Effect of anisodamine injection-assisted azithromycin sequential therapy on serum indexes in children with mycoplasma pneumonia

Huan Zhang*

Second District of General Pediatrics, Huizhou Municipal Central Hospital, Huizhou City, Guangdong Province, 51600

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 17 December 2015
Accepted 18 November 2015
Available online 20 November 2015

Keywords:
Azithromycin
Anisodamine injection
Mycoplasma pneumonia

ABSTRACT

Objective: To analyze the effect of anisodamine injection-assisted azithromycin sequential therapy on serum indexes in children with mycoplasma pneumonia. Methods: A total of 218 cases of children with bronchial pneumonia were randomly divided into observation group and control group, each group included 109 cases, control group received azithromycin therapy alone, observation group received anisodamine injection-assisted azithromycin sequential therapy, and then differences in serum Clara cell secretory protein (CCSP), intercellular adhesion molecule-1 (sICAM-1), interleukin-17A (IL-17A), CD3+, CD4+, CD8+, monocyte chemoattractant protein-4 (MCP-4), macrophage-derived chemokine (MCP), cysteinyl leukotrienes (CysLTs), pulmonary surfactant protein-A (SP-A), pulmonary surfactant protein-B (SP-B), pulmonary surfactant protein-C (SP-C) and pulmonary surfactant protein-D (SP-D) levels of two groups were compared after treatment. Results: After treatment, serum CCSP level of observation group was higher than that of control group, and levels of sICAM-1 and IL-17A were lower than those of control group; after treatment, CD3+ and CD4+ levels of observation group were higher than those of control group, and CD8+ level was lower than that of control group; serum MCP-4, MDC and CysLTs levels of observation group were lower than those of control group; after treatment, serum SP-A, B, C and D levels of observation group were lower than those of control group. Conclusion: Anisodamine injection-assisted azithromycin sequential therapy for children with mycoplasma pneumonia can reduce inflammatory injury of lung and airway, balance cellular immune function and promote the rehabilitation of illness.

1. Introduction

Mycoplasma pneumonia is caused by mycoplasma infection and clinically called primary atypical pneumonia, children are the high risk group of mycoplasma pneumonia because of the weak natural defense, and the disease accounts for 50%-60% of pediatric respiratory diseases. Mycoplasma pneumonia in children is mainly characterized by high fever and persistent severe cough, and may also be associated with complications of blood system, digestive system and other non-pulmonary organs, and without timely treatment, there will be severe consequences[1,2]. Azithromycin is a macrolide antibiotic and the most important type of antibiotic for treatment of mycoplasma pneumonia, but its long-term intravenous infusion can increase the incidence of adverse reactions, and therefore, azithromycin sequential therapy is currently more advocated. Anisodamine belongs to cholinergic receptor blocker and also play a positive role in relieving spasm of the smooth muscles and meanwhile alleviating airway inflammation[3]. In the research, children with mycoplasma pneumonia treated in our hospital from July 2010 to July 2014 were selected as research subjects, and the effect of anisodamine injection-assisted azithromycin sequential therapy on serum indexes in children with mycoplasma pneumonia was analyzed, hereby reported as follows.
2. Subjects and methods

2.1. Research subjects

A total of 218 cases of children with bronchial pneumonia were randomly divided into observation group and control group, each group with 109 cases; observation group included 59 male cases and 50 female cases who were 2-11 years old with average (6.18±0.92) years; control group included 57 male cases and 52 female cases who were 3-12 years old with average (6.64±0.87) years. Both groups were excluded of infectious diseases of other organs, didn’t have allergic reactions to azithromycin or anisodamine injection, didn’t receive hormone medication within 30 d, and signed informed consent forms after families were informed of treatment and research process.

2.2. Treatment methods

Both groups received routine treatment such as cough suppressing and panting calming, phlegm dispelling and nutritional support. Control group received azithromycin therapy alone, and details were as follows: dissolving azithromycin for injection (Xi’an Lijun Pharmaceutical Co., Ltd., Approval No.: H20020269) 10 mg/kg•d in 5% glucose liquid 250 mL, intravenous drip, continuous medication for 3 d, and if illness was relieved, switching to oral administration of azithromycin 10 mg/kg•d for continuous 5 d. Observation group received same usage and dosage of azithromycin as that of control group with additional assistant therapy of anisodamine injection, and details were as follows: intravenous injection of raceanisodamine hydrochloride injection (Topfond Pharmaceutical Co., Ltd., Approval No.: H41023403) 0.2-0.3 mg/kg/time, 1-2 times every day for continuous 5 d.

2.3. Specimen collecting

5 d after treatment, 3 mL of fasting peripheral blood was drawn, let stand for 20 min at room temperature and then centrifuged for 5 min with the speed of 3 000 r/min, intravenous drip, continuous medication for 3 d, and if illness was relieved, switching to oral administration of azithromycin 10 mg/kg•d for continuous 5 d. Observation group received same usage and dosage of azithromycin as that of control group with additional assistant therapy of anisodamine injection, and details were as follows: intravenous injection of raceanisodamine hydrochloride injection (Topfond Pharmaceutical Co., Ltd., Approval No.: H41023403) 0.2-0.3 mg/kg/time, 1-2 times every day for continuous 5 d.

2.4. Observation indexes

Double sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect Clara cell secretory protein (CCSP), intercellular adhesion molecule-1 (sICAM-1) and interleukin-17A (IL-17A) levels after treatment, and specific procedures were conducted in strict accordance with the kit instructions. Flow cytometer was used to detect T-cell subset levels after treatment, including CD3+, CD4+ and CD8+, and antibodies used were mouse anti-human CD3FITC, CD4PerCP and CD8 PE. ELISA was used to detect serum monocyte chemoattractant protein-4 (MCP-4), macrophage-derived chemokine (MCP) and cysteinyl leukotrienes (CysLTs) levels after treatment. SP-A, B, C and D kits were used to detect serum pulmonary surfactant protein-A (SP-A), pulmonary surfactant protein-B (SP-B), pulmonary surfactant protein-C (SP-C) and pulmonary surfactant protein-D (SP-D) contents after treatment.

2.5. Statistical methods

SPSS 21.0 software was used to statistically analyze above data, measurement data between two groups was by $t$ test, correlation analysis was by linear regression, and obtained results were considered to be statistically significant at a level of $P<0.05$.

3. Results

3.1. Serum CCSP, sICAM-1 and IL-17A

ELISA was used to detect serum CCSP, sICAM-1 and IL-17A levels of two groups after treatment, and results were as follows: after treatment, serum CCSP level of observation group was higher than that of control group, sICAM-1 and IL-17A levels were lower than those of control group, differences between two groups were statistically significant ($P<0.05$), and details were shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TSB (μmol/L)</th>
<th>DB (μmol/L)</th>
<th>TCB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>AT</td>
<td>BT</td>
</tr>
<tr>
<td>Observation</td>
<td>168.63±23.45</td>
<td>19.75±1.28</td>
<td>80.11±3.26</td>
</tr>
<tr>
<td>Control</td>
<td>167.29±21.34</td>
<td>29.69±2.13</td>
<td>80.54±3.07</td>
</tr>
</tbody>
</table>

$P>0.05$ $P<0.05$ $P>0.05$ $P<0.05$ $P>0.05$ $P<0.05$

BT: Before treatment; AT: After treatment.

3.2. Serum T-cell subset levels

After two groups of children with mycoplasma pneumonia received different treatment options, serum T-cell levels were detected, and results were as follows: after treatment, CD3+ and CD4+ levels of observation group were higher than those of control group, CD8+ level was lower than that of control group, differences between two groups were statistically significant ($P<0.05$), and details were shown in Figure 1.
3.3. Serum MCP-4, MDC and CysLTs levels

ELISA was used to detect serum of two groups after treatment, and results were as follows: serum MCP-4, MDC and CysLTs levels of observation group were lower than those of control group, differences between two groups were statistically significant ($P<0.05$), and details were shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCP-4 (pg/mL)</th>
<th>MDC (pg/mL)</th>
<th>CysLTs (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>46.81±3.92</td>
<td>632.17±48.24</td>
<td>853.81±78.62</td>
</tr>
<tr>
<td>Control</td>
<td>85.05±6.93</td>
<td>1092.84±89.82</td>
<td>1211.46±109.83</td>
</tr>
<tr>
<td>$t_2$</td>
<td>7.134</td>
<td>9.824</td>
<td>11.523</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.4. Serum alveolar surfactant protein contents

After two groups of children with mycoplasma pneumonia received different treatment options, serum alveolar surfactant protein contents were detected, and results were as follows: after treatment, serum SP-A, B, C and D levels of observation group were lower than those of control group, differences were statistically significant ($P<0.05$), and details were shown in Figure 2.

![Figure 2. Serum alveolar surfactant protein contents of two groups after treatment.](image)

4. Discussion

Mycoplasma pneumonia is one of the most common pulmonary infectious diseases in children and mostly treated with macrolide antibiotics, among which azithromycin is the mostly widely used in clinical treatment. Azithromycin inhibits the synthesis of bacterial protein through combination of special target of pathogen 50s subunit 23SrRNA. But with the increasing application of azithromycin, its clinical effect presents a trend of gradual decline, and after many children receive azithromycin therapy alone, the effect is not ideal[4,5]. Anisodamine is cholinergic receptor blocker that can relieve spasm of the smooth muscles and play an active role in the acute phase of mycoplasma pneumonia, and specific mechanisms may contain the following several points: 1) anisodamine stabilizes lysosomal membrane in acute phase of inflammation and clears peroxide; 2) it relieves spasm of the smooth muscles as well as platelet aggregation and release, and decreases endothelial cell damage and blood perfusion of inflammatory tissue. It is currently believed that anisodamine combined with antibiotics can play a coordinating role, but it needs further study to determine levels of which types of factors are changed by them.

Clara cell secretory protein (CCSP) is secreted by Clara cell, it is an important endogenous anti-inflammatory factor and it has anti-inflammatory, immunomodulatory and cell protective function. Study has shown that in models of acute pulmonary infection, CCSP content decreases in bronchoalveolar lavage fluid, indicating that CCSP is involved in the regulation of early onset of pneumonia[6]. Interleukin-17A (IL-17A) can regulate the expression of various cytokines, chemokines and adhesion factors, and study has shown that IL-17A can activate and recruit NEU to the airway and enhance airway inflammation, and may increase the incidence of long-term asthma in children. Intercellular adhesion molecule-1 (sICAM-1) is soluble adhesion molecule that comes off adhesion molecules on surface of leukocytes, vascular endothelial cells and so on and enters into the bloodstream, and it plays an important role in the process of endothelial cell adhesion and movement towards the outside of vessels. sICAM-1 has pro-inflammatory property and can induce migration of eosinophils[7,8]. In the research, after observation group received anisodamine injection-assisted azithromycin sequential therapy, serum CCSP, sICAM-1 and IL-17A levels were detected, and results showed that CCSP level was higher and levels of sICAM-1 and IL-17A were lower, indicating that anisodamine injection could increase body’s anti-inflammatory ability and decrease the migration of eosinophils.

There is universal immune injury, especially cellular immune function disorder in children with mycoplasma infection, including excessive activation of helper lymphocytes in peripheral blood, shift state of Th2-type factors and declined proportion of CD4+/CD8+, which directly result in decreased natural ability of children to resist pathogens and also influence the effect of drug treatment. Azithromycin is a new macrolide antibiotic and most studies showed that its effect in treatment of mycoplasma pneumonia is significant[9]. Anisodamine can play a positive role in acute phase of mycoplasma pneumonia and promote the disappearance of symptoms and signs in children. In the research, after observation group received anisodamine combined with azithromycin therapy, serum CD3+ and CD4+ levels increased and CD8+ level decreased, indicating that disorder of T-cell subset levels was improved and also showing that combined drug therapy could enhance the cellular immune function in children, and it might be one of the internal mechanisms for the treatment option to exert the clinical efficacy[10].

Monocyte chemoattractant protein-4 (MCP-4) is mainly from mononuclear cells and epithelial cells, and it can selectively recruit Th2 cells to areas with inflammation and cause Th2 cell-mediated immune inflammatory injury. Macrophage-derived chemokine (MDC) is mainly from macrophages and dendritic cells from mononuclear cells, and its receptor is expressed in Th2 cells and can result in Th2 cell-mediated immune inflammatory response[11,12].
Both MCP-4 and MDC have potent function of recruiting eosinophils to airway, and a variety of toxin proteins and cytokines, etc released by them can cause further bronchial mucosal epithelium damage and inflammatory cell infiltration. Cysteinyl leukotrienes (CysLTs) are leukotrienes C4, D4 and E4 collectively, belong to arachidonic acid metabolites, are both the most potent bronchoconstrictor and inducer of mucous hypersecretion, and can cause bronchial mucosa hypersecretion and hinder ciliary movement. In the research, serum MCP-4, MDC and CysLTs levels of observation group decreased after treatment, indicating that anisodamine injection-assisted azithromycin sequential therapy could relieve inflammatory injury of lung and trachea, reduce the recruitment of inflammatory cells in airway and thus alleviate the condition[13].

Pulmonary surfactant (PS) is synthesized and secreted by AEC- II and made up of 90% phospholipid and 10% SP-A, B, C and D. SP-A and D have the effect of removing bacteria as well as apoptotic and necrotic cells, reducing immune response, eliminating inflammation, and so on, and SP-B and C can be combined with dipalmitoyl phosphatidyl choline in phospholipid to reduce pulmonary surface tension. Recent studies have shown that in the early stage of infection in young rats, number of mitochondria and lamellar bodies in cytoplasm increases significantly, indicating that both synthesis and secretion of all SP subsets increase in acute injury[14,15]. When the host is infected with mycoplasma, alveolar epithelial cells and the surrounding capillaries receive varying degrees of damage, the capillary permeability increases, and therefore, after mycoplasma infection, increasingly synthesized and released SP-A, B, C and D can infiltrate from alveolar space to permeability-increased capillaries and finally enter into the blood with blood flow, thus resulting in dramatically increased SP-A, B, C and D levels in serum of children with mycoplasma pneumonia. In the research, serum SP-A, B, C and D levels of observation group were lower after treatment, indicating that adding anisodamine injection could ease acute lung injury in the process of mycoplasma pneumonia, facilitate the early recovery of pulmonary function and reduce the incidence of complications such as pulmonary dysfunction.

To sum up, it is concluded that anisodamine injection-assisted azithromycin sequential therapy for children with mycoplasma pneumonia can reduce inflammatory injury of lung and airway, balance cellular immune function and promote the rehabilitation of illness, and it’s worth popularization in clinical practice in the future.

References


