The cerebral protective effect of mouse nerve growth factor combined with early mild hypothermia therapy for patients with severe craniocerebral injury and the influence on the inflammatory and stress reaction indexes

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Objective: To study the cerebral protective effect of mouse nerve growth factor combined with early mild hypothermia therapy for patients with severe craniocerebral injury and the influence on the inflammatory and stress reaction indexes. Methods: 68 patients with severe craniocerebral injury who were treated in our hospital between May 2012 and February 2016 were collected and then divided into the control group (n = 38) who received mild hypothermia therapy and the observation group (n = 30) who received mouse nerve growth factor combined with early mild hypothermia therapy after the treatment and laboratory test results were reviewed. Immediately after admission and after 2 weeks of treatment, automatic biochemical analyzer was used to detect the contents of nerve injury indexes; enzyme-linked immunosorbent assay (ELISA) was used to detect the contents of neurotransmitters and inflammatory factors; RIA method was used to detect the contents of stress hormones. Results: Before treatment, the nerve injury indexes, neurotransmitters, inflammation factors and stress hormones were not statistically different between two groups of patients (P > 0.05); after treatment, peripheral blood nerve function indexes myelin basic protein (MBP), ischemia modified albumin (IMA) and neuron-specific enolase (NSE) levels of observation group were lower than those of control group while brain-derived neurotrophic factor (BDNF) level was higher than that of control group (P < 0.05); peripheral blood peptide neurotransmitters vasopressin (VAP), dynorphin (Dny-A) and neuropeptide (NPY) as well as amino acid neurotransmitters amino acid neurotransmitters glutamate (Glu) and aspartic acid (Asp) contents of observation group were lower than those of control group while γ-aminobutyric acid (GABA) level was higher than that of control group (P < 0.05); serum inflammatory factors C-reactive protein (CRP), nuclear factor-κB (NF-κB) and interleukin-1β (IL-1β) contents as well as stress hormones angiotensin II (AngII), cortisol (Cor), corticotrophin-releasing hormone (CRH) and norepinephrine (NE) contents of observation group were lower than those of control group (P < 0.05). Conclusion: Mouse nerve growth factor combined with early mild hypothermia therapy can protect the brain function and inhibit the systemic inflammatory and stress reaction in patients with severe craniocerebral injury.

1. Introduction

Head injury is severe in patients with severe craniocerebral injury, and intracranial hematoma compresses local nerve tissue and promotes edema, which further increases neurological damage. Early mild hypothermia therapy is the important way to optimize the outcome of patients with craniocerebral injury, which protects the brain by reducing brain metabolism, reducing neuronal intracellular calcium overload, decreasing excitatory amino acid and oxygen free radical secretion and a series of other functions[1,2]. Studies have also pointed out that the effect of mild hypothermia therapy alone is limited in reversing neurologic injury, and exogenous neuroprotective factors are needed to expand its curative effect. Mouse nerve growth factor (mNGF) is a small molecular protein from mouse submandibular gland purification, belongs to the neurotrophic factor, and has been proven excellent in enhancing neurotransmitter activity, promoting damaged nerve
fiber regeneration and other aspects[3,4]. mNGF can promote neural function in patients with brain injury, its combination with mild hypothermia treatment is expected to break the current bottleneck in the treatment of patients with severe craniocerebral injury, and this new treatment compatibility is studied from brain protection, inflammatory response and stress response.

2. Materials and methods

2.1. Clinical information

68 patients with severe craniocerebral injury who were treated in our hospital between May 2012 and February 2016 were included, and the patients’ family members signed the informed consent. After the treatment and laboratory test results were reviewed, they were divided into the control group (n=38) who received mild hypothermia therapy and the observation group (n=30) who received mouse nerve growth factor combined with early mild hypothermia therapy. Control group included 20 male cases and 18 female cases, they were 27–69 years old, and the interval between injury and admission was 1–6 h and (3.16±0.74) h in average; observation group included 16 male cases and 14 female cases, they were 28–67 years old, and the time interval between injury and admission was 1–5 h and (3.07±0.68) h in average. Two groups of patients were not statistically different in gender, age and admission time interval distribution (P>0.05), and the hospital ethics committee approved the study.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) with clear trauma history and clinical manifestations; (2) with clear intracranial hemorrhage after skull CT; (3) without previous history of cerebral hemorrhage or cerebral infarction; (4) without drug taking history of aspirin, warfarin or other drugs that affected blood coagulation function; (5) surviving after treatment. Exclusion criteria: (1) with congenital cerebrovascular malformation; (2) with basic cognitive dysfunction; (3) associated with systemic infectious diseases; (4) pregnant or breastfeeding women.

2.3. Therapy

Both groups of patients accepted routine treatment for patients with craniocerebral injury, including the control of intracranial pressure, anti-infection, brain cell nutrition, dehydration, etc. The control group, on the basis of conventional treatment, received the early mild hypothermia treatment, specifically as follows: medical semiconductor ice blanket machine (Changchun Changjiang Sci-Tech Industrial Co., LTD.) was used for continuous cooling, and lyric cocktail and muscle relaxants, etc were used together. Assistant ice compress could be done on head and neck, armpits, groin and so on to eventually control the anal temperature between 33 °C and 35 °C, and the therapy lasted for 1 week. Physical cooling was stopped, re-warming was gradually conducted (increasing body temperature by 1 °C every 4 h, and stopping re-warming until anal temperature reached 36 °C), and the treatment lasted for 2 weeks. Observation group of patients, on the basis of routine therapy, received mouse nerve growth factor combined with early mild hypothermia therapy, specifically as follows: deep intramuscular injection of mouse nerve growth factor for injection (trade name NOBEX, from Xiamen Beidazhilu Biological Engineering Co., LTD., approved by S20060052, 18 μg/pcc, 1 pc/time, for 2 weeks in a row. Mild hypothermia treatment was the same as that of control group.

2.4. Observation indexes

2.4.1. Neurological function indexes

Immediately after admission and after 2 weeks of treatment, 1.5 mL of fasting peripheral venous blood was extracted from two groups of patients at the same point in time, and automatic biochemical analyzer (Zhuhai Senlong Biotech Co., LTD., Senlo8030) was used to determine nerve injury index contents in it, including myelin basic protein (MBP), brain-derived neurotrophic factor (BDNF), ischemia modified albumin (IMA) and neuron-specific enolase (NSE). The contents of neurotransmitters in it were determined by enzyme-linked immunosorbent assay (ELISA), including peptide neurotransmitters: vasopressin (VAP), dynorphin (Dny-A) and neuropeptide (NPY); amino acid neurotransmitters glutamate (Glu), γ-aminobutyric acid (GABA) and aspartic acid (Asp).

2.4.2. Inflammatory and stress response

Immediately after admission and after 2 weeks of treatment, 1.5 mL of peripheral venous blood was extracted from two groups of patients in the same way and centrifuged at low speed to get supernatant and freeze it in -80 °C refrigerator for test, and specific detection indexes were as follows: (1) inflammatory factors: ELISA kit instructions were followed to detect serum inflammatory factors C-reactive protein (CRP), nuclear factor-κB (NF-κB) and interleukin-1β (IL-1β) contents, ELISA kits were purchased from Sigma Company in the United States, and the article number were TN182, YS291 and IU631. (2) Stress hormones: RIA kits were used to detect the contents of stress hormones, including angiotensin || (AngII), cortisol (Cor), corticotrophin-releasing hormone (CRH) and norepinephrine (NE). RIA kits were purchased from Abcam Company, and the article number was YB092, SB391 and MA351.

2.5. Statistical analysis

Data in study was input in statistical software SPSS15.0 by specially-assigned personnel, measurement data was in terms of \( \bar{x} \pm s \), separate comparison before treatment and after treatment was by \( t \) test, comparison before and after treatment was by paired \( t \) test and \( P<0.05 \) indicated statistical differences.
3. Results

3.1. Nerve injury indexes

Comparison of peripheral blood nerve injury indexes MBP, BDNF, IMA and NSE levels between two groups of patients was as follows: before treatment, differences in peripheral blood nerve injury indexes MBP, BDNF, IMA and NSE levels were not statistically different between two groups of patients ($P > 0.05$); after treatment, peripheral blood nerve injury indexes MBP, IMA and NSE levels of both groups were lower than those before treatment while BDNF levels were higher than those before treatment, and differences within group were statistically significant before and after treatment ($P < 0.05$). After treatment, peripheral blood nerve injury indexes MBP, IMA and NSE levels of observation group were lower than those of control group while BDNF level was higher than that of control group, and differences between groups were statistically significant after treatment ($P < 0.05$), shown in Table 1.

3.2. Neurotransmitters

Comparison of peripheral blood peptide neurotransmitters VAP, Dny-A and NPY as well as amino acid neurotransmitters Glu, GABA and Asp contents of observation group were lower than those of control group while BDNF level was higher than that of control group patients, and differences between groups were statistically significant after treatment ($P < 0.05$), shown in Table 1.

### Table 1
Comparison of peripheral blood nerve injury indexes levels before and after treatment (\(\bar{x} \pm s\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>MBP (ng/L)</th>
<th>BDNF (ng/mL)</th>
<th>IMA (µg/L)</th>
<th>NSE (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>30</td>
<td>Before treatment</td>
<td>0.64±0.08</td>
<td>4.12±0.45</td>
<td>74.38±8.19</td>
<td>12.17±1.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>0.38±0.04</td>
<td>6.73±0.74</td>
<td>56.72±6.16</td>
<td>8.42±0.91</td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>Before treatment</td>
<td>0.63±0.07</td>
<td>4.09±0.46</td>
<td>73.47±7.59</td>
<td>12.09±1.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>0.47±0.05</td>
<td>5.62±0.63</td>
<td>64.15±6.83</td>
<td>9.71±0.98</td>
</tr>
</tbody>
</table>

Compared with same group before treatment, *$P < 0.05$; compared with control group after treatment, **$P < 0.05$.

### Table 2
Comparison of peripheral blood neurotransmitter contents before and after treatment (\(\bar{x} \pm s\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>Peptide neurotransmitters</th>
<th>Amino acid neurotransmitters</th>
<th>Glu (µmol/L)</th>
<th>GABA (µmol/L)</th>
<th>Asp (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VAP (ng/L)</td>
<td>Dny-A (ng/L)</td>
<td>NPY (µg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation group</td>
<td>30</td>
<td>Before treatment</td>
<td>22.17±2.84</td>
<td>61.27±6.58</td>
<td>225.38±25.76</td>
<td>92.46±9.73</td>
<td>4.28±0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>9.54±0.98</td>
<td>54.02±5.88</td>
<td>178.93±20.54</td>
<td>80.52±8.98</td>
<td>6.09±0.64</td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>Before treatment</td>
<td>21.85±2.54</td>
<td>60.85±6.57</td>
<td>227.52±24.19</td>
<td>92.87±9.54</td>
<td>4.23±0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>14.37±1.98</td>
<td>57.32±5.98</td>
<td>193.26±24.37</td>
<td>86.49±8.93</td>
<td>5.48±0.57</td>
</tr>
</tbody>
</table>

Compared with same group before treatment, *$P < 0.05$; compared with control group after treatment, **$P < 0.05$.

### Table 3
Comparison of serum inflammatory factor contents before and after treatment (\(\bar{x} \pm s\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>CRP (mg/L)</th>
<th>NF-κB (ng/mL)</th>
<th>IL-1β (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>30</td>
<td>Before treatment</td>
<td>34.28±0.09</td>
<td>125.84±14.76</td>
<td>43.81±5.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>11.35±1.64</td>
<td>83.77±9.15</td>
<td>17.43±1.85</td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>Before treatment</td>
<td>34.17±3.85</td>
<td>126.75±13.95</td>
<td>42.95±4.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>18.93±2.03</td>
<td>104.85±12.23</td>
<td>28.95±3.42</td>
</tr>
</tbody>
</table>

Compared with same group before treatment, *$P < 0.05$; compared with control group after treatment, **$P < 0.05$.
3.4. Stress hormones

Comparison of serum stress hormones AngII, Cor, CRH and NE contents between two groups of patients was as follows: before treatment, differences in serum stress hormones AngII, Cor, CRH and NE contents were not statistically different between two groups of patients (P>0.05); after treatment, serum stress hormones AngII, Cor, CRH and NE contents of both groups were lower than those before treatment, and differences within group were statistically significant before and after treatment (P<0.05). After treatment, serum stress hormones AngII, Cor, CRH and NE contents of observation group were lower than those of control group, and differences between groups were statistically significant after treatment (P<0.05), shown in Table 4.

4. Discussion

Early mild hypothermia therapy is considered to be one of the most reliable ways to treat patients with craniocerebral injury, which reduces brain metabolism, decreases the toxic effects of harmful substances (excitatory amino acids, oxygen free radical, etc.) on neurons and changes apoptosis-related gene expression to finally suppress neuronal necrosis/apoptosis process[5]. The role of mild hypothermia in blocking the deterioration of neurological function after traumatic brain injury has been approved, but it cannot reverse the function of damaged neurons, and exogenous nutrients are necessary to further protect the brain[6]. mNGF is neurotrophic agent and nerve regeneration agent, it has been confirmed in the animal experiments that mNGF can improve the rat limb movement dysfunction caused by toxic peripheral neuropathy, and it prompts the damaged nerve function recovery through the effect such as inhibiting the myelin swelling and reducing neural degeneration[7]. In the study, mNGF combined with mild hypothermia therapy was applied in patients with severe craniocerebral injury in our hospital in order to define the value of mNGF for intervening with brain injury and systemic inflammatory stress response.

Both brain injury itself and hematoma compression can affect the function of neurons in lesion area, and cause cell membrane and blood-brain barrier dysfunction, and a lot of nerve damage factors are detected in the peripheral blood[8]. MBP is the main protein of myelin sheath, it is neuron-specific, it is hardly expressed in peripheral blood under physiological status, and in the case of nerve injury and blood-brain barrier damage, MBP enters into the blood to stimulate autoimmune body production and induce autoimmune inflammatory response[9]. IMA is a new type of ischemia marker, its terminal amino acid sequence changes when the albumin crosses through the ischemic tissue, and the N-terminal 2-4 amino acids change and form the IMA[10]. NSE exists in nerve and neuroendocrine tissue, it is seldom expressed in serum and other tissue organs, and when the neurons are damaged, it crosses through the dysfunctional blood brain barrier and enters into the peripheral blood. BDNF belongs to the family of neurotrophic factors, it can maintain neuron survival and growth, and the increase of BDNF expression in brain tissue is a sign that the damaged nerve cells get repair[11,12]. In the study, the nerve injury factor levels in circulating blood of two groups of patients were detected, and it was found that compared with control group, the observation group were with lower MBP, IMA and NSE levels, and higher BDNF level after 2 weeks of treatment (P<0.05), confirming that the nerve damage in patients reduces after mNGF was added in the treatment.

After traumatic brain injury, the neurotransmitter expression changes, peptide neurotransmitters VAP, Dny-A and NPY contents can reflect the degree of nerve damage, high levels of VAP and NPY may contract blood vessels to ensure the brain blood supply, Dny-A can reduce intracranial hypertension, therefore the above neurotransmitter levels rise reactively in the acute phase of cerebral injury, and the degree of concrete increase is in line with brain damage severity[13]. Glu, GABA and Asp belong to amino acid neurotransmitters, Glu and Asp are the excitatory amino acids, GABA is inhibitory amino acid, and the two are in dynamic equilibrium under physiological conditions[14,15]. After the occurrence of intracranial injury, Glu and Asp are massively released and produce neurotoxicity, and GABA levels reactively reduce. In the study, above neurotransmitter levels were detected, and it was found that compared with control group, the observation group were with lower peripheral blood VAP, Dny-A, NPY, Glu and Asp contents, and higher GABA content (P<0.05), confirming that adding mNGF to mild hypothermia therapy can effectively balance the neurotransmitter levels and inhibit neurotoxic neurotransmitter production, and this is the one of the important mechanisms for it to protect the brain.

Brain hematoma compression on local nerve tissue can directly induce the release of massive inflammatory mediators, and it can also cause microglial cell and leukocyte infiltration, activate the classic complement system and prompt other inflammatory factor synthesis to indirectly increase brain injury[16]. CRP can early reflect the severity of craniocerebral injury, and its level fluctuates with the illness. NF-κB is an inflammation regulator, and can further

<table>
<thead>
<tr>
<th>Table 4</th>
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<tbody>
<tr>
<td>Comparison of serum stress hormone contents before and after treatment (±S).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>AngII (ng/mL)</th>
<th>Cor (ng/mL)</th>
<th>CRH (ng/mL)</th>
<th>NE (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>30</td>
<td>Before</td>
<td>68.25±7.11</td>
<td>251.83±28.75</td>
<td>28.64±3.05</td>
<td>412.48±48.59</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>After</td>
<td>47.14±5.03</td>
<td>189.74±20.55</td>
<td>15.78±1.95</td>
<td>360.57±39.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>Before</td>
<td>68.16±7.09</td>
<td>250.96±27.85</td>
<td>28.73±3.19</td>
<td>409.72±43.85</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>After</td>
<td>57.55±6.14</td>
<td>224.75±25.92</td>
<td>21.04±2.67</td>
<td>382.61±40.57</td>
</tr>
</tbody>
</table>

* Compared with same group before treatment, *P*<0.05; compared with control group after treatment, **P**<0.05.
activate IL-1β and other pro-inflammatory factors and induce the inflammatory cascade reaction\cite{7}. In the study, serum inflammatory factor levels of two groups of patients were detected, and it was found that compared with control group, the observation group were with lower serum CRP, NF-xkB and IL-1β contents after 2 weeks of treatment (P<0.05), indicating that mNGF combined with early mild hypothermia treatment can effectively inhibit inflammation in patients with severe head trauma. Stress is the systemic nonspecific adaptive reaction caused by internal and external stimuli, it is mostly characterized by the increased stress hormones AngII, Cor, CRH and NE contents, the elevated blood pressure, increased vascular resistance and high protein decomposition in the patients, and it is not conducive to the recovery of trauma and neural function\cite{18,19}. It was found in the study that compared with control group, the observation group were with lower serum AngII, Cor, CRH and NE contents after 2 weeks of treatment (P<0.05), confirming that mNGF combined with early mild hypothermia treatment can effectively inhibit the systemic stress response and further injury of neuron function in the patients.

Thus it shows that mNGF combined with early mild hypothermia therapy can play an active role in brain protection and also inhibit inflammatory stress reaction in patients with severe craniocerebral injury, has a positive therapeutic value, and is worthy of popularization and application in clinical practice in the future.

References


