Curative effect of singulair combined with methylprednisolone in treatment of mycoplasma pneumonia and the effect on inflammatory response and peripheral blood lymphocyte subset content

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ABSTRACT

Objective: To study the effect of Singulair combined with methylprednisolone treatment on inflammatory response and peripheral blood lymphocyte subset content in children with mycoplasma pneumonia. Methods: 74 children with severe mycoplasma pneumonia who were treated in Huai’an Second People’s Hospital between June 2014 and October 2016 were selected as the research subjects and randomly divided into two groups, the intervention group received routine treatment combined with Singulair and methylprednisolone, and control group received routine treatment. Before and after treatment, serum was collected to detect the contents of inflammatory cytokines and corresponding cytokines of T lymphocyte subsets, and peripheral blood was collected to determine the contents of T cell subsets. Results: 3 d and 7 d after treatment, serum MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ and IL-17 contents as well as peripheral blood Th1 and Th17 contents of both groups were significantly lower than those before treatment while serum IL-4 and TGF-β contents as well as peripheral blood Treg and Th2 contents were significantly higher than those before treatment, and serum MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ and IL-17 contents as well as peripheral blood Th1 and Th17 contents of intervention group were significantly lower than those of control group while serum IL-4 and TGF-β contents as well as peripheral blood Treg and Th2 contents were significantly higher than those of control group. Conclusion: Singulair combined with methylprednisolone treatment of mycoplasma pneumonia can inhibit the inflammatory response and regulate the balance of Th1/Th2 and Th17/Treg cells.

1. Introduction

Mycoplasma pneumoniae (MP) is a common pathogenic bacterium that causes community-acquired pneumonia in children. The condition is self-limiting in most children with mycoplasma pneumonia, but the illness will aggravate and bronchiolitis obliterans will occur in some children after macrolide antibiotic treatment, and severe cases may develop into severe pneumonia and endanger the safety of life[1,2]. Activation of systemic inflammatory response is the feature of severe pneumonia, and the abnormal secretion of various inflammatory mediators is an important part causing the disease progression[3]. Methylprednisolone is an intermediate-acting glucocorticoid preparation that inhibits inflammatory and immune response, and singulair is leukotriene receptor antagonist that can antagonize the inflammatory response mediated by leukotriene[4,5]. In the following study, singulair combined with methylprednisolone was used for treatment of children with mycoplasma pneumonia, and the changes of inflammatory reaction and peripheral blood lymphocyte subset contents were specifically analyzed.
2. Research subjects and methods

2.1 Research subjects

74 children with severe mycoplasma pneumonia who were treated in Huai’an Second People’s Hospital between June 2014 and October 2016 were selected as the research subjects, all children are with the clinical symptoms of fever, cough and wheezing, X-ray film conformed to the features of mycoplasma pneumonia, and serum mycoplasma IgM was positive. Random number table was used to divide the 74 children into two groups, each with 37 cases. Intervention group were treated with routine treatment combined with Singulair and methylprednisolone, including 22 male cases and 15 female cases that were 5-12 years old; control group received routine treatment, including 21 male cases and 16 female cases that were 5-13 years old. There was no significant difference in general information between the two groups of patients (P>0.05).

2.2 Therapy

Both groups of patients were given anti-febrile, phlegm-reducing, anti-infection and other conventional treatment, Mucosolvan was used to reduce phlegm, and anti-infection treatment adopted azithromycin (approved by: H20030983, Shenyang Jinlong Pharmaceutical Co., Ltd.) 10 mg/kg/d in 100 mL of 5% glucose injection, by intravenous drip for 5 d. Intervention group, on the basis of routine treatment, received Singulair combined with methylprednisolone treatment, and the method was as follows: methylprednisolone (approved by: H61023180, Xi’an Lijun Pharmaceutical Co., Ltd.) 2 mg/kg/d in 100 mL of 5% glucose injection, by intravenous drip, for 5 d; oral administration of Singulair (approved by: H20083330, Shandong Lunan Beite Pharmaceutical Co., Ltd.), 4 mg for children 2-5 years old and 5 mg for children 6-12 years old, 1 time/night. Both groups were treated for seven consecutive days.

2.3 Serum cytokine content detection methods

Before treatment as well as 3 d and 7 d after treatment, 3 mL of peripheral venous blood was collected from two groups of children respectively and centrifuged to separate serum, and then enzyme-linked immunosorbent assay kit was used to detect MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ, IL-4, IL-17 and TGF-β contents.

2.4 Peripheral blood T cell subset content detection methods

Before treatment as well as 3 d and 7 d after treatment, 3 mL of peripheral venous blood was collected from two groups of children respectively and anti-coagulated with EDTA to incubate the monoclonal antibody of CD4, IFN-γ, IL-4, IL-17 and TGF-β, and then the Th1 (CD4+IFN-γ), Th2 (CD4+IL-4), Th17 (CD4+IL-17) and Treg (CD4+TGF-β) contents were determined in flow cytometer.

2.5 Statistical methods

SPSS 21.0 software was used for statistical analysis, comparison of serum cytokine contents and peripheral blood T cell contents between two groups was by t test, and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum inflammation index contents

Before treatment as well as 3 d and 7 d after treatment, analysis of serum inflammation indexes MCP-1 (ng/L), PCT (μg/L), ICAM-1 (μg/L), CXCL8 (ng/L) and CRP (mg/L) contents between two groups of patients was as follows: before treatment, differences in serum MCP-1, PCT, ICAM-1, CXCL8 and CRP contents were not statistically significant between two groups of patients (P>0.05); 3 d and 7 d after treatment, serum MCP-1, PCT, ICAM-1, CXCL8 and CRP contents of both groups were significantly lower than those before treatment (P<0.05); 3 d and 7 d after treatment, serum MCP-1, PCT, ICAM-1, CXCL8 and CRP contents of both groups were significantly lower than those before treatment (P<0.05), shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>MCP-1</th>
<th>PCT</th>
<th>ICAM-1</th>
<th>CXCL8</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>37</td>
<td>Before treatment</td>
<td>174.5±22.3</td>
<td>16.9±2.2</td>
<td>352.3±52.4</td>
<td>93.5±11.2</td>
<td>38.9±6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>96.4±13.6</td>
<td>8.3±1.1</td>
<td>201.3±34.6</td>
<td>48.6±6.2</td>
<td>17.6±2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 d after treatment</td>
<td>52.9±11.2</td>
<td>4.2±0.7</td>
<td>142.6±22.7</td>
<td>30.2±4.7</td>
<td>9.5±1.1</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>Before treatment</td>
<td>177.1±20.5</td>
<td>17.2±2.3</td>
<td>357.1±42.1</td>
<td>95.1±11.8</td>
<td>39.6±4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>129.7±17.3</td>
<td>12.8±1.9</td>
<td>285.2±33.6</td>
<td>75.2±9.3</td>
<td>23.7±3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 d after treatment</td>
<td>93.5±11.7</td>
<td>9.5±1.1</td>
<td>219.3±27.4</td>
<td>57.5±7.4</td>
<td>14.2±1.9</td>
</tr>
</tbody>
</table>

△: comparison between intervention group and control group, P<0.05; ▲: comparison between before treatment and after treatment, P<0.05.
Peripheral blood T lymphocyte subsets-corresponded cytokine contents of two groups of patients before and after treatment.

Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TH1</th>
<th>TH2</th>
<th>TH17</th>
<th>Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>37</td>
<td>Before</td>
<td>17.5±2.41</td>
<td>5.6±0.84</td>
<td>4.4±0.67</td>
<td>0.7±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after</td>
<td>10.3±1.88</td>
<td>7.9±0.95</td>
<td>2.1±0.35</td>
<td>1.4±0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 d after</td>
<td>7.5±0.93</td>
<td>9.5±1.15</td>
<td>1.5±0.20</td>
<td>1.9±0.22</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>Before</td>
<td>17.9±2.52</td>
<td>5.4±0.86</td>
<td>4.3±0.69</td>
<td>0.8±0.10</td>
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<td></td>
<td></td>
<td>3 d after</td>
<td>13.9±2.15</td>
<td>6.7±0.85</td>
<td>3.4±0.52</td>
<td>1.1±0.18</td>
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<td></td>
<td></td>
<td>7 d after</td>
<td>10.2±1.72</td>
<td>7.9±0.85</td>
<td>2.5±0.36</td>
<td>1.3±0.20</td>
</tr>
</tbody>
</table>

*: comparison between intervention group and control group, P<0.05; #: comparison between before treatment and after treatment, P<0.05.

3.2 Peripheral blood T lymphocyte subset and corresponding cytokine contents

Before treatment as well as 3 d and 7 d after treatment, analysis of peripheral blood TH1, TH2, TH17 and Treg contents between two groups of patients was as follows: before treatment, differences in peripheral blood TH1, TH2, TH17 and Treg contents were not statistically significant between two groups of patients (P>0.05); 3 d and 7 d after treatment, peripheral blood TH1 and TH17 contents of both groups were significantly lower than those before treatment while TH2 and Treg contents were significantly higher than those before treatment (P<0.05), and peripheral blood IFN-γ and IL-4 contents of intervention group were significantly lower than those before treatment (P<0.05), shown in Table 2.

Before treatment as well as 3 d and 7 d after treatment, analysis of peripheral blood T lymphocyte subsets-corresponded cytokines IFN-γ (μg/L), IL-4 (μg/L), IL-17 (ng/L) and TGF-β (μg/L) contents between two groups of patients was as follows: before treatment, differences in peripheral blood IFN-γ, IL-4, IL-17 and TGF-β contents were not statistically significant between two groups of patients (P>0.05); 3 d and 7 d after treatment, peripheral blood IFN-γ and IL-17 contents of both groups were significantly lower than those before treatment while IL-4 and TGF-β contents were significantly higher than those before treatment (P<0.05), and peripheral blood IFN-γ and IL-17 contents of intervention group were significantly lower than those of control group while IL-4 and TGF-β contents were significantly higher than those of control group (P<0.05), shown in Table 3.

Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-17</th>
<th>TGF-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>37</td>
<td>Before</td>
<td>5.9±0.78</td>
<td>0.4±0.10</td>
<td>84.4±10.25</td>
<td>1.4±0.19</td>
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<tr>
<td></td>
<td></td>
<td>3 d after</td>
<td>2.5±0.34</td>
<td>0.7±0.11</td>
<td>36.4±6.24</td>
<td>3.6±0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 d after</td>
<td>1.9±0.22</td>
<td>0.9±0.13</td>
<td>20.3±3.84</td>
<td>5.0±0.77</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>Before</td>
<td>6.0±0.91</td>
<td>0.4±0.07</td>
<td>86.2±10.77</td>
<td>1.5±0.20</td>
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<tr>
<td></td>
<td></td>
<td>3 d after</td>
<td>3.9±0.53</td>
<td>0.5±0.08</td>
<td>59.6±8.36</td>
<td>2.7±0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 d after</td>
<td>2.7±0.42</td>
<td>0.7±0.09</td>
<td>36.4±5.28</td>
<td>3.3±0.51</td>
</tr>
</tbody>
</table>

*: comparison between intervention group and control group, P<0.05; #: comparison between before treatment and after treatment, P<0.05.

4. Discussion

Mycoplasma pneumoniae is a kind of pathogen without cell wall, and it will cause bronchial and capillary bronchial epithelial damage and airway inflammation, and lead to the increase of airway secretions and the obstruction of capillary bronchus after it infects the respiratory tract. Macrolide antibiotics are the common drugs for the treatment of mycoplasma pneumoniae infection, most children can achieve ideal curative effect, but the illness develops rapidly in some children with severe mycoplasma pneumonia, and the macrolide antibiotic therapy alone is difficult to obtain exact curative effect[6-7]. Excessive activation of the airway inflammatory response is the important link causing severe mycoplasma pneumonia progression, and the combination of drugs with different mechanisms of action to suppress the inflammatory reaction is the key to the treatment of severe mycoplasma pneumonia[8]. Singulair is a type of leukotriene receptor antagonist, which is able to antagonize the combination of leukotriene with its receptor as well as the activation of downstream inflammatory response[9]; methylprednisone is an intermediate-acting glucocorticoid with significant anti-inflammatory and immunosuppressive effects[10]. Studies have reported the positive value of singulair and methylprednisolone for mycoplasma pneumonia treatment, but it is not yet clear about the efficacy of joint application of two drugs for the treatment of mycoplasma pneumonia. In the study, the curative effect of singulair combined with methylprednisolone for mycoplasma pneumoniae treatment was analyzed from the aspects of inflammatory response and immune response.

Overactivation of inflammatory response is an important...
characteristic of severe mycoplasma pneumonitis, and also an important link in the development of the disease. During the activation of inflammatory response, the secretion of MCP-1, PCT, ICAM-1, CXCL8, CRP and other inflammatory mediators increases significantly. MCP-1 and CXCL8 are the important chemokines that can promote the inflammatory cell chemotaxis to inflammatory lesions and increase the number of inflammatory cells infiltrated in the lesion[11]; PCT is a precursor of calcitonin, and a variety of parenchymal cells in the body massively express PCT under the action of pro-inflammatory medium and secrete it into the blood circulation, which can sensitively reflect the degree of inflammation[12]; ICAM-1 is an important adhesion molecule that can mediate the adhesion between inflammatory cells and vascular endothelium and promote inflammatory cell infiltration to the inflammatory region[13]; CRP is a non-specific acute phase protein synthesized by hepatocytes, and the activation of inflammatory response is consistent with the secretion of CRP. In order to define the singulair combined with methylprednisolone therapy on the inflammatory response in the development of mycoplasma pneumonia, the levels of above inflammatory mediators were analyzed in the study, and the result showed that serum MCP-1, PCT, ICAM-1, CXCL8 and CRP contents of both groups significantly decreased after treatment, and serum MCP-1, PCT, ICAM-1, CXCL8 and CRP contents of intervention group after treatment were significantly lower than those of control group. It means that routine anti-infection, phlegm-reducing and other symptomatic treatment can inhibit the inflammatory response to a certain extent, and the joint use of singulair and methylprednisolone can more effectively inhibit the inflammatory response in the development and changes of mycoplasma pneumonia.

The abnormal activation of inflammatory response in children with severe mycoplasma pneumonia is closely related to the disorder of the immune response. The CD4+T subset in the T cell subsets is the important subset that resists and eliminates the pathogens, which can be divided into Th1, Th2, Th17, Treg and other subsets according to the different patterns to secrete cytokines. Th1 cells mainly secrete INF-γ, IL-2, TNF-α and other cytokines[14,15], and Th17 cells mainly secrete IL-17, IL-22 and IL-23, are mainly involved in cellular immune response and can remove pathogens[16]; the IL-4, IL-5, TGF-β and IL-10 secreted by Th2 and Treg have the effect of inhibiting inflammatory response, and can antagonize the differentiation, maturation and secretory function of Th1 and Th17[17,18]. During the progression of mycoplasma pneumonia, Th1 and Th17 are significantly activated, and the cytokines secreted by them are on the one hand, conducive to eliminating pathogens, and on the other hand can also cause tissue damage; The activation of Th2 and Treg can inhibit the function of Th1 and Th17 after the pathogen is cleared to prevent tissue damage. In the study, analysis of above CD4+T cell subset contents in the peripheral blood showed that peripheral blood Th1 and Th17 contents of both groups significantly decreased while Th2 and Treg contents significantly increased after treatment, and peripheral blood Th1 and Th17 contents of intervention group were significantly lower than those of control group while Th2 and Treg contents were significantly higher than those of control group. Further analysis of CD4+T cell subsets-corresponded cytokine levels showed that peripheral blood IFN-γ and IL-17 contents of both groups significantly decreased while IL-4 and TGF-β contents significantly increased after treatment, and peripheral blood IFN-γ and IL-17 contents of intervention group were significantly lower than those of control group while IL-4 and TGF-β contents were significantly higher than those of control group. This means that the conventional anti-infection, phlegm-reducing and other symptomatic treatment can regulate immune response to a certain extent, and the combination of singulair and methylprednisolone can more effectively regulating the balance of Th1/Th2 and Th17/Treg cells.

Singulair combined with methylprednisolone has more exact curative effect for the treatment of mycoplasma pneumonia, and can restrain the inflammatory response during disease progression and adjust the balance of Th1/Th2 and Th17/Treg cells.

References


[16] Shao L, Cong Z, Li X, Zou H, Cao L, Guo Y. Changes in levels of IL-9, IL-17, IFN-γ, dendritic cell numbers and TLR expression in peripheral blood in asthmatic children with Mycoplasma pneumoniae infection. *Int J Clin Exp Pathol* 2015; 8(5): 5263-5272.
