Curative effect of small-dose glucocorticoids + azithromycin treatment of lobar pneumonia
Li Zhang

Pediatrics Department, the First People’s Hospital of Ziyang, Ziyang 641300, Sichuan Province, China

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ABSTRACT

Objective: To study the effect of small-dose glucocorticoids + azithromycin therapy on inflammatory response and stress response in children with lobar pneumonia. Methods: Children with mycoplasma pneumoniae lobar pneumonia who were treated in the First People’s Hospital of Ziyang between January 2015 and January 2018 were chosen as the research subjects and randomly divided into two groups: GCs group were treated with low-dose glucocorticoids + azithromycin, and control group were treated with azithromycin. The inflammatory response indicators and stress response indicators in serum and peripheral blood were measured before treatment as well as 3 d and 5 d after treatment. Results: Compared with those of same group before treatment, peripheral blood TLR4, NF-κB, NOX2, p38MAPK, iNOS, GRP78, PERK, ATF4, CHOP and Caspase-12 expression intensity as well as serum TNF-α, HMGB-1, ICAM-1, NO, MDA and 8-OHdG levels of both groups were significantly decreased after treatment, and peripheral blood TLR4, NF-κB, NOX2, p38MAPK, iNOS, GRP78, PERK, ATF4, CHOP and Caspase-12 expression intensity as well as serum TNF-α, HMGB-1, ICAM-1, NO, MDA and 8-OHdG levels of GCs group after treatment were significantly lower than those of control group. Conclusion: Small-dose glucocorticoid + azithromycin therapy is more effective than azithromycin therapy to suppress the inflammatory and stress response in children with lobar pneumonia.

1. Introduction

Pneumonia is a common respiratory disease in children. Lobar pneumonia is a common type of pneumonia, it is urgent and serious, and it needs timely symptomatic and supportive treatment. Streptococcus pneumoniae and Mycoplasma pneumoniae are common pathogens causing lobar pneumonia; the lobar pneumonia caused by Mycoplasma pneumoniae has been rising in recent years, this kind of pneumonia has long course, and the antibiotic treatment alone is with poor effect and difficult to quickly control lung inflammation[1]. Glucocorticoid is a strongly anti-inflammatory adrenal cortical hormone, and the intermediate-acting glucocorticoid treatment of lobar pneumonia can inhibit the aggregation of inflammatory cells in the alveoli and stabilize lysosomal membrane structure to relieve the inflammation and stress in the course of lobar pneumonia[2,3]. Study has shown that glucocorticoid treatment of children with lobar pneumonia can promote the improvement of clinical symptoms and signs[4], but it is not yet clear about the change of inflammatory and stress response in course of the disease. In the following studies, we specifically analyzed the therapeutic effect of small-dose glucocorticoid + azithromycin treatment of children with lobar pneumonia from the perspectives of inflammatory response and stress response.

2. General information and research methods

2.1 General case information

Children with Mycoplasma pneumoniae lobar pneumonia who were treated in the First People’s Hospital of Ziyang between...
January 2015 and January 2018 were selected as the research subjects, all children were diagnosed with lobar pneumonia by clinical symptoms and signs as well as imageological examination, and the mycoplasma pneumoniae nucleic acid test was positive; the children who were treated by antibacterial agents or immunoregulatory medicines and the children combined with congenital disease were excluded. A total of 56 children were enrolled and divided into two groups by random number table method, each with 28 cases. There were 16 males and 12 females in the GCs group, they were 2-9 years old, and the onset time was 13 h-3 d; there were 15 males and 13 females in the control group, they were 2-10 years old, and the onset time was 15 h-3 d. There was no significant difference in the general data between the two groups ($P>0.05$).

### 2.2 Research methods

#### 2.2.1 Therapy

After admission, both groups of children received routine oxygen uptake, physical cooling, calming panting and suppressing cough, and other symptomatic and supportive treatments. Control group received azithromycin for infection on the basis of the above symptomatic and supportive treatment, and the method was as follows: intravenous drip of azithromycin injection 5 mg/kg + saline injection 250 mL was performed for 5 days in a row, stopped for 4 days and then changed to oral administration of same dose of azithromycin for 5 consecutive days. GCs group received azithromycin and glucocorticoids on the basis of the above symptomatic and supportive treatment, and the method was as follows: azithromycin treatment was the same as that of control group, intravenous drip of methylprednisolone sodium succinate 2 mg/kg was conducted 2 times/day for 5 d in a row, and then changed to oral administration of same dose of prednisone, 1 time/day, and the medication was stopped 3 d after the body temperature was normal.

#### 2.2.2 Laboratory detection methods

The 5-6 mL of peripheral venous blood was collected before treatment as well as 3 d and 5 d after treatment. 3-4 mL of venous blood was centrifuged to separate serum, the Elisa kit instructions were followed for laboratory operations and determination of the contents of MDA, 8-OhdG; the rest of the venous blood was taken, anti-coagulated with EDTA and sub-packed with 0.1 mL/part to incubate the fluorescent antibody of TLR4, NF-κB, NOX2, p38MAPK, iNOS, GRP78, PERK, ATF4, CHOP and Caspase-12, and flow cytometer was used to determine their expression intensity.

### 2.3 Statistical methods

Software SPSS 22.0 was used to input data, the differences in measurement data between groups were analyzed by t test and $P<0.05$ showed statistical significance in the differences.

### 3. Results

#### 3.1 Inflammation markers in serum and peripheral blood

Analysis of inflammation markers TLR4, NF-κB, TNF-α (ng/L), HMGB-1 (ng/L) and ICAM-1 (μg/L) in peripheral blood and serum of the two groups of children was as follows: peripheral blood TLR4 and NF-κB expression intensity as well as serum TNF-α, HMGB-1 and ICAM-1 levels of both groups were significantly different between before and after treatment ($P<0.05$); peripheral blood TLR4 and NF-κB expression intensity as well as serum TNF-α, HMGB-1 and ICAM-1 levels were not significantly different between the two groups before treatment ($P>0.05$) whereas they were significantly different after treatment ($P<0.05$), and peripheral blood TLR4 and NF-κB expression intensity as well as serum TNF-α, HMGB-1 and ICAM-1 levels of GCs group were lower than those of control group.

#### 3.2 Oxidative stress markers in peripheral blood and serum

Analysis of oxidative stress markers NOX2, p38MAPK, iNOS, NO (μmol/L), MDA (μmol/L) and 8-OhdG (μg/L) in peripheral blood and serum of the two groups of children was as follows: peripheral blood NOX2, p38MAPK and iNOS expression intensity as well as serum NO, MDA and 8-OhdG levels of both groups were significantly different between before and after treatment ($P<0.05$); peripheral blood NOX2, p38MAPK and iNOS expression intensity as well as serum NO, MDA and 8-OhdG levels were not significantly

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TLR4</th>
<th>NF-κ B</th>
<th>TNF-α</th>
<th>HMGB-1</th>
<th>ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCs group</td>
<td>28</td>
<td>Before treatment</td>
<td>1.03±0.14</td>
<td>0.99±0.14</td>
<td>129.30±16.20</td>
<td>168.40±20.30</td>
<td>252.30±33.50</td>
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<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.58±0.07*</td>
<td>0.52±0.06*</td>
<td>70.30±9.30*</td>
<td>106.40±12.70*</td>
<td>136.30±13.70*</td>
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<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.39±0.05*</td>
<td>0.38±0.04*</td>
<td>54.70±7.40*</td>
<td>82.30±10.50*</td>
<td>103.60±12.90*</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>Before treatment</td>
<td>1.01±0.14</td>
<td>1.02±0.15</td>
<td>130.60±17.10</td>
<td>170.10±19.40</td>
<td>254.20±34.70</td>
</tr>
<tr>
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<td></td>
<td>3 d after treatment</td>
<td>0.73±0.08*</td>
<td>0.69±0.08*</td>
<td>98.30±11.50*</td>
<td>139.50±17.40*</td>
<td>189.30±22.60*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.59±0.06*</td>
<td>0.54±0.05*</td>
<td>76.40±9.30*</td>
<td>116.30±14.70*</td>
<td>146.40±18.40*</td>
</tr>
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</table>

* Comparison between before and after treatment within the two groups, $P<0.05$; ** Comparison between the two groups after treatment, $P<0.05$; *** Comparison between the two groups after treatment, $P<0.05$. 

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for children with lobar pneumonia, we first analyzed the changes in the inflammation of the lungs to clarify the value of glucocorticoid + azithromycin therapy. Glucocorticoids can rapidly reduce alveolar exudate and the powerful anti-inflammatory effects of glucocorticoids have been increasingly used in the treatment of lobar pneumonia, and the powerful anti-inflammatory effects of azithromycin monotherapy on infection is poor. In recent years, antibiotic for pediatric lobar pneumonia, but the control effect of azithromycin is lower than those of control group.

### 3.3 Endoplasmic reticulum stress markers in peripheral blood

Analysis of endoplasmic reticulum stress markers GRP78, PERK, ATF4, CHOP and Caspase-12 expression intensity of both groups were significantly different before and after treatment (P<0.05); peripheral blood GRP78, PERK, ATF4, CHOP and Caspase-12 expression intensity of both groups were significantly different before and after treatment (P<0.05); peripheral blood GRP78, PERK, ATF4, CHOP and Caspase-12 expression intensity were not significantly different between the two groups before treatment (P>0.05) whereas they were significantly different after treatment (P<0.05), and peripheral blood GRP78, p38MAPK and iNOS expression intensity as well as serum NO, MDA and 8-OhdG levels of GCs group were lower than those of control group.

### Discussion

Mycoplasma pneumoniae is a common pathogen in children with lobar pneumonia, and it causes much alveolar exudate and high risk of heart, liver, brain and other important organ injury, so it requires active treatment. Azithromycin is a common antibiotic for pediatric lobar pneumonia, but the control effect of azithromycin monotherapy on infection is poor. In recent years, glucocorticoids have been increasingly used in the treatment of lobar pneumonia, and the powerful anti-inflammatory effects of glucocorticoid can rapidly reduce the exudation of the lungs and reduce the inflammation of the lungs. In the above studies, in order to clarify the value of glucocorticoid + azithromycin therapy for children with lobar pneumonia, we first analyzed the changes in the inflammatory response before and after treatment. TLR4 is an important receptor molecule mediating inflammatory reaction in the process of pathogen infection, which belongs to the pattern recognition receptor, and can recognize Mycoplasma pneumoniae to start intracellular signal transduction and activate transcription factor NF-κB; activated NF-κB can initiate expression of TNF-α, HMGB-1, ICAM-1 and other inflammatory cytokines[7,8]. TNF-α and HMGB-1 have pro-inflammatory activity; they are secreted by activated mononuclear macrophages, and can mediate the cascade amplification activation of inflammatory response after pathogen infection[9,10]; ICAM-1 has intercellular adhesion activity, promote the adhesion between inflammatory cells and alveolar epithelium, and increase the infiltration of inflammatory cells in the lungs[11]. Comparison of above inflammation markers between the two groups showed that peripheral blood TLR4 and NF-κB expression intensity as well as serum TNF-α, HMGB-1 and ICAM-1 levels of both groups were decreased after treatment, and the decrease of above inflammation markers in GCs group was more significant than that in control group. This shows that glucocorticoid + azithromycin is more effective than azithromycin alone to inhibit the inflammation amplification and activation mediated by TLR4/NF-κB in the course of lobar pneumonia.

The excessive activation of neutrophils by pathogens in the course of lobar pneumonia would increase the expression of myeloperoxidase in cells, and then increase the production of peroxide through the activity of the catalyzing enzyme and mediate the excessive activation of oxidative stress reaction in the course of pneumonia. Glucocorticoid has strong anti-inflammatory activity; it can restrain the activation and infiltration of a variety of inflammatory cells, and it is not only beneficial to prevent the amplification and activation of inflammatory response, but also helps to reduce the production of peroxide and inhibit the activation of oxidative stress reaction. NOX2 is the NOX family member that is highly expressed in the lung tissue, it catalyzes the NAPDH electron transfer to oxygen molecules to increase the superoxide anion generation, and superoxide anions are strongly oxidizing and can mediate the oxidative stress injury of local tissue[12,13].

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>NOX2</th>
<th>p38MAPK</th>
<th>iNOS</th>
<th>NO</th>
<th>MDA</th>
<th>8-OhdG</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCs group</td>
<td>28</td>
<td>Before treatment</td>
<td>1.04±0.15</td>
<td>0.99±0.13</td>
<td>1.03±0.16</td>
<td>1.04±0.14</td>
<td>1.01±0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.62±0.08*</td>
<td>0.59±0.06*</td>
<td>0.52±0.08*</td>
<td>0.60±0.08*</td>
<td>0.51±0.07*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.47±0.06*</td>
<td>0.41±0.06*</td>
<td>0.35±0.05*</td>
<td>0.45±0.06*</td>
<td>0.34±0.05*</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>Before treatment</td>
<td>1.06±0.12</td>
<td>1.01±0.14</td>
<td>1.01±0.14</td>
<td>1.02±0.16</td>
<td>1.03±0.17</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.79±0.09*</td>
<td>0.76±0.09*</td>
<td>0.76±0.09*</td>
<td>0.76±0.09*</td>
<td>0.71±0.08*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.63±0.07*</td>
<td>0.58±0.07*</td>
<td>0.61±0.07*</td>
<td>0.59±0.07*</td>
<td>0.60±0.07*</td>
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</tr>
</tbody>
</table>

1: Comparison between before and after treatment within the two groups, *P*<0.05; 2: Comparison between the two groups after treatment, *P*<0.05.

### Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>GRP78</th>
<th>PERK</th>
<th>ATF4</th>
<th>CHOP</th>
<th>Caspase-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCs group</td>
<td>28</td>
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<td></td>
<td>3 d after treatment</td>
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<td>0.52±0.08*</td>
<td>0.60±0.08*</td>
<td>0.51±0.07*</td>
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<td>0.35±0.05*</td>
<td>0.45±0.06*</td>
<td>0.34±0.05*</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>Before treatment</td>
<td>1.06±0.12</td>
<td>1.01±0.14</td>
<td>1.01±0.14</td>
<td>1.02±0.16</td>
<td>1.03±0.17</td>
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<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.79±0.09*</td>
<td>0.76±0.09*</td>
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<td>0.59±0.07*</td>
<td>0.60±0.07*</td>
</tr>
</tbody>
</table>

1: Comparison between before and after treatment within the two groups, *P*<0.05; 2: Comparison between the two groups after treatment, *P*<0.05.
p38MAPK is the MAPKs signaling pathway family member that mediates oxidative stress response in local lung tissue, it induces the expression of iNOS and catalyzes the generation of NO through downstream signal transduction, and NO can further induce the generation of oxygen free radicals and nitrogen free radicals and mediate oxidative stress reaction[14]. During the excessive activation of oxidative stress, the lipids and proteins in local lung tissue are injured after having oxidation reaction with superoxide anions, oxygen free radicals, nitrogen free radicals and other strong oxidants, and generate the corresponding products MDA anions, oxygen free radicals, nitrogen free radicals and other.

Activation of oxidative stress, the lipids and proteins in local lung tissue, it induces oxidative stress reaction and mediate oxidative stress reaction mediated by NOX2 and p38MAPK in the course of lobar pneumonia. Glucocorticoid has a wide range of biological activities in the body, and the activity of stable lysosomal membrane can reduce the tissue damage in the process of inflammation and stress. The changes of lysosomal membrane stability in the course of lobar pneumonia are closely related to the activation of endoplasmic reticulum stress response. Endoplasmic reticulum is involved in protein biosynthesis and folding process in cells, and the changes of the lysosome membrane structure stability will cause the misfolded or unfolded proteins to increase and accumulate continuously in the endoplasmic reticulum, which will change the biological function of endoplasmic reticulum and produce endoplasmic reticulum stress[17]. Molecular chaperone GRP78 plays the role of a sensor in endoplasmic reticulum stress process, which can activate transcription factor ATF6 through the downstream PERK signal transduction to increase the expression of target genes CHOP and Caspase-12 and cause cell dysfunction[18]. We analyzed the changes of endoplasmic reticulum stress markers before and after the treatment in the study, and the results showed that peripheral blood NOX2, p38MAPK and iNOS expression intensity as well as serum NO, MDA and 8-OhdG levels of both groups were decreased after treatment, and the decrease of above oxidative stress markers in GCs group was more significant than that in control group. It shows that glucocorticoid + azithromycin is more effective than azithromycin to inhibit the oxidative stress response mediated by NOX2 and p38MAPK in the course of lobar pneumonia.

The research of the study confirms that low-dose glucocorticoid + azithromycin treatment of children with lobar pneumonia is more effective than azithromycin treatment alone to inhibit the activation of endoplasmic reticulum stress process, which can activate transcription factor ATF6 through the downstream PERK signal transduction to increase the expression of target genes CHOP and Caspase-12 and cause cell dysfunction[18]. We analyzed the changes of endoplasmic reticulum stress markers before and after the treatment in the study, and the results showed that peripheral blood NOX2, p38MAPK and iNOS expression intensity as well as serum NO, MDA and 8-OhdG levels of both groups were decreased after treatment, and the decrease of above endoplasmic reticulum stress markers in GCs group was more significant than that in control group after treatment. This indicates that glucocorticoid + azithromycin is more effective than azithromycin alone in inhibiting endoplasmic reticulum stress in the course of lobar pneumonia.

The research of the study confirms that low-dose glucocorticoid + azithromycin treatment of children with lobar pneumonia is more effective than azithromycin treatment alone to inhibit the activation of inflammatory response, oxidative stress response and endoplasmic reticulum stress reaction in the course of disease.

References