte, mast cell and eosinophil dysfunction, the excessive secretion of pro-inflammatory factors and the enhancement of protease activity mediated by multiple signaling pathways played an important role in the course of bronchial asthma[1,2]. β2 receptor agonist, glucocorticoid and aminophylline are the common drugs for clinical treatment of acute attack of bronchial asthma, which can not only effectively relax the airway smooth muscle and inhibit airway inflammation, but mostly achieve good curative effect. However, there are still some patients with acute attack of asthma whose condition cannot be controlled in time after conventional drug treatment, and severe cases can cause respiratory failure and be life-threatening, so other different drugs are also needed to control the condition as soon as possible. Mailuoning injection, made from honeysuckle, radix scrophulariae, twotooth achyranthes root, dendrobe and other Chinese medicines, has anti-inflammatory and antioxidant activity, and has been proven to be able to improve the airway function in patients with bronchial asthma[3]. In the following studies, we specifically analyzed the effect of Mailuoning injection combined with conventional medical treatment for acute attack of bronchial asthma on inflammatory factor and protease levels as well as signal molecule expression.
2. Case information and research methods

2.1 General information

Patients with acute attack of bronchial asthma who were treated in Traditional Chinese Medicine Hospital of Shunyi District Beijing between January 2015 and January 2018 were chosen as the research subjects, all the patients were in accordance with the diagnosis for acute attack of bronchial asthma, and the patients who used glucocorticoids or other immunomodulators 3 months before inclusion were ruled out. A total of 102 patients were enrolled and divided into two groups by random number table, each with 51 cases. There were 28 males and 23 females in the Mailuoning group, who were 28-44 years old; there were 29 males and 22 females in the control group, who were 29-42 years old. There was no significant difference in the general data between the two groups (P>0.05).

2.2 Therapy

After admission, both groups were treated according to the routine procedures for acute attack of bronchial asthma, including oxygen uptake, aerosol inhalation of β 2 receptor agonist and glucocorticoid, aminophylline, and antibiotics, etc. On the basis of the conventional treatment, Mailuoning group were treated with Mailuoning injection, and the method was as follows: 20 mL of Mailuoning injection was added to 500 mL of 5% glucose injection, which was provided by intravenous drip, 2 times/d. Both groups were treated for seven days in a row.

2.3 Laboratory index detection

Before treatment and 3 d after treatment, analysis of serum proteases before and after treatment. 8-10 mL of cubital venous blood was collected from the two groups of patients and divided into two parts. One part was centrifuged to separate serum, and the contents of IL-18, IL-33, MIP-1 α, S1P, Eotaxin, MC-CP, ADAM33, MMP9 and TIMP1 were determined according to the instructions of Elisa kit. The other part was anti-coagulated with EDTA and then used directly to incubate the fluorescence antibodies of TIM3, Galectin-9, GATA-3, ROR γ t and Foxp3, and then the expression intensity of the corresponding molecules was detected on the flow cytometer.

Table 1.

Changes in serum inflammatory factors before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>IL-18</th>
<th>IL-33</th>
<th>MIP-1 α</th>
<th>S1P</th>
<th>Eotaxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mailuoning</td>
<td>51</td>
<td>Before treatment</td>
<td>361.2±33.9</td>
<td>221.3±29.3</td>
<td>55.31±6.72</td>
<td>2.31±0.34</td>
<td>346.2±42.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>183.5±22.3 *</td>
<td>141.2±18.3 *</td>
<td>28.38±3.41 *</td>
<td>1.22±0.18 *</td>
<td>201.2±23.6 *</td>
</tr>
<tr>
<td>Control group</td>
<td>51</td>
<td>Before treatment</td>
<td>364.1±37.2</td>
<td>220.8±28.7</td>
<td>54.89±6.23</td>
<td>2.28±0.31</td>
<td>348.1±45.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>245.2±32.4</td>
<td>189.2±22.1</td>
<td>39.51±4.47</td>
<td>1.83±0.23</td>
<td>283.4±33.2 *</td>
</tr>
</tbody>
</table>

*: comparison between before and after treatment within group, P<0.05; *: comparison between groups after treatment, P<0.05.

Table 2.

Changes in serum proteases before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>MC-CP</th>
<th>ADAM33</th>
<th>MMP9</th>
<th>TIMP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mailuoning</td>
<td>51</td>
<td>Before treatment</td>
<td>825.2±89.5</td>
<td>42.39±5.84</td>
<td>334.1±42.6</td>
<td>12.31±1.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>562.3±56.2 *</td>
<td>20.12±2.83 *</td>
<td>170.2±20.3 *</td>
<td>19.48±2.25 *</td>
</tr>
<tr>
<td>Control group</td>
<td>51</td>
<td>Before treatment</td>
<td>828.1±93.6</td>
<td>42.11±5.37</td>
<td>332.8±46.2</td>
<td>12.03±1.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>673.5±82.5</td>
<td>29.38±3.85</td>
<td>231.2±29.3</td>
<td>15.38±2.15 *</td>
</tr>
</tbody>
</table>

*: comparison between before and after treatment within group, P<0.05; *: comparison between groups after treatment, P<0.05.

2.4 Statistical analysis

Software SPSS 18.0 was used to input the experimental data, the differences in data between groups were analyzed by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum inflammatory factor levels

Before treatment and 3 d after treatment, analysis of serum inflammatory factors IL-18 (pg/mL), IL-33 (pg/mL), MIP-1 α (pg/mL), S1P (μmol/L) and Eotaxin (ng/mL) levels in the two groups of patients was as follows: serum IL-18, IL-33, MIP-1 α, S1P and Eotaxin levels were not significantly different between the two groups of patients before treatment (P>0.05) while these levels were significantly different after treatment (P<0.05). Serum IL-18, IL-33, MIP-1 α, S1P and Eotaxin levels of Mailuoning group were lower than those of control group; compared with inflammatory factors of same group before treatment, serum IL-18, IL-33, MIP-1 α, S1P and Eotaxin levels of both groups of patients were significantly lower after treatment (P<0.05).

3.2 Serum protease levels

Before treatment and 3 d after treatment, analysis of serum proteases MC-CP (pg/mL), ADAM33 (pg/mL), MMP9 (ng/mL) and TIMP1 (pg/mL) levels in the two groups of patients was as follows: serum MC-CP, ADAM33, MMP9 and TIMP1 levels were not significantly different between the two groups of patients before treatment (P>0.05) while these levels were significantly different after treatment (P<0.05). Serum MC-CP, ADAM33 and MMP9 levels of Mailuoning group were lower than those of control group whereas TIMP1 level was higher than that of control group; compared with proteases of same group before treatment, serum MC-CP, ADAM33 and MMP9 levels of both groups of patients were significantly lower whereas TIMP1 level were significantly higher after treatment (P<0.05).
### 3.3 Peripheral blood signal molecule expression

Before treatment and 3 d after treatment, analysis of peripheral blood signal molecules TIM3, Galectin-9, GATA-3, ROR γ γ and Foxp3 expression in the two groups of patients was as follows: peripheral blood TIM3, Galectin-9, GATA-3, ROR γ γ and Foxp3 expressions were not significantly different between the two groups of patients before treatment (P>0.05) while they were significantly different after treatment (P<0.05). Peripheral blood TIM3, Galectin-9, GATA-3 and ROR γ γ expressions of Mailuoning group were lower than those of control group whereas Foxp3 expression was higher than that of control group; compared with signal molecules of same group before treatment, peripheral blood TIM3, Galectin-9, GATA-3 and ROR γ γ expressions of both groups of patients were significantly lower whereas Foxp3 expression was significantly higher after treatment (P<0.05).

### 4. Discussion

Mailuoning injection is a drug made from honeysuckle, radix scrophulariae, twotooth achyranthes root, dendrobe and other Chinese medicines. It has been proven to be able to improve the airway function of patients with acute attack of bronchial asthma[3], but there is no report about its influence on the excessive activation of airway inflammation in the course of asthma. IL-18 and IL-33 are the interleukin family members that are closely related to the airway inflammation and airway hyperresponsiveness, and both cytokines can significantly induce Th2 cell activation and increase the Th2 type immune response in airway, which can increase the release of histamine, leukotrienes and other inflammatory mediators and activate the airway inflammation[4,5]. MIP-1 α is the β chemokine synthesized and secreted by the macrophages, which can promote the chemotactic movement of eosinophils, mast cells and so on to airway smooth muscle, and facilitate the activation of inflammation in the airway[6]. S1P is an inflammatory cytokine produced during sphingomyelin metabolism, which can serve as the extracellular matrix to start the sphingosine kinase signaling pathways, induce mast cell degranulation in airway and amplify the airway inflammation[7,8]. Eotaxin belongs to the chemokine CC family, and can act on the CCR3 receptor on the surface of eosinophils, promote cell infiltration to airway and promote the airway inflammation mediated by eosinophils[9]. Analysis of the changes in corresponding inflammatory factors before and after Mailuoning injection treatment showed that compared with the inflammatory factors of same group before treatment, serum IL-18, IL-33, MIP-1 α, S1P and Eotaxin levels of both groups of patients significantly decreased after treatment, and serum IL-18, IL-33, MIP-1 α, S1P and Eotaxin levels of Mailuoning group after treatment were significantly lower than those of control group. It means that Mailuoning combined with conventional treatment can effectively reduce the release of inflammatory factors and inhibit the over-activation of airway inflammation in patients with acute asthma attack.

During the repeated attack of bronchial asthma, the persistent activation of the inflammatory response can enhance the activity of a variety of proteases, resulting in the abnormal degradation and accumulation of collagen, elastin and other compositions in airway smooth muscle interstitium, and the airway remodeling. In addition, changes in the airway smooth muscle interstitium compositions are also conducive to local infiltration of inflammatory cells and promote the amplification and activation of inflammatory response. MC-CP is a carboxypeptidase produced by the activated mast cells, which is involved in the hydrolysis of various protein and polypeptide carboxyl terminals, and it is beneficial to the large infiltration of mast cells in the airway smooth muscle. ADAM33 is a member of the ADAMs family that has disintegrin domain, metalloproteinase domain and growth factor domain, and can not only promote the hydrolysis of proteins in airway smooth muscle interstitium, but also facilitate fibroblast and other cell proliferation and intensify the airway remodeling[10,11]. MMP9 is the MMPs family member that has strong hydrolysis effect on the proteins in airway smooth muscle interstitium, and it causes the excessive hydrolysis of collagen, laminin, elastin and other airway smooth muscle interstitium elements, resulting in the occurrence of airway remodeling[12,13]; TIMP1 is the specific inhibitor of MMP9, which can reduce the hydrolysis activity of MMP9 on protease and delay the occurrence of airway remodeling. Analysis of the changes of corresponding proteases in serum before and after Mailuoning injection treatment showed that compared with the proteases of same group before treatment, serum MC-CP, ADAM33 and MMP9 levels of both groups of patients significantly decreased whereas TIMP1 levels significantly increased after treatment, and serum MC-CP, ADAM33 and MMP9 levels of Mailuoning group after treatment were lower than those of control group whereas TIMP1 level was higher than that of control group. This means that both two different therapies can inhibit the activity of a variety of proteases in patients with acute asthma attack, but Mailuoning combined with
conventional therapy has stronger inhibitory effect on the activity of proteases than conventional therapy, and can inhibit the airway remodeling mediated by proteases.

The excessive secretion of inflammatory cytokines and the excessive increase of protease activity in the course of bronchial asthma are closely related to the abnormal differentiation of CD4+ T cell subsets Th1, Th2, Th17 and Treg. The shift of Th1/Th2 balance to Th2 and the shift of Th17/Treg balance to Th17 are the important characteristics of bronchial asthma[14]. Multiple signaling molecules on the cell surface play a key regulatory role in the differentiation of CD4+T cells into different subsets. TIM3 is a member of the TIM gene family, and can interact with the ligand Galectin-9 to realize the negative regulation on Th1 cells and facilitate the shift of Th1/Th2 balance to Th2[15]; GATA-3 is the zinc finger protein GATA family member specifically expressed in Th2 cells, which can effectively induce CD4+T cells to differentiate into Th2 subtype and play an important role in bronchial asthma[16,17]. RORγt and Foxp3 are the specific signal molecules of Th17 cells and Treg cells respectively, the former can increase the secretion of IL-17 and promote the activation of airway inflammation after inducing Th17 differentiation and maturation, and the latter can exert immunosuppressive effect and hinder the development of bronchial asthma after inducing Treg differentiation and maturation[18,19]. Analysis of the changes in peripheral blood immune cell signaling molecules before and after Mailuoning injection treatment showed that compared with signal molecules of same group before treatment, peripheral blood TIM3, Galectin-9, GATA-3 and RORγt expression of both groups of patients significantly decreased whereas Foxp3 expression significantly increased after treatment; peripheral blood TIM3, Galectin-9, GATA-3 and RORγt expression of Mailuoning group was lower than those of control group whereas Foxp3 expression was higher than that of control group. It shows that Mailuoning combined with conventional treatment can be more effective than conventional treatment to modulate the differentiation of CD4+T cell subsets, and correct the disorder of CD4+T cell subsets in the course of bronchial asthma.

Above all, it can be concluded that compared with conventional treatment, Mailuoning injection combined with conventional medical treatment for acute attack of bronchial asthma can more effectively suppress the inflammatory response, reduce the protease activity, adjust the signal molecule expression and correct the CD4+T cell subset disorder.

References