Effect of monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate on serum indexes and immune response in patients with Parkinson’s disease

Shi-Ping Sun¹, Ping Huang¹, Zhi-Qiang Xu²

¹Neurology Department, Second People’s Hospital of Banan District Chongqing, Chongqing, 400054, China
²Neurology Department, Daping Hospital of Third Military Medical University, Chongqing, 400054, China

ARTICLE INFO

Objective: To study the effect of monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate on serum indexes and immune response in patients with Parkinson’s disease. Methods: A total of 94 patients with Parkinson’s disease diagnosed and treated in our hospital between March 2012 and December 2014 were selected and randomly divided into observation who received monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate treatment group and control group who received monosialotetrahexosylganglioside sodium monotherapy, and the levels of CD4⁺ T lymphocyte subsets in peripheral blood as well as the levels of cytokines and nerve injury molecules in serum were determined. Results: CD4⁺CD45RA⁺ naive T cell and CD4⁺CD25highFoxp3⁺ regulatory T cell levels in peripheral blood of observation group were significantly higher than those of control group while CD4⁺CD45RO⁺ memory T cell level in peripheral blood was significantly lower than that of control group; serum IFN-γ, TNF-α and IL-17 as well as NSE and S100β levels were significantly lower than those of control group while serum IL-4 and IL-10 as well as Aβ1-42 and BDNF levels were significantly higher than those of control group. Conclusions: Monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate treatment of PD can adjust the immune function and reduce the inflammatory injury and immune injury of the neurons.

1. Introduction

Parkinson’s disease (PD) is a common degenerative disease of central nervous system, and its characteristic pathological changes are dopaminergic neuron loss and Lewy body formation in midbrain substantia nigra compacta. There will be static tremor, gait and posture change, cognitive impairment, abnormal mental state and so on in patients with PD. The incidence of PD has been increasing in recent years, which greatly impact the patients as well as the families and the society[1,2]. At present, the pathogenesis of PD is not entirely clear, and specific clinical treatment is also short.

Abnormal immune response, T lymphocyte infiltration within the midbrain nigra and the resulting abnormal cytokine secretion are the important links in the pathogenesis of PD, and are closely related to the neuronal degeneration and loss in patients with PD[3,4]. Monosialotetrahexosylganglioside sodium has brain damage repair effect, and the cerebroprotein hydrolysate is a kind of peptidergic neurotrophic drug[5,6]. In the following study, monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate was used to treat Parkinson’s disease, and the serum indexes and immune response indexes were analyzed.

2. Materials and methods

2.1. Clinical information
A total of 94 patients with Parkinson's disease diagnosed and treated in our hospital between March 2012 and December 2014 were selected for study, all patients conformed to the diagnostic criteria for Parkinson's disease, and patients with secondary Parkinson's syndrome and Parkinsonism-plus syndrome as well as Alzheimer's disease, cerebral infarction and cerebral hemorrhage were excluded. After signing informed consent, the included patients were randomly divided into observation group and control group, 47 cases in each group. Observation group received monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate treatment, 28 cases were male and 19 cases were female, they were (64.9±8.9) years old and the course of disease was (5.42±0.84) years; control group received monosialotetrahexosylganglioside sodium treatment, 26 cases were male and 21 cases were female, they were (63.5±8.5) years old and the course of disease was (5.51±0.79) years. The two groups of patients showed no significant difference in general information.

2.2. Research methods

2.2.1. Treatment methods

Control group received monosialotetrahexosylganglioside sodium treatment, the initial dose of 500-1 000 mg in 250 mL saline, intravenous drip, the dose of 200 mg/d from the second day in 100 mL saline, intravenous drip, for consecutive 18 weeks. Observation group received monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate therapy, the method of intravenous drip of monosialotetrahexosylganglioside sodium was the same as that of control group, and they received cerebroprotein hydrolysate for injection in 100 mL of saline, intravenous drip, for consecutive 18 weeks.

2.2.2. Clinical sample collection methods

18 weeks after treatment, 8-10 mL of cubital venous blood was collected from two groups and divided into two, one was anticoagulated with EDTA and then used for flow cytometry detection; the other was let stand at room temperature for 20 min, anticoagulated with EDTA and then used for flow cytometry detection; the other was let stand at room temperature for 20 min, anticoagulated with EDTA and then used for flow cytometry was collected from two groups and divided into two, one was 18 weeks after treatment, 8-10 mL of cubital venous blood

2.2.3. Flow cytometry detection

2.2.4. Serum cytokine level detection

Serum specimens were taken, enzyme-linked immunosorbent assay kits were used to detect interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), IL-4, IL-10, neuron specific enolase (NSE), β-amylloid 1-42 (Aβ 1-42), S100β and brain-derived neurotrophic factor (BDNF) levels, and all steps were conducted according to the kit instruction.

2.3. Statistical methods

SPSS 19.0 software was used to input and process data, measurement data was in terms of mean±sd, comparison between two groups was performed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. T lymphocyte subset levels in peripheral blood

Percentage of CD4+CD45RA naïve T cells and CD4+CD25highFoxp3+ regulatory T cells among CD4+ T cells in peripheral blood of observation group were significantly higher than those of control group while percentage of CD4+CD45RO+ memory T cells and CD4+CD25highFoxp3+ regulatory T cells among all CD4+ T cells were determined by flow cytometer. Cubital venous blood anticoagulated with EDTA was collected, 100 μL of whole blood sample was added each of the three reaction tubes, 10 μL each of FITC-labeled CD4 monoclonal antibody, PerCP-labeled CD25 monoclonal antibody and PE4-labeled Foxp3 monoclonal antibody were added in the first tube, 10 μL each of FITC-labeled CD4 monoclonal antibody and PE4-labeled CD45RO monoclonal antibody were added in the second tube, and 10 μL each of FITC-labeled CD4 monoclonal antibody and PE4-labeled CD45RA monoclonal antibody were added in the third tube. After incubation at room temperature away from light for 30 min, 2 mL of hemolysin was added, the samples were continuously incubated away from light for 10 min and centrifuged for 5 min at 1 500 r/min, the supernatant was abandoned, the remaining was washed with PBS twice and then re-suspended with 300 μL of fixer, and then the percentage of CD4+CD45RA+ naïve T cells, CD4+CD45RO+ memory T cells and CD4+CD25highFoxp3+ regulatory T cells among all CD4+ T cells were determined by flow cytometer.

Table 2

Serum cytokine levels of two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>Pro-inflammatory factors</th>
<th>Anti-inflammatory factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF-α (ng/mL)</td>
<td>IFN-γ (pg/mL)</td>
</tr>
<tr>
<td>Observation</td>
<td>47</td>
<td>17.64±2.62</td>
<td>14.28±2.91</td>
</tr>
<tr>
<td>Control</td>
<td>47</td>
<td>25.62±4.09</td>
<td>27.62±4.18</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
lower than that of control group \((P<0.05)\), shown in Table 1.

Table 1
T lymphocyte subset levels in peripheral blood of two groups after treatment (107).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>CD4(^+)CD45RA(^-)</th>
<th>CD4(^+)CD45RO(^-)</th>
<th>CD4(^+)CD25(^{high}) Foxp3(^{+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>47</td>
<td>6.86±0.81</td>
<td>2.62±0.45</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>Control</td>
<td>47</td>
<td>4.07±0.57</td>
<td>3.18±0.52</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>(t)</td>
<td></td>
<td>8.918</td>
<td>6.694</td>
<td>9.392</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.2. Serum cytokine levels

Serum IFN-\(\gamma\), TNF-\(\alpha\) and IL-17 levels of observation group were significantly lower than those of control group while serum IL-4 and IL-10 levels were significantly higher than those of control group \((P<0.05)\), shown in Table 2.

3.3. Serum nerve injury molecule levels

Serum NSE and S100\(\beta\) levels of observation group were significantly lower than those of control group while A\(\beta\)1-42 and BDNF levels were significantly higher than those of control group \((P<0.05)\), shown in Table 3.

4. Discussion

Dopaminergic neuron loss in substantia nigra compacta of patients with PD can cause recognition and memory function impairment as well as implicit memory and everyday memory retention, which not only cause adverse effects on the patients’ daily life and work, but will also increase the burden on the families and society[7,8]. Nourishing nerves and promoting neuron repair is the key to PD treatment. Monosialotetrahexosylganglioside sodium is a glycosphingolipid containing sialic acid, can repair nerve damage, alleviate nerve cell edema and protect the nerve function, and is used in the treatment of hypoxic-ischemic encephalopathy and hyperbilirubinemia-induced brain injury, and in recent years, studies have also reported the positive value of the drug for PD treatment. Cerebroprotein hydrolysate is a kind of active peptide hydrolysate extracted from fresh pig brain tissue, it is the nervous system-specific neurotrophic substance, and it can freely cross through the blood brain barrier, induce differentiation of neurons and protect neurons from toxin damage[9,10]. Existing animal study has confirmed that cerebroprotein hydrolysate has therapeutic effect on PD animal models[11]. Nevertheless, the value of the combined therapy for PD treatment has not been confirmed in clinical study at present.

In recent years, research on the pathogenesis of PD believes that immune dysfunction is an important link in the onset of PD, a large number of CD4\(^+\)T lymphocytes infiltrate in midbrain nigra of patients with PD, and there is also the disorder of CD4\(^+\)T lymphocyte subset levels in patients with PD. Decreased CD4\(^+\)CD45RA\(^-\) naive T cell and CD4\(^+\)CD25\(^{high}\)Foxp3\(^{+}\) regulatory T cell percentage and increased CD4\(^+\)CD45RO\(^-\) memory T cell ratio in CD4\(^+\)T lymphocyte subsets is an important characteristic of PD patients. CD4\(^+\)CD45RO\(^-\) memory T cell is also known as Th17, and as effector cell of immune response, it can secrete IL-17 and cause dopaminergic neuron damage, and its content increase could promote the development of PD[12]; CD4\(^+\)CD25\(^{high}\)Foxp3\(^{+}\) regulatory T cells have inhibitory effect on immune response and inflammatory response, can reduce the synthesis and secretion of pro-inflammatory factors, and have protective effect on neurons, and its content decrease can cause weakened neuroprotective effect and promote the development of PD[13]. In the study, analysis of T lymphocyte subset levels in peripheral blood of two groups of patients after treatment proved that the percentage of naive T cells and regulatory T cells in peripheral blood of observation group were significantly higher than those of control group while the percentage of memory T cells was significantly lower than that of control group. This indicates that monosialotetrahexosylganglioside sodium combined with cerebroprotein hydrolysate treatment can more effectively rectify immune response disorder in patients with PD.

Regulatory T cells can suppress the activation of CD4\(^+\)CD45RO\(^-\) memory T cell and inhibit the secretion of pro-inflammatory factor IL-17 through cell surface Foxp3, and can also synthesize inhibitory cytokine IL-10 to regulate the differentiation and maturation of a variety of T lymphocyte subsets and inhibit the immune response mediated by CD4\(^+\)T lymphocytes[14]. In the development of PD, in addition to the regulatory T cell and memory T cell imbalance, the proportion of Th1/Th2 cells is also abnormal. Both Th1 and Th2 are differentiated from CD4\(^+\)T cells, the former can secrete IFN-\(\gamma\), TNF-\(\alpha\) and other pro-inflammatory factors and cause dopaminergic neuron loss, and the latter can secrete IL-4 and inhibit the dopaminergic neuron injury mediated by Th1 cells. In the study, analysis of the levels of cytokines in serum of two groups of patients with PD after treatment showed that serum IFN-\(\gamma\), TNF-\(\alpha\) and IL-17 levels of observation group were significantly lower than those of control group while serum IL-4 and IL-10 levels were significantly lower than those of control group (\(P<0.05\)), shown in Table 2.

Table 3
Serum nerve injury molecule levels of two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>NSE (ng/mL)</th>
<th>A(\beta)1-42 (ng/mL)</th>
<th>S100(\beta) (ng/mL)</th>
<th>BDNF (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>47</td>
<td>14.29±1.96</td>
<td>1.72±0.18</td>
<td>2.03±0.35</td>
<td>27.75±4.22</td>
</tr>
<tr>
<td>Control</td>
<td>47</td>
<td>23.22±4.28</td>
<td>1.04±0.14</td>
<td>3.76±0.51</td>
<td>18.76±2.95</td>
</tr>
<tr>
<td>(t)</td>
<td></td>
<td>7.492</td>
<td>8.592</td>
<td>8.114</td>
<td>7.069</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
higher than those of control group. This further confirms that mono-
sialotetrahexosylganglioside sodium combined with cerebroprotein
hydrolysate treatment can adjust the balance of immune function and
the secretion of cytokines in patients with PD.

To further clarify the protective effect of monosialotetrahexosylgan-
glioside sodium combined with cerebroprotein hydrolysate therapy
on dopaminergic neurons in patients with PD, serum levels of nerve
injury molecules were detected in the study. NSE is a metabolic
enzyme involved in glucose alcoholysis in brain tissue, its expression
increases in the case of brain tissue damage and it will be released
from cells and lead to higher serum NSE levels[15]. S-100 β is an
acid calcium-binding protein with relatively rich content in glial
cells, and the nerve damage caused by immune and inflammatory
response can lead to the release of S-100 β into serum[16]. Unlike
NSE and S-100B, A β 1-40 transport within the central nervous
system will cause the morphological change and functional damage
of neurons as well as lower A β 1-40 level in the peripheral blood[17].
In addition, the levels of BDNF significantly reduce in serum and
cerebrospinal fluid of patients with PD, BDNF has promoting effect
on dopaminergic neuron survival, differentiation and maturation, it is an important nutritional factor within the nervous system, and
its content decrease is closely related to neuronal damage[18]. In
the study, analysis of the serum levels of the four molecules confirmed
that serum NSE and S100 β levels of observation group were
significantly lower than those of control group while A β 1-42 and
BDNF levels were significantly higher than those of control group.
This means that monosialotetrahexosylganglioside sodium combined
with cerebroprotein hydrolysate therapy has better protective effect
on dopaminergic neurons in patients with PD than monosialotetrahe-
xsylganglioside sodium monotherapy.

In conclusion, monosialotetrahexosylganglioside sodium combined
with cerebroprotein hydrolysate treatment of PD can adjust the
immune function and reduce the inflammatory injury and immune
injury of the neurons.

References

female patients with Parkinson’s disease: the importance of gender-
Parkinson’s tremor: An effective connectivity fMRI study. J Neurosci
subsets in Parkinson’s disease patients with constipation. Int J Clin Exp
in peripheral immune system of Parkinson’s disease model mice.
improves neurological outcome in rats after acute stroke: A prospective,
randomized, blinded, and placebo-controlled study. Int J Stroke 2016;
pharmacodynamic synergism between neuropeptides and lithium in
the neurotrophic and neuroprotective action of cerebrolysin. Zh Nevrol
of life in incident Parkinson’s disease: The role of attention. Parkinsonism
movement disorders: Recommendations of the international Parkinson
and movement disorder society task force. Mov Disord 2016; 31 (4):
436-457.
Cerebrolysin improves the survival of neural stem cell grafts in an APP
54-67.
Alzheimer’s disease: a meta-analysis of randomized controlled clinical
total potential of cerebrolysin in 6-OHDA rat model of Parkinson’s disease.
[12] Jiang LG, Mu ZX, Sun BQ. The significance of Treg/Th17 balance and
the associated cytokines in the peripheral blood of Parkinson’s disease. J
subsets in Parkinson’s disease patients with constipation. Int J Clin Exp
[14] Levite M. Dopamine and T cells: dopamine receptors and potent
effects on T cells, dopamine production in T cells, and abnormalities in
the dopaminergic system in T cells in autoimmune, neurological and
levels in patients with different subtypes of mild cognitive impairment.
S100B in Parkinson’s Disease: A glimpse into sleep-related
[17] Liang RB, Lei J, Zhang XN. Level of serum β- amyloid protein 1-42
and its relationship with the disease severity of patients with Parkinson
BDNF with cognitive deficits in people with Parkinson’s disease. J