Effect of salvianolate therapy on peripheral blood Bcl-2, BAX and Caspase-3 expression in patients with cerebral ischemic stroke and their correlation with neural function

Gui-Bin Wen

Neurology Department, Zigong Fourth People’s Hospital in Sichuan Province, Zigong City, Sichuan Province, 643000

ARTICLE INFO

Article history:
Received 16 Apr 2017
Received in revised form 17 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Cerebral ischemic stroke
Salvianolate
Apoptosis
Neural function

ABSTRACT

Objective: To study the peripheral blood Bcl-2, BAX and Caspase-3 expression in patients with cerebral ischemic stroke before and after salvianolate therapy and their correlation with neural function. Methods: A total of 89 patients with cerebral ischemic stroke who received salvianolate therapy in Neurology Department of Zigong Fourth People’s Hospital between May 2014 and April 2016 were studied. Before treatment as well as 2 weeks and 4 weeks after treatment, the peripheral blood apoptotic molecules Bcl-2, BAX and Caspase-3 expression as well as serum neural cytokine and oxidative stress product levels were determined respectively. Results: 2 weeks and 4 weeks after treatment, peripheral blood Bcl-2 mRNA expression as well as serum BDNF, NGF, VEGF and IGF-1 levels were significantly higher than those before treatment while BAX and Caspase-3 mRNA expression as well as serum MDA, 8-OHdG and 8-iso-PGF2α levels were significantly lower than those before treatment; 4 weeks after treatment, peripheral blood Bcl-2 mRNA expression as well as serum BDNF, NGF, VEGF and IGF-1 levels was significantly higher than those 2 weeks after treatment while BAX and Caspase-3 mRNA expression as well as serum MDA, 8-OHdG and 8-iso-PGF2α levels were significantly lower than those 2 weeks after treatment. Peripheral blood Bcl-2 mRNA expression was positively correlated with serum BDNF, NGF, VEGF and IGF-1 levels, and negatively correlated with serum MDA, 8-OHdG and 8-iso-PGF2α levels; peripheral blood BAX and Caspase-3 mRNA expression were negatively correlated with serum BDNF, NGF, VEGF and IGF-1 levels, and positively correlated with serum MDA, 8-OHdG and 8-iso-PGF2α levels. Conclusion: Salvianolate treatment of cerebral ischemic stroke can increase the anti-apoptotic molecule expression and inhibit the pro-apoptotic molecule expression to improve the neural function.

1. Introduction

Ischemic stroke is a clinical common type of stroke that has high morbidity and mortality, and the rising incidence in recent years[1]. Ischemia hypoxia damage to brain tissue can not only cause neurological damage, but can also affect the patients’ quality of life and increase the burden on society and family. Restoring brain tissue reperfusion is the key measure in the treatment of cerebral infarction, and the adjuvant anticoagulation, lipid-lowering, hypoglycemic and antihypertensive drugs can improve cerebral circulation and reduce ischemia hypoxia damage to nerve function[2,3]. Salvianolate is the magnesium lithospermate extracted from Chinese herbal medicine salvia miltiorrhiza, it has the pharmacological effects such as improving microcirculation, anti-inflammation, anti-oxidation and removing oxygen free radicals, and it can exert therapeutic effect on multiple pathological links in ischemic stroke. Studies have shown that salvianolate can improve the curative effect of ischemic cerebral stroke and improve the recovery of neural function[4], but the specific mechanism remains to be further elucidated. Cell apoptosis is an important part of the brain tissue injury after ischemia hypoxia, and the Bcl-2 and BAX can adjust Caspase-3 activity through the
mitochondrial pathway so as to regulate apoptosis process. In the following study, the peripheral blood Bcl-2, BAX and Caspase-3 expression in patients with cerebral ischemic stroke before and after salvianolate therapy and their correlation with neural function were analyzed.

2. Materials and methods

2.1 Research design

Prospective self-controlled clinical research was designed, and the study was approved by the hospital ethics committee and obtained the informed consent from the included patients.

2.2 Research subjects

Patients with cerebral ischemic stroke who were treated in the Neurology Department of Zigong Fourth People’s Hospital between May 2014 and April 2016 were selected as the research subjects of this prospective study, and the inclusion criteria were as follows: (1) diagnosed with ischemic cerebral stroke after head CT and MRI; (2) the time between onset and admission <48 h; (3) accepting adjuvant salvianolate therapy. Patients with transient cerebral ischemic attack, patients with cerebral hemorrhage and patients associated with heart, liver and kidney dysfunction were ruled out. 89 patients were selected, including 54 male cases and 35 female cases that were 45-67 years old.

2.3 Research methods

2.3.1 Therapy

Patients received basic symptomatic treatment immediately after admission, including early thrombolysis treatment, aspirin enteric-coated tablets and clopidogrel bisulfate for antiplatelet, atorvastatin for lipid lowering, angiotensin converting enzyme inhibitor/angiotensin II receptor inhibitor/calcium channel antagonist for pressure lowering, insulin for glucose lowering, etc., and meantime, they received salvianolate treatment as follows: 200 mg salvianolate in 250 mL saline, by intravenous drip, 1 time/d.

2.3.2 Peripheral blood apoptosis molecule expression detection methods

Before treatment as well as 2 weeks and 4 weeks after treatment, 5 mL of cubital venous blood was collected from the patients respectively, added in Ficoll-hypzue lymphocyte separation medium and centrifuged, the obtained peripheral blood mononuclear cells were washed with PBS and centrifuged twice to extract total RNA with Trizol lysis buffer, the RNA was synthesized into cDNA via reverse transcription, fluorescence quantitative PCR was used to amplify the Bcl-2, BAX and Caspase-3, the reference gene was β -actin, β -actin amplification was used for the standardization before and after treatment, and the Bcl-2, BAX and Caspase-3 amplification were calculated.

2.3.3 Serum neural function index level detection methods

Before treatment as well as 2 weeks and 4 weeks after treatment, 5 mL of cubital venous blood was collected from the patients respectively and centrifuged to separate serum, and the enzyme-linked immunosorbent assay kits were used to determine the malondialdehyde (MDA), 8-hydroxy deoxyguanosine (8-OHdG), 8-iso prostaglandins F2 (8-iso-PGF2 α ), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF-1) levels.

2.3.4 Statistical methods

SPSS 17.0 software was used to input and analyze data, comparison among different points in time was by repeated measures analysis of variance, and $ P<0.05 $ indicated statistical significance in differences.

3. Results

3.1 Peripheral blood apoptosis molecules Bcl-2, BAX and Caspase-3 mRNA expression before and after treatment

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of peripheral blood apoptosis molecules Bcl-2, BAX and Caspase-3 mRNA expression was as follows: 2 weeks and 4 weeks after treatment, peripheral blood Bcl-2 mRNA expression were significantly higher than those before treatment while BAX and Caspase-3 mRNA expression were significantly lower than

<table>
<thead>
<tr>
<th>Points in time</th>
<th>n</th>
<th>Bcl-2</th>
<th>BAX</th>
<th>Caspase-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>89</td>
<td>1.03±0.15</td>
<td>0.93±0.13</td>
<td>0.97±0.18</td>
</tr>
<tr>
<td>2 weeks after treatment</td>
<td>89</td>
<td>1.89±0.25</td>
<td>0.65±0.09</td>
<td>0.61±0.08</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>89</td>
<td>2.78±0.34</td>
<td>0.44±0.07*</td>
<td>0.39±0.05*</td>
</tr>
</tbody>
</table>

*: compared with before treatment, $ P<0.05 $; †: compared with 2 weeks after treatment, $ P<0.05 $.
those before treatment; 4 weeks after treatment, peripheral blood Bcl-2 mRNA expression was significantly higher than that 2 weeks after treatment while BAX and Caspase-3 mRNA expression were significantly lower than those 2 weeks after treatment. Differences in pair-wise comparison of peripheral blood apoptosis molecules Bcl-2, BAX and Caspase-3 mRNA expression were statistically significant among different points in time ($P<0.05$).

### 3.2 Serum oxidative stress products MDA, 8-OHdG and 8-iso-PGF2α levels before and after treatment

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of serum oxidative stress products MDA (nmol/L), 8-OHdG (pg/mL) and 8-iso-PGF2α (pg/mL) was as follows: 2 weeks and 4 weeks after treatment, serum MDA, 8-OHdG and 8-iso-PGF2α levels were significantly lower than those before treatment; 4 weeks after treatment, serum MDA, 8-OHdG and 8-iso-PGF2α levels were significantly lower than those 2 weeks after treatment. Differences in pair-wise comparison of serum MDA, 8-OHdG and 8-iso-PGF2α levels were statistically significant among different points in time ($P<0.05$).

<table>
<thead>
<tr>
<th>Points in time</th>
<th>n</th>
<th>MDA</th>
<th>8-OHdG</th>
<th>8-iso-PGF2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>89</td>
<td>13.51±2.23</td>
<td>598.66±81.29</td>
<td>535.21±77.65</td>
</tr>
<tr>
<td>2 weeks after treatment</td>
<td>89</td>
<td>7.53±0.94</td>
<td>373.41±46.74</td>
<td>221.54±34.52</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>89</td>
<td>4.25±0.52</td>
<td>231.24±32.56</td>
<td>145.61±22.85</td>
</tr>
</tbody>
</table>

*: compared with before treatment, $P<0.05$; #: compared with 2 weeks after treatment, $P<0.05$.

---

### 3.3 Serum nerve cytokines BDNF, NGF, VEGF and IGF-1 levels before and after treatment

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of serum nerve cytokines BDNF (ng/mL), NGF (ng/mL), VEGF (pg/mL) and IGF-1 (ng/mL) was as follows: 2 weeks and 4 weeks after treatment, serum BDNF, NGF, VEGF and IGF-1 levels were significantly higher than those before treatment; 4 weeks after treatment, serum BDNF, NGF, VEGF and IGF-1 levels was significantly higher than those 2 weeks after treatment. Differences in pair-wise comparison of serum BDNF, NGF, VEGF and IGF-1 levels were statistically significant among different points in time ($P<0.05$).

<table>
<thead>
<tr>
<th>Points in time</th>
<th>n</th>
<th>BDNF</th>
<th>NGF</th>
<th>VEGF</th>
<th>IGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>89</td>
<td>1.03±0.16</td>
<td>0.78±0.10</td>
<td>2231.5±26.74</td>
<td>121.42±17.65</td>
</tr>
<tr>
<td>2 weeks after treatment</td>
<td>89</td>
<td>1.83±0.25</td>
<td>1.21±0.24</td>
<td>278.76±31.45</td>
<td>178.76±22.36</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>89</td>
<td>2.82±0.41</td>
<td>2.36±0.36</td>
<td>346.54±51.58</td>
<td>242.15±32.59</td>
</tr>
</tbody>
</table>

*: compared with before treatment, $P<0.05$; #: compared with 2 weeks after treatment, $P<0.05$.

---

### 3.4 Correlation between peripheral blood apoptosis molecule mRNA expression and serum indexes

Pearson test analysis of the correlation between peripheral blood apoptosis molecule mRNA expression and serum indexes showed that peripheral blood Bcl-2 mRNA expression was positively correlated with serum BDNF, NGF, VEGF and IGF-1 levels, and negatively correlated with serum MDA, 8-OHdG and 8-iso-PGF2α levels; peripheral blood BAX and Caspase-3 mRNA expression were negatively correlated with serum BDNF, NGF, VEGF and IGF-1 levels, and positively correlated with serum MDA, 8-OHdG and 8-iso-PGF2α levels.

### 4. Discussion

Salvianolate is a drug that has the activity of improving microcirculation, anti-inflammation, anti-oxidation, removing oxygen free radicals and so on, and it is used as the adjuvant therapy for ischemic cerebral stroke[5,6]. The drug can act on multiple pathological links of ischemic cerebral stroke, relieve nerve function damage, and improve the prognosis. However, the mechanism for salvianolate to exert the above effects is not clear. Activated apoptosis process is an important factor for ischemia hypoxia to cause brain tissue damage, and the Bcl-2/BAX is the key molecule to regulate mitochondrial apoptosis. Cerebral ischemia hypoxia can affect the mitochondrial function and activate mitochondrial apoptosis. The Bcl-2 can inhibit the opening of transition pore on mitochondrial membrane so as to reduce cytochrome C release from mitochondria into the cytoplasm; BAX is able to form dimers with Bcl-2 to antagonize the function of the Bcl-2 so as to increase the cytochrome C release from mitochondria into the cytoplasm[7,8].
After entering the cytoplasm, cytochrome C can cause Caspase-3 activation through cascade activation reaction, and eventually mediate apoptosis. In order to define the effect of salvianolate on cell apoptosis during recovery in patients with cerebral ischemic stroke, peripheral blood apoptosis molecule expression levels were analyzed in the study before and after the treatment, and the results showed that at 2 weeks and 4 weeks after treatment, peripheral blood Bcl-2 mRNA expression was increasing while BAX and Caspase-3 mRNA expression was decreasing. This means that salvianolate treatment of ischemic cerebral stroke can adjust the expression of mitochondrial apoptosis molecules Bcl-2 and BAX, increase anti-apoptosis molecule Bcl-2 expression and inhibit pro-apoptosis molecule BAX expression so as to reduce Caspase-3 activation and inhibit cell apoptosis after cerebral infarction.

The activation of apoptosis in patients with ischemic cerebral stroke is associated with the activation of oxidative stress caused by ischemia hypoxia, and ischemia hypoxia will cause the respiratory chain coupling loss and the massive production of oxygen free radicals in mitochondria, and then causes oxidizing reaction of a variety of compositions in cells through the oxygen free radicals accumulated in local tissue[9,10]. MDA and 8-iso-PGF2α are the products after the lipid components in cell membrane and biological membrane structure react with oxygen free radicals[11,12], and 8-OHdG is the product after the DNA in nucleus reacts with oxygen free radicals[13]. MDA, 8-OHdG and 8-iso-PGF2α levels in blood circulation can reflect the generation of oxygen free radicals and the degree of oxidative stress reaction. In the study, analysis of above serum oxidative stress product levels before and after treatment showed that 2 weeks and 4 weeks after treatment, the serum MDA, 8-OHdG and 8-iso-PGF2α levels were negatively correlated with peripheral blood Bcl-2 mRNA expression and positively correlated with peripheral blood BAX and Caspase-3 mRNA expression. This means that the inhibiting effect of salvianolate on cell apoptosis and the promoting effect on nerve cytokines in patients with cerebral ischemic stroke are interactional. The increased nerve cytokine secretion can promote neuron restoration and inhibit cell apoptosis; inhibited cell apoptosis is advantageous to the neuron growth, which will secrete nerve cytokines.

As the increase of apoptosis during recovery in patients with ischemic cerebral infarction, which is characterized by further reduction of the generation of free radicals. This means that salvianolate can increase the secretion of nerve cytokines during recovery in patients with ischemic cerebral infarction, which is beneficial to the recovery of neural function. Further analysis of the correlation between apoptosis molecule expression and nerve cytokine levels showed that serum BDNF, NGF, VEGF and IGF-1 levels were positively correlated with peripheral blood Bcl-2 mRNA expression and negatively correlated with peripheral blood BAX and Caspase-3 mRNA expression. This means that the inhibiting effect of salvianolate on cell apoptosis and the promoting effect on nerve cytokines in patients with cerebral ischemic stroke are interactional. The increased nerve cytokine secretion can promote neuron restoration and inhibit cell apoptosis; inhibited cell apoptosis is advantageous to the neuron growth, which will secrete nerve cytokines.

Based on the analysis of above serum index and apoptosis molecule expression, it is believed that salvianolate can inhibit apoptosis in patients with ischemic cerebral stroke, which is characterized by increasing the anti-apoptosis molecule expression and inhibiting pro-apoptosis molecule expression; the salvianolate inhibition on cell apoptosis is associated with decreasing oxidative stress and increasing nerve cytokine secretion.

References


