Efficacy of adjuvant zinc gluconate therapy for infantile rotavirus enteritis diarrhea

Jian-Ning Zhang

Department of Pediatrics, Baoji Central Hospital in Shaanxi Province, Baoji, Shaanxi Province, 721008, China

Objective: To study the efficacy of adjuvant zinc gluconate therapy for infantile rotavirus enteritis diarrhea. Methods: Children who were hospitalized in Baoji Central Hospital due to rotavirus enteritis diarrhea between March 2014 and April 2017 were selected as the research subjects and divided into the observation group who received zinc gluconate combined with conventional treatment and the control group who received conventional treatment. The contents of inflammatory cytokines, immunoglobulin and complement in serum, the contents of T cell subsets in peripheral blood and the contents of intestinal flora in feces were determined before treatment and after 10 d of treatment. Results: after 10 d of treatment, TNF-α, IL-1β, IL-8, sIL-2R, DAO and D-lactic acid contents in serum, CD8+ contents in peripheral blood as well as Escherichia coli contents in feces of both groups were lower than those before treatment whereas C3, C4, IgA and IgG contents in serum, CD3+ and CD4+ contents in peripheral blood as well as bifidobacterium and lactobacillus contents in feces were higher than those before treatment, and TNF-α, IL-1β, IL-8, sIL-2R, DAO and D-lactic acid contents in serum, CD8+ content in peripheral blood as well as Escherichia coli content in feces of observation group were lower than those of control group whereas C3, C4, IgA and IgG contents in serum, CD3+ and CD4+ contents in peripheral blood as well as bifidobacterium and lactobacillus contents in feces were higher than those of control group. Conclusion: Adjuvant zinc gluconate therapy for infantile rotavirus enteritis diarrhea can inhibit the inflammatory response and improve the immune response and intestinal mucosal barrier function.

1. Introduction

Rotavirus (RV) infection is a common infectious disease in infants and young children and mainly occurs in the autumn and winter. After rotavirus infection, it mainly affects the small intestinal epithelial cells, it causes epithelial cell damage and can lead to clinical symptoms such as abdominal pain and diarrhea, and therefore, it is also known as rotavirus enteritis[1]. At present, rotavirus enteritis is the most common type of viral enteritis in infants and young children, the infants and young children have poor immunity and high risk of rotavirus infection, most of the children with rotavirus enteritis have short course of disease, the clinical symptoms are self-limiting and the self-healing rate is high[2,3]. However, the illness is progressively aggravated in some children with rotavirus enteritis, persistent diarrhea can occur and cause dehydration and electrolyte disorder, and severe cases can be life-threatening. Study on rotavirus enteritis in recent years has shown that the deficiency of a variety of trace elements in the children is closely related to the disease deterioration, zinc is of important value for maintaining the integrity of the intestinal epithelial cellular structure and function, and zinc deficiency is closely related to the progression of rotavirus enteritis[4]. In the following studies, zinc gluconate was used to for zinc supplementation and the efficacy of adjuvant zinc gluconate therapy for infantile rotavirus enteritis diarrhea was specifically analyzed.

2. Materials and methods

2.1. General case information

Children who were hospitalized in Baoji Central Hospital due to rotavirus enteritis diarrhea between March 2014 and April 2017 were selected as the research subjects, and all children were hospitalized for acute diarrhea, with disease course < 48 h, 3 months—3 years old, and proven to be rotavirus enteritis positive by the stool RV antigen test after admission. Children with bacterial dysentery and those combined with bacterial infection were excluded. A total of 96 children were enrolled, and the random number table method was used to divide them into two groups,
each with 48 cases. There were 29 males and 19 females in the observation group, and they were 3 months to 35 months years old; there were 27 males and 21 females in the control group, and they were 3 months to 36 months years old. There was no significant difference in the general data between the two groups ($P>0.05$).

2.2. Therapy

Both groups of patients received symptomatic and supportive treatment after admission, including anti-diarrheal by montmorillonite powder, regulating intestinal flora with probiotics, fluid infusion and electrolyte supplementation, children combined with myocardial injury received myocardial nutrients, those combined with liver damage received liver-protective medicines, and those combined with fever received antipyretics and physical cooling. On the basis of the above symptomatic and supportive treatment, observation group received zinc supplementation, which was as follows: children of 3–6 months old were given 70 mg of zinc glucosate particles, administered after dissolved, once a day, children of 6 months to 3 years old were given 140 mg of zinc glucosate particles, administered after dissolved, once a day, and they were treated for 10 consecutive days.

2.3. Clinical index detection

2.3.1. Serum index detection

Before treatment and after 10 d of treatment, 2-3 mL of cubital venous blood was collected from two groups of children and centrifuged to separate serum, and enzyme-linked immunosorbent assay kit was used to determine serum TNF-$\alpha$, IL-1 $\beta$, IL-8, sIL-2R, C3, C4, IgA and IgG levels.

2.3.2. Peripheral blood index detection

Before treatment and after 10 d of treatment, 0.5-1 mL of cubital venous blood was collected from two groups of children, joined by EDTA for anticoagulation to incubate the fluorescent antibody of CD3, CD4 and CD8 away from light for 20 min and joined by permeabilization reagent for another 15 min of incubation, then the cells were washed with PBS and centrifuged twice, finally PBS was used to re-suspend the cells and flow cytometer was adopted to determine the contents of CD3+, CD4+ and CD8+.

2.3.3. Feces index detection

Before treatment and after 10 d of treatment, the feces specimens were obtained from two groups of children, the genomic DNA kit was used to separate and extract the genomic DNA in the feces, the genomic DNA kit were obtained from two groups of children, the genomic DNA kit was designed for PCR amplification, and the amplification curve was referred to calculate the contents of bifidobacterium, lactobacillus and E. coli.

2.4. Statistical methods

SPSS 17.0 software was adopted to input data, the data analysis between two groups before and after treatment was by t test and the difference was statistically significant if $P<0.05$.

3. Results

3.1. Changes of inflammatory cytokine contents in serum

Before treatment and after 10 d of treatment, analysis of serum inflammatory cytokines TNF-$\alpha$ (ng/mL), IL-1 $\beta$ (pg/mL), IL-8 (pg/mL) and sIL-2R (pg/mL) between two groups of children was shown in Table 1: before treatment, serum TNF-$\alpha$, IL-1 $\beta$, IL-8 and sIL-2R contents were not significantly different between two groups of children ($P>0.05$); after 10 d of treatment, TNF-$\alpha$, IL-1 $\beta$, IL-8 and sIL-2R contents in serum of both groups were lower than those before treatment ($P<0.05$), and TNF-$\alpha$, IL-1 $\beta$, IL-8 and sIL-2R contents in serum of observation group were lower than those of control group ($P<0.05$).

3.2. Changes of immunoglobulin and complement contents in serum as well as T cell subset contents in peripheral blood

Before treatment and after 10 d of treatment, analysis of immunoglobulin IgA and IgG contents as well as complement C3 and C4 contents in serum between two groups of children was shown in Table 2: before treatment, serum C3, C4, IgA and IgG contents were not significantly different between two groups of children ($P>0.05$); after 10 d of treatment, C3, C4, IgA and IgG contents in serum of both groups were higher than those before treatment ($P<0.05$), and C3, C4, IgA and IgG contents in serum of observation group were higher than those of control group ($P<0.05$).

Before treatment and after 10 d of treatment, analysis of T cell subsets CD3+, CD4+ and CD8+ contents in peripheral blood between two groups of children was shown in Table 3: before treatment, CD3+, CD4+ and CD8+ contents in peripheral blood were not significantly different between two groups of children ($P>0.05$); CD3+ and CD4+ contents in peripheral blood of both groups were higher than those before treatment whereas CD8+ contents were lower than those before treatment ($P<0.05$), and CD3+ and CD4+ contents in peripheral blood of both groups were higher than those before treatment whereas CD8+ contents were lower than those before treatment ($P<0.05$), and CD3+ and CD4+ contents in peripheral blood of both groups were higher than those before treatment whereas CD8+ contents were lower than those before treatment ($P<0.05$).

### Table 1.

Comparison of inflammatory cytokine contents in serum before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TNF-$\alpha$ (pg/mL)</th>
<th>IL-1 $\beta$ (pg/mL)</th>
<th>IL-8 (pg/mL)</th>
<th>sIL-2R (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>48</td>
<td>Before treatment</td>
<td>3.80±0.52</td>
<td>142.3±19.3</td>
<td>529.2±72.5</td>
<td>51.4±8.1</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>Before treatment</td>
<td>1.62±0.24$^a$</td>
<td>64.3±8.9$^a$</td>
<td>262.1±33.9$^a$</td>
<td>25.2±3.9$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>3.89±0.58</td>
<td>143.6±20.3</td>
<td>531.4±68.7</td>
<td>52.1±7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>2.51±0.37$^a$</td>
<td>99.3±11.5$^a$</td>
<td>384.9±51.8$^a$</td>
<td>36.7±5.2$^a$</td>
</tr>
</tbody>
</table>

$^a$: comparison between before and after treatment within group, $P<0.05$; $^b$: comparison between two groups after treatment, $P<0.05$.

### Table 2.

Comparison of immunoglobulin and complement contents in serum before and after treatment (g/L).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>IgA</th>
<th>IgG</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>48</td>
<td>Before treatment</td>
<td>8.96±1.05</td>
<td>0.91±0.12</td>
<td>0.93±0.12</td>
<td>0.52±0.08</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>Before treatment</td>
<td>13.21±1.84$^a$</td>
<td>1.55±0.19$^a$</td>
<td>1.89±0.24$^a$</td>
<td>0.93±0.12$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>8.89±1.06</td>
<td>0.95±0.12</td>
<td>0.93±0.12</td>
<td>0.54±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>11.25±1.57$^a$</td>
<td>1.24±0.17$^a$</td>
<td>1.35±0.18$^a$</td>
<td>0.69±0.09$^a$</td>
</tr>
</tbody>
</table>

$^a$: comparison between before and after treatment within group, $P<0.05$; $^b$: comparison between two groups after treatment, $P<0.05$. 
high fever, vomiting and diarrhea in the course of rotavirus enteritis, that the adjuvant zinc gluconate therapy can shorten the duration of diarrhea aggravation, so it is necessary to pay attention to zinc deficiency can cause damage to the body and is directly related to the catalytic activity of enzymes, and is involved in the composition of various metabolic enzymes in the body and is a kind of chemotactic cytokine that can cause neutrophil and lymphocyte chemotaxis and infiltration in inflammatory regions and can mediate the cascade activation of inflammatory response and kill pathogens. IL-8 is a kind of chemotactic cytokine that can cause neutrophil and lymphocyte chemotaxis and infiltration in inflammatory regions and can start the cascade activation of inflammatory response. The analysis of the changes in serum contents of above inflammatory cytokines before and after treatment showed that TNF-α, IL-1β, IL-8 and sIL-2R contents in serum of both groups after treatment were lower than those before treatment, and TNF-α, IL-1β, IL-8 and sIL-2R contents in serum of observation group were lower than those of control group. This means that both conventional symptomatic and supportive treatment and zinc gluconate combined with symptomatic and supportive treatment can effectively inhibit the inflammation in the course of rotavirus enteritis, and adjuvant zinc gluconate treatment can more significantly reduce the inflammatory cytokine release and inhibit the inflammatory response than routine symptomatic and supportive treatment.

In the course of rotavirus enteritis, zinc deficiency can directly affect the differentiation of various immune cells and cause immune response disorders. Immunoglobulin IgA and IgG are the immunoregulatory molecules secreted by activated B lymphocytes, which mediate the humoral immune process and can participate in the removal of pathogens; complement C3 and C4 are the immunoreactive molecules that are involved in regulating phagocytosis in antiviral immunity. Zinc deficiency can cause deficiency of immunoglobulin and complement contents in peripheral blood of observation group were higher than those of control group whereas CD8+ content was lower than that of control group (P<0.05).

3.3. Changes of intestinal mucosal barrier molecule contents in serum as well as intestinal flora contents in feces

Before treatment and after 10 d of treatment, analysis of intestinal mucosal barrier molecules DAO (mg/L) and D-lactic acid (mg/L) contents in serum as well as the intestinal flora contents bifidobacterium (1gcopies/g), lactobacillus (1gcopies/g) and E. coli (1gcopies/g) in feces between two groups of children was shown in Table 4: before treatment, DAO and D-lactic acid contents in serum as well as bifidobacterium, lactobacillus and E. coli contents in feces were not significantly different between two groups of children (P>0.05); after 10 d of treatment, DAO and D-lactic acid contents in serum as well as E. coli contents in feces of both groups were lower than those before treatment whereas bifidobacterium and lactobacillus contents in feces were higher than those before treatment (P<0.05), and DAO and D-lactic acid contents in serum as well as E. coli content in feces of observation group were lower than those of control group whereas bifidobacterium and lactobacillus contents in feces were higher than those of control group (P<0.05).

4. Discussion

Rotavirus enteritis is the most common type of viral enteritis in early childhood and also the most common cause of early childhood diarrhea, the disease is self-limiting and the self-healing rate is high in most children with rotavirus enteritis, but the disease is severe in a small number of patients, there are dehydration and water electrolyte disorder in the course of disease and they will endanger the safety of life[5-7]. In recent years, the research on rotavirus enteritis has shown that zinc deficiency is closely related to the aggravation of diarrhea and the deterioration of the disease[8]. Zinc is involved in the composition of various metabolic enzymes in the body and is directly related to the catalytic activity of enzymes, and it can guarantee the structural and functional integrity of intestinal mucosal epithelial cells. Zinc deficiency can cause damage to the structure and function of intestinal mucosal epithelial cells and lead to diarrhea aggravation, so it is necessary to pay attention to zinc supplementation in the course of rotavirus enteritis. Zinc gluconate is a clinical common zinc preparation, and studies have confirmed that the adjuvant zinc gluconate therapy can shorten the duration of high fever, vomiting and diarrhea in the course of rotavirus enteritis, reduce the incidence of liver and heart damage and improve the overall effect[9]. However, it is still not clear about the effects of adjuvant zinc gluconate therapy on inflammatory response and immune response in patients with rotavirus enteritis.

In the course of rotavirus enteritis, rotavirus infection will lead to the inflammation activation in the body and cause the secretion of various inflammatory cytokines[10]. TNF-α and IL-1β are pro-inflammatory cytokines that change significantly in the early stage of inflammatory response, and they can mediate the cascade activation of inflammatory response and kill pathogens[11]. IL-8 is a kind of chemotactic cytokine that can cause neutrophil and lymphocyte chemotaxis and infiltration in inflammatory regions and can start the cascade activation of inflammatory response[12]. sIL-2R is a soluble form of the IL-2 receptor, which will be massively released into the blood circulation and enhance the pro-inflammatory effect of IL-2 during the continuous activation of the inflammatory response. The analysis of the changes in serum contents of above inflammatory cytokines before and after treatment showed that TNF-α, IL-1β, IL-8 and sIL-2R contents in serum of both groups after treatment were lower than those before treatment, and TNF-α, IL-1β, IL-8 and sIL-2R contents in serum of observation group were lower than those of control group after treatment. This means that both conventional symptomatic and supportive treatment and zinc gluconate combined with symptomatic and supportive treatment can effectively inhibit the inflammation in the course of rotavirus enteritis, and adjuvant zinc gluconate treatment can more significantly reduce the inflammatory cytokine release and inhibit the inflammatory response than routine symptomatic and supportive treatment.
cell subset contents in peripheral blood before and after treatment showed that C3, C4, IgA and IgG contents in serum as well as CD3+ and CD4+ contents in peripheral blood of both groups after treatment were higher than those before treatment whereas CD8+ contents in peripheral blood were lower than those before treatment, and C3, C4, IgA and IgG contents in serum as well as CD3+ and CD4+ contents in peripheral blood of observation group were higher than those of control group whereas CD8+ content in peripheral blood was lower than that of control group after treatment. This shows that adjuvant zinc gluconate therapy is more effective than conventional symptomatic and supportive treatment in improving the immune response.

After rotavirus infection, it can directly attack the epithelial cells of the intestinal mucosa, which causes the damage of epithelial cells and will affect the integrity of intestinal mucosal barrier function. DAO and D-lactic acid are the specific catalytic enzyme and metabolite in intestinal mucosal epithelial cells respectively, which are massively released into the blood circulation during cell damage and can reflect the damage degree of intestinal mucosa barrier[10]. The intestinal tract is where the bacteria aggregate in the body. In physiological conditions, there are mainly bifidobacteria, lactobacillus and other probiotics, and the pathogenic bacteria such as escherichia coli are in an inhibited state; in the case of intestinal lactobacillus and other probiotics, and the pathogenic bacteria such as escherichia coli are in an inhibited state; in the case of intestinal mucosal epithelial damage, the reproduction of bifidobacterium, lactobacillus and other probiotics will be inhibited, and the E. coli will multiply abundantly and transfer into the blood circulation to become the source of systemic inflammatory response[17,18]. DAO and D-lactic acid contents in serum as well as E. coli contents in feces of both groups after treatment were lower than those before treatment whereas bifidobacterium and lactobacillus contents in feces were higher than those before treatment, and DAO and D-lactic acid contents in serum as well as E. coli content in feces of observation group were lower than those of control group whereas bifidobacterium and lactobacillus contents in feces were higher than those of control group after treatment. This indicates that adjuvant zinc gluconate therapy can more effectively improve the intestinal mucosal barrier function and correct the intestinal flora disturbance than conventional symptomatic and supportive treatment.

Above all, it can be concluded the adjuvant zinc gluconate therapy is more effective than conventional symptomatic and supportive treatment to improve the condition of infantile rotavirus enteritis diarrhea, which is specifically characterized by inhibiting the inflammatory response and improving the immune response and intestinal mucosal barrier function.

References