Effects of methylprednisolone combined with montelukast on immune function and cytokines in children with recurrent Henoch–Schonlein purpura

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ABSTRACT

Objective: To study the effects of methylprednisolone combined with montelukast on immune function and cytokines in children with recurrent Henoch-Schonlein purpura. Methods: Children who were diagnosed with recurrent Henoch-Schonlein purpura in Zigong Third People’s Hospital between September 2015 and August 2017 were selected as the research subjects and randomly divided into the intervention group who received methylprednisolone combined with montelukast therapy and the control group who received hydrocortisone therapy. The levels of Th1/Th2 and Th17/Treg immunity indexes in peripheral blood as well as cytokines in serum were measured before treatment as well as 4 and 8 weeks after treatment. Results: 4 weeks and 8 weeks after treatment, Th1 and Treg cell contents as well as Th2 and Th17 cell contents as well as GATA-3 and RORγt mRNA expression in peripheral blood of both groups of patients were significantly higher than those before treatment whereas Th2 and Th17 cell contents as well as GATA-3 and RORγt mRNA expression in peripheral blood of intervention group were significantly higher than those of control group whereas Th2 and Th17 cell contents as well as GATA-3 and RORγt mRNA expression in peripheral blood and NF-κB, OPN, IL-33, MK and HMGB1 contents in serum were significantly lower than those of control group. Conclusion: methylprednisolone combined with montelukast treatment of recurrent Henoch-Schonlein purpura can regulate the immune function and inhibit the cytokine secretion.

1. Introduction

Henoch-Schonlein purpura (HSP) is the autoimmune disease with the basic pathological characteristic of systemic small vasculitis, and its main clinical manifestation is the nonthrombocytopenic purpura accompanied by the symptoms such as arthralgia, abdominal pain and proteinuria[1,2]. Immune response is disorder, CD4+T cellular immune subset imbalance and abnormal cytokine secretion are the main pathological links causing small vessel damage in the course of HSP, and to correct the immune response disorder is also the main means for the clinical treatment of HSP[3]. Glucocorticoid is an endogenous hormone with immunomodulatory effect, methylprednisolone and hydrocortisone are the common glucocorticoid preparations, and methylprednisolone is better than hydrocortisone in the affinity to glucocorticoid receptor and the anti-inflammatory activity, so its value of HSP treatment is more outstanding[4]. Montelukast is a kind of selective leukotriene receptor antagonist, which can antagonize the pro-inflammatory effect mediated by leukotriene and exert anti-inflammatory activity[5]. In the following studies, we analyzed the effects of methylprednisolone combined with montelukast on immune function and cytokines in children with recurrent Henoch-Schonlein purpura.
2. Information and methods

2.1 Genera case information

Children who were diagnosed with recurrent Henoch-Schonlein purpura in Zigong Third People’s Hospital between September 2015 and August 2017 were selected as the research subjects, all children conformed to the diagnosis of recurrent Henoch-Schonlein purpura, and those with thrombocytopenic purpura, other blood system diseases or autoimmune diseases were eliminated. A total of 92 children were enrolled in the study, and random number table method was used to divide them into the intervention group (n=46) who received methylprednisolone combined with montelukast therapy and the control group (n=46) who received hydrocortisone therapy. There were 28 males and 18 females in the intervention group, and they were 7-15 years old; there were 26 males and 20 females in the control group, and they were 8-14 years old. There was no significant difference in the general data between the two groups (P>0.05).

2.2 Therapy

Both groups of children received anti-infection, antihistamine, vitamin C, calcium and other conventional symptomatic supportive treatment, observation group received methylprednisolone combined with montelukast therapy and the method was as follows: 1-2 mg/kg methylprednisolone sodium succinate for injection was added in 100 mL of saline, which was by intravenous drip, 2 times/d; Montelukast Sodium Chewable Tablets 5 mg, by chewing, 1 time/night. Control group received hydrocortisone on the basis of symptomatic support therapy, and the method was as follows: 5-10 mg/kg of Hydrocortisone Succinate injection was added in 100 mL of saline, by intravenous drip, 2 times/d. The hormones of both groups were used for 5 d in a row and then taken orally, and the dosage was gradually reduced.

2.3 Peripheral blood index detection

Before treatment as well as 4 weeks and 8 weeks after treatment, 6-8 mL of peripheral blood was collected respectively and centrifuged to separate serum, and the enzyme-linked immunosorbent assay kit was used to determine the contents of NF-κB, OPN, IL-33, MK and HMGB1.

2.4 Serum index detection

Before treatment as well as 4 weeks and 8 weeks after treatment, 6-8 mL of peripheral blood was collected respectively and centrifuged to separate serum, and the enzyme-linked immunosorbent assay kit was used to determine the contents of NF-κB, OPN, IL-33, MK and HMGB1.

2.5 Statistical methods

Software SPSS 22.0 was used to input the data, the differences in measurement data between two groups were analyzed by t test and the difference was statistically significant if P<0.05.

3. Results

3.1 Peripheral blood Th1/Th2 immune response

Before treatment as well as 4 weeks and 8 weeks after treatment, analysis of Th1 and Th2 cell contents as well as transcription factors T-bet and GATA-3 expression in peripheral blood between the two groups of patients was as follows: before treatment, Th1 and Th2 cell contents as well as T-bet and GATA-3 mRNA expression in peripheral blood were not significantly different between the two groups of patients (P>0.05); 4 weeks and 8 weeks after treatment, Th1 cell contents and T-bet mRNA expression in peripheral blood of both groups of patients were significantly higher than those before treatment whereas Th2 cell contents and GATA-3 mRNA expression were significantly lower than those before treatment (P<0.05), and Th1 cell contents and T-bet mRNA expression in peripheral blood of intervention group were significantly higher than those of control group whereas Th2 cell contents and GATA-3 mRNA expression were significantly lower than those of control group (P<0.05).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>Th1</th>
<th>Th2</th>
<th>T-bet</th>
<th>GATA-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention group</td>
<td>46</td>
<td>Before treatment</td>
<td>7.85±0.92</td>
<td>12.73±1.85</td>
<td>1.02±0.17</td>
<td>1.04±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>10.24±1.37*</td>
<td>9.92±1.16*</td>
<td>1.66±0.24*</td>
<td>0.71±0.09*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 weeks after treatment</td>
<td>13.29±1.77*</td>
<td>7.86±0.93*</td>
<td>2.04±0.32*</td>
<td>0.45±0.07*</td>
</tr>
<tr>
<td>Control group</td>
<td>46</td>
<td>Before treatment</td>
<td>7.91±0.89</td>
<td>12.93±1.92</td>
<td>1.03±0.15</td>
<td>1.02±0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>8.82±1.24*</td>
<td>11.38±1.74*</td>
<td>1.36±0.19*</td>
<td>0.84±0.12*</td>
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<tr>
<td></td>
<td></td>
<td>8 weeks after treatment</td>
<td>10.83±1.47*</td>
<td>9.73±1.13*</td>
<td>1.59±0.22*</td>
<td>0.73±0.08*</td>
</tr>
</tbody>
</table>

*: comparison between before and after treatment within group, P<0.05; *, comparison between two groups after treatment, P<0.05.
Comparison of peripheral blood Th17/Treg immune response before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>Th17</th>
<th>Treg</th>
<th>ROR γ t</th>
<th>FoxP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>46</td>
<td>Before treatment</td>
<td>3.05±0.45</td>
<td>0.73±0.11</td>
<td>0.98±0.11</td>
<td>1.04±0.17</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>Before treatment</td>
<td>3.11±0.47</td>
<td>0.75±0.09</td>
<td>1.01±0.15</td>
<td>1.02±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>2.14±0.32*</td>
<td>1.15±0.16*</td>
<td>0.67±0.09*</td>
<td>1.83±0.25*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 weeks after treatment</td>
<td>1.65±0.22*</td>
<td>1.44±0.18*</td>
<td>0.42±0.08*</td>
<td>2.44±0.35*</td>
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<td>4 weeks after treatment</td>
<td>2.67±0.41*</td>
<td>0.89±0.11*</td>
<td>0.81±0.12</td>
<td>1.45±0.19</td>
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<td></td>
<td>8 weeks after treatment</td>
<td>2.03±0.32*</td>
<td>1.12±0.16</td>
<td>0.69±0.08</td>
<td>1.91±0.27</td>
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</tbody>
</table>

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

Comparison of serum cytokines before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>NF-κ B</th>
<th>OPN</th>
<th>IL-33</th>
<th>MK</th>
<th>HMGB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>46</td>
<td>Before treatment</td>
<td>3.29±0.35</td>
<td>10.92±1.85</td>
<td>372.1±46.9</td>
<td>293.5±33.7</td>
<td>16.52±2.32</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>Before treatment</td>
<td>16.12±2.03*</td>
<td>4.67±0.68*</td>
<td>231.4±30.9</td>
<td>189.3±22.6</td>
<td>7.05±0.89*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>20.28±3.35*</td>
<td>6.49±0.81*</td>
<td>293.5±32.6*</td>
<td>225.1±28.5*</td>
<td>10.21±1.44*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 weeks after treatment</td>
<td>28.61±3.94*</td>
<td>8.93±1.08*</td>
<td>332.6±42.6*</td>
<td>264.4±33.2*</td>
<td>13.03±1.74*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>23.36±3.03*</td>
<td>7.42±0.93*</td>
<td>294.6±33.9*</td>
<td>216.8±25.8*</td>
<td>10.93±1.73*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 weeks after treatment</td>
<td>22.6±0.47*</td>
<td>7.42±0.93*</td>
<td>294.6±33.9*</td>
<td>216.8±25.8*</td>
<td>10.93±1.73*</td>
</tr>
</tbody>
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*: comparison between before and after treatment within group, P<0.05; #: comparison between two groups after treatment, P<0.05.

3.2 Peripheral blood Th17/Treg immune response

Before treatment as well as 4 weeks and 8 weeks after treatment, analysis of Th17 and Treg cell contents as well as transcription factors ROR γ t and FoxP3 expression in peripheral blood between the two groups of patients was as follows: before treatment, Th17 and Treg cell contents as well as ROR γ t and FoxP3 mRNA expression in peripheral blood were not significantly different between the two groups of patients (P>0.05); 4 weeks and 8 weeks after treatment, Th17 cell contents and ROR γ t mRNA expression in peripheral blood of both groups of patients were significantly lower than those before treatment whereas Treg cell contents and FoxP3 mRNA expression were significantly higher than those before treatment (P<0.05), and Th17 cell contents and ROR γ t mRNA expression in peripheral blood of intervention group were significantly lower than those of control group whereas Treg cell contents and FoxP3 mRNA expression were significantly higher than those of control group (P<0.05).

3.3 Serum cytokine contents

Before treatment as well as 4 weeks and 8 weeks after treatment, analysis of cytokines NF-κ B (μmol/L), OPN (ng/mL), IL-33 (pg/mL), MK (pg/mL) and HMGB1 (ng/mL) contents in serum between the two groups of patients was as follows: before treatment, NF-κ B, OPN, IL-33, MK and HMGB1 contents in serum were not significantly different between the two groups of patients (P>0.05); 4 weeks and 8 weeks after treatment, NF-κ B, OPN, IL-33, MK and HMGB1 contents in serum of both groups of patients were significantly lower than those before treatment (P<0.05), and NF-κ B, OPN, IL-33, MK and HMGB1 contents in serum of intervention group were significantly lower than those of control group (P<0.05).

4. Discussion

HSP is an autoimmune disease with the basic pathological characteristic of small vasculitis, and the immune response disorder, increased autoantibody formation and cytokine secretion disorder can cause small blood vessel damage and lead to skin purpura, arthralgia, abdominal pain and other clinical symptoms[6,7]. Glucocorticoid preparations methylprednisolone and hydrocortisone are the most common drugs for clinical treatment of autoimmune diseases, and their action with glucocorticoid receptors can inhibit the immune response and inflammatory response. The affinity of methylprednisolone to glucocorticoid receptor is higher than that of hydrocortisone, and the anti-inflammatory activity mediated by it is also stronger than that by hydrocortisone; it has been reported that methylprednisolone therapy for HSP is more effective than hydrocortisone to improve the clinical symptoms[8,9]. In recent years, the value of leukotriene receptor antagonist montelukast for autoimmune diseases has received more and more attention, and montelukast can antagonize the inflammatory response mediated by leukotriene to reduce the release of inflammatory mediators, and thus alleviate the immune and inflammatory damage that autoimmune disorder causes to one’s own tissue. Related study has confirmed that methylprednisolone combined with montelukast treatment of recurrent HSP is more effective than monotherapy to shorten the duration of clinical symptoms and correct the immunoglobulin disorder[10], but there is no report on the change of CD4+T cellular immune response and cytokine secretion in the body. In the above studies, the value of methylprednisolone combined with montelukast for recurrent HSP was mainly analyzed from the perspectives of CD4+T cellular immune response and cytokine secretion.
Th1 and Th2 were the earliest discovered CD4+T cell subsets, and the balance of Th1/Th2 immune response has regulatory effects on humoral immune responses and cellular immune responses. Th1 cells mainly secrete cytokines such as IL-2, TNF-α and IFN-γ, and enhance cellular immune response and delayed-type hypersensitivity[11]; Th2 cells mainly secrete cytokines such as IL-4, IL-5 and IL-10, participate in the activation of B cells as well as the synthesis and secretion of autoantibodies, and can enhance the humoral immune response[12]. T-bet and GATA3 are the transcription factors that play an important role in the differentiation from CD4+T cells to Th1 and Th2 subsets, and they mediate the differentiation and maturation of Th1 and Th2 respectively[13]; when Th1/Th2 balance is shifted to Th2, the humoral immunity is excessively enhanced and the autoantibody generation increases, causing the occurrence of small vasculitis in HSP. In order to define the effect of methylprednisolone combined with montelukast therapy on immune function of children with recurrent HSP, the changes of Th1/Th2 immune response before and after treatment were analyzed in the study, and the results showed that Th1 cell contents and T-bet mRNA expression of both groups of patients significantly increased whereas Th2 cell contents and GATA-3 mRNA expression significantly decreased after treatment, and Th1 cell contents and T-bet mRNA expression of intervention group were higher than those of control group whereas Th2 cell contents and GATA-3 mRNA expression significantly increased after treatment, and Th17 cell contents and RORγt mRNA expression of both groups of patients significantly decreased whereas Treg cell contents and FoxP3 mRNA expression significantly increased after treatment, and Th17 cell contents and RORγt mRNA expression of intervention group were lower than those of control group whereas Treg cell contents and FoxP3 mRNA expression were higher than those of control group after treatment. This means that after glucocorticoid treatment, the Th17 immune responses is restrained and the Treg immune response is enhanced in children with recurrent HSP, and methylprednisolone combined with montelukast therapy is more effective than hydrocortisone therapy to correct the Th17/Treg immune response disorder and make Th17/Treg immune response shift to Treg.

The pathological essence of HSP is the small vasculitis mediated by immune response disorder and excessive inflammation activation, and the Th1/Th2 and Th17/Treg immune response disorders in the body are closely related to the excessive inflammation activation and excessive cytokine release. NF-κB is a transcription factor involved in regulating the expression of various cytokines in the process of inflammation cascade amplification, and it is combined with the inhibitory factor IκB and in a quiescent condition under physiological condition; in the case of immune response disorder, NF-κB is dissociated with IκB and becomes activated free state, and then it transfers into the nucleus and initiates the transcription of various cytokines. OPN and HMGB1 are the important cytokines regulated by NF-κB, and the mass expression and release of them can mediate the cascade activation of inflammatory response[18]; IL-33 is a newly discovered multifunctional cytokine in recent years, which is not only involved in the activation of inflammatory response, but is also related to the over-activation of Th2 cells and the shifting of Th1/Th2 to Th2[19]; MK is the cytokine involved in Th17/Treg regulation, which can hinder the differentiation and maturation of Treg and make Th17/Treg shift to Th1[20]. The analysis of the changes in serum contents of above cytokines after treatment showed that NF-κB, OPN, IL-33, MK and HMGB1 contents in serum of both groups of patients significantly decreased after treatment, and NF-κB, OPN, IL-33, MK and HMGB1 contents in serum of intervention group were significantly lower than those of control group after treatment. This means that after glucocorticoid treatment, the secretion of various cytokines is restrained in children with recurrent HSP, and methylprednisolone combined with montelukast therapy is more effective than hydrocortisone therapy to inhibit the secretion of cytokines.

To sum up, it can be concluded in the study that methylprednisolone combined with montelukast treatment of recurrent Henoch-Schonlein purpura can regulate the Th1/Th2 and Th17/Treg immune responses, and make the immune responses shift to the Th2 and Treg respectively; it can also inhibit the secretion of various cytokines.
References


