Neuroprotective effect of butylphthalide combined with aspirin and clopidogrel antiplatelet therapy on progressive cerebral infarction

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ABSTRACT

Objective: To analyze the neuroprotective effect of butylphthalide combined with aspirin and clopidogrel antiplatelet therapy on progressive cerebral infarction. Methods: A total of 86 patients with progressive cerebral infarction were randomly divided into observation group and control group (n=43), control group received aspirin and clopidogrel antiplatelet therapy, observation group received butylphthalide combined with aspirin and clopidogrel antiplatelet therapy, and then the differences in platelet function, blood coagulation function, middle cerebral artery blood flow state and nerve function index levels were compared between two groups after treatment. Results: After 1 course of treatment, the relative content of P-selectin and GPIIb/IIIa on platelet surface as well as TXB2, vWF and D-D content in plasma of observation group were significantly lower than those of control group, while 6-Keto-PGF1α content in plasma was significantly higher than that of control group); thrombelastogram indexes R and K value were higher than those of control group while MA, Angle and CI value were lower than those of control group; middle cerebral artery PSV, EDV, Vm and PI were higher than those of control group while RI value was lower than that of control group; nerve function indexes BDNF and H 2S content in plasma were higher than those of control group while NSE, MBP, S100B and MMP-9 content were lower than those of control group (P<0.05). Conclusions: Butylphthalide combined with aspirin and clopidogrel antiplatelet therapy can effectively optimize the platelet and blood coagulation function in patients with progressive cerebral infarction, promote the middle cerebral artery blood flow recovery and exert positive neuroprotective effect.

1. Introduction

Progressive cerebral infarction (PCI) refers to the ischemic stroke aggravated within 7d after onset, and at present, many scholars have limited the timing to within 48h after onset. Related statistics show that PCI is with high mortality rate and is currently one of the most intractable diseases in the cerebrovascular discipline[1,2]. The fundamental mechanism of PCI is the new vascular stenosis or original narrow vascular occlusion, which further aggravates local cerebral ischemia and increases neurological impairment. Platelet activation is one of the core links of PCI, aspirin and clopidogrel is the most common clinical dual antiplatelet therapy, and it can positively inhibit platelet aggregation and microthrombosis[2]. But antiplatelet therapy alone has little reversing effect on the existing neuron damage in patients, and many scholars recommend adding cerebral protection drugs such as butylphthalide. The effective component of butylphthalide is 3-n-butylphathilde, and it has multiple functions of inhibiting platelet aggregation, improving microcirculation, reducing neuron calcium overload, optimizing mitochondria function and reducing brain cell injury[2-3]. In the study, butylphthalide was added in PCI treatment, and the differences in platelet function, middle cerebral artery hemodynamics and nerve function index content were compared between the two groups after treatment in order to clarify the effect of butylphthalide treatment on the treatment effect in patients with PCI.

2. Materials and methods
2.1. General information

A total of 86 patients with progressive cerebral infarction treated in our hospital between December 2013 and December 2015 were included, and the inclusion criteria were: (1) diagnosed with cerebral infarction by head MRI; (2) with onset for the first time; (3) with the time between onset and admission ≥ 48 h, and with deteriorated condition during treatment; (4) excluded of cerebral hemorrhage, hemorrhage after infarction and other cerebral re-infarction in intracranial artery; (5) the patients’ family signed informed consent. Exclusion criteria were: (1) acute systemic infection; (2) with severe cardiopulmonary dysfunction; (3) with severe electrolyte disorder; (4) with congenital intracranial vascular anomaly; (5) allergic to drugs such as butylphthalide, aspirin and clopidogrel; (6) with incomplete clinical data. All patients conformed to the above inclusion criteria, and according to the different treatment methods, they were divided into observation group and control group (n=43). Control group included 23 male cases and 20 female cases, they were 48-73 years old and (60.49±8.93) years old in average, and the history of concomitant diseases was: 29 cases with hypertension, 17 cases with diabetes and 9 cases with coronary heart disease; observation group included 24 male cases and 19 female cases, they were 47-72 years old and (61.53±8.76) years old in average, and the history of concomitant diseases was: 31 cases with hypertension, 19 cases with diabetes and 10 cases with coronary heart disease. The two groups of patients showed no statistically difference in the distribution of gender, age and concomitant diseases (P>0.05) and they were comparable.

2.2. Treatment methods

Both groups of patients received conventional symptomatic treatments such as regulating lipid by statins, lowering blood pressure, regulating blood glucose and electrolyte disorder and neurotrophic drugs. Control group received routine treatment + aspirin and clopidogrel treatment, specifically as follows: oral administration of 300 mg of clopidogrel and 100 mg of aspirin on the 1 d, once every day, oral administration of 75 mg of clopidogrel and 100 mg of aspirin on the 2-14 d, once every day. Observation group received conventional treatment + butylphthalide + aspirin and clopidogrel treatment, specifically as follows: slow intravenous drip of butylphthalide and sodium chloride injection 25 mg, single drip duration 50 min, 2 times every day, time interval between two medication ≥ 6 h, 14 d as 1 course of treatment. The dosage and methods of aspirin and clopidogrel treatment were the same as those of control group.

2.3. Observation indexes

2.3.1. Platelet function and thrombelastogram

After 1 course of treatment, 10 mL of fasting peripheral venous blood was collected, 6 mL of it was added in anticoagulation tube containing 0.2 mL of sodium citrate, and flow cytometer was used to determine the relative content of P-selectin and GP IIb/IIIa on platelet membrane. A total of 2 mL of peripheral venous blood was not anticoagulated to separate plasma, and enzyme-linked immunosorbent assay was used to determine TXB2, 6-Keto-PGF1α, vWF and D-dimer (D-D) levels. As for the last 2 mL of peripheral venous blood, thromboelastography was used to determine the reaction time (R), maximum amplitude (MA), clot formation rate (Angle), clot formation time (K), and coagulation index (CI).

2.3.2. Hemodynamic parameters

After a course of treatment, color Doppler ultrasonic diagnostic instrument was used to determine middle cerebral artery peak systolic velocity (PSV), end-diastolic velocity (EDV), mean blood flow velocity (Vm), pulsatility index (PI) and resistance index (RI) via the temporal window.

2.3.3. Nerve function-related indexes in plasma

After 1 course of treatment, 2 mL of fasting peripheral venous blood was collected and centrifuged to separate plasma, and enzyme-linked immunosorbent assay was used to detect nerve function-related indexes, including neuron-specific enolase (NSE), brain-derived neurotrophic factor (BDNF), myelin basic protein (MBP), S100B protein (S100B), hydrogen sulfide (H2S) and matrix metalloproteinase-9 (MMP-9).

2.4. Statistical methods

Data in the study was input in statistical software SPSS23.0, measurement data was performed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Platelet function

The relative content of P-selectin and GP IIb/IIIa on platelet surface as well as TXB2, vWF and D-D content in plasma of observation group were significantly lower than those of control group, 6-Keto-PGF1α content in plasma was significantly higher than that of control group (P<0.05), shown in Table 1.

3.2. Thrombelastogram parameters

Peripheral blood R and K value of observation group were significantly higher than those of control group while MA, Angle

2.3. Observation indexes

The values and statistical significance of platelet function indexes in both groups after treatment are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>Platelet surface molecules</th>
<th>Plasma molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P-selectin</td>
<td>GP IIb/IIIa</td>
</tr>
<tr>
<td>Observation</td>
<td>43</td>
<td>8.23±0.11</td>
<td>13.24±1.77</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>13.67±1.94</td>
<td>19.53±2.28</td>
</tr>
<tr>
<td>t</td>
<td>8.342</td>
<td>7.982</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
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</table>
and CI value were significantly lower than those of control group ($P<0.05$), shown in Table 2.

### 3.3. Hemodynamic parameters

PSV, EDV, Vm and PI of observation group were higher than those of control group while RI value was significantly lower than that of control group ($P<0.05$), shown in Table 3.

### 3.4. Nerve function-related indexes in plasma

BDNF and H$_2$S content in plasma of observation group were significantly higher than those of control group while NSE, MBP, S100B and MMP-9 content were significantly lower than those of control group ($P<0.05$), shown in Table 4.

#### Table 2
Comparison of thrombelastogram parameter value between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>R (min)</th>
<th>MA (mm)</th>
<th>Angle (%)</th>
<th>K (min)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>43</td>
<td>5.2±0.63</td>
<td>59.38±6.11</td>
<td>67.34±7.19</td>
<td>2.11±0.24</td>
<td>0.56±0.07</td>
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<tr>
<td>Control group</td>
<td>43</td>
<td>4.89±0.52</td>
<td>63.42±7.09</td>
<td>71.63±8.43</td>
<td>1.76±0.18</td>
<td>1.13±0.14</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>5.839</td>
<td>7.192</td>
<td>8.384</td>
<td>5.793</td>
<td>6.293</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>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

#### Table 3
Comparison of hemodynamic parameters between two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>PSV (cm/s)</th>
<th>EDV (cm/s)</th>
<th>Vm (cm/s)</th>
<th>PI</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>43</td>
<td>84.2±9.41</td>
<td>38.94±4.12</td>
<td>56.3±6.12</td>
<td>1.03±0.14</td>
<td>0.45±0.05</td>
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<tr>
<td>Control group</td>
<td>43</td>
<td>77.5±8.03</td>
<td>30.75±3.62</td>
<td>34.9±4.09</td>
<td>0.91±0.09</td>
<td>0.62±0.07</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>8.394</td>
<td>7.283</td>
<td>9.172</td>
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<td>6.394</td>
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<td>$P$</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</table>

#### Table 4
Comparison of nerve function-related index content in plasma between two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>NSE (μg/L)</th>
<th>BDNF (ng/mL)</th>
<th>MBP (μg/L)</th>
<th>S100B (μg/L)</th>
<th>H$_2$S (μmol/L)</th>
<th>MMP-9 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>43</td>
<td>13.17±1.43</td>
<td>3.64±0.32</td>
<td>2.58±0.32</td>
<td>0.71±0.08</td>
<td>378.94±41.52</td>
<td>65.38±7.11</td>
</tr>
<tr>
<td>Control group</td>
<td>43</td>
<td>20.65±2.75</td>
<td>2.71±0.35</td>
<td>3.47±0.42</td>
<td>1.18±0.13</td>
<td>281.33±32.67</td>
<td>79.62±8.41</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>6.835</td>
<td>5.743</td>
<td>7.384</td>
<td>5.192</td>
<td>12.842</td>
<td>8.394</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>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

#### 4. Discussion

PCI belongs to the clinical severe illness, both morbidity and mortality of patients are high, and there is no consensus on the clinical treatment of the disease. One of the important pathogenesis of PCI is that the primary site vessels spread and generate new stenosis or vascular occlusion, it is mainly related to vascular spasm and platelet aggregation, and therefore, the antiplatelet therapy is of great significance in preventing or reducing PCI condition[4]. Both aspirin and clopidogrel are the clinical common antiplatelet agents, and aspirin can cause the platelet cyclooxygenase acetylation and inhibit platelet aggregation in vivo; clopidogrel selectively inhibits ADP combination with platelet receptor and inhibit glycoprotein GP IIb/IIIa complex activation so as to finally inhibit platelet aggregation[5,6]. Aspirin combined with clopidogrel belongs to the potent antiplatelet therapy, and study has found the therapy plays a positive role in reducing the recurrence of thrombotic events in patients with cerebral infarction. Antiplatelet therapy plays an indirect role in the protection of the nerve cell function, and due to the severe neurologic injury in patients with PCI, some scholars put forward that the targeted cerebral protection drugs should be added on the basis of dual antiplatelet therapy in order to promote the recovery of patients' neural function. Butylphthalide belongs to new cerebral protection drug, foreign study has confirmed that it can exert neuronprotective effect in multiple links, so it was used together with antiplatelet therapy in the study, the effect of combined therapy on regulating platelet, blood coagulation function, hemodynamic parameters and neural function-related parameters was mainly observed.

Platelet activation and aggregation is a key link in the process of PCI, and positive antiplatelet therapy is expected to ease PCI condition and recover part of the blood flow in blood vessels with infarction. In the study, platelet function indexes of the two groups were detected after treatment, and it was found that P-selectin, GPIIb/IIIa, TXB2, vWF and D-D content of observation group were lower while 6-Keto-PGF1α content was higher after treatment. P-selectin is a specific marker reflecting platelet activation and release reaction, and it can mediate the platelet activation and start as well as expand the thrombosis. GP IIb/IIIa complex is a reliable sign of platelet activation, and is the common pathway of platelet aggregation[7]. vWF and D-D synthesis increases in endothelial function impairment, prompting platelet adhesion and thrombosis; TXB2 and 6-Keto-PGF1α are the metabolites of TXA2 and PGI2, TXB2 can strongly promote the platelet aggregation and vasoconstriction, and 6-Keto-PGF1α can strongly dilate blood vessels and inhibit platelet aggregation[8,9]. The above study shows that the platelet function of observation group is further suppressed after adding butylphthalide therapy, domestic scholars have also reported the effect of butylphthalide on inhibiting platelet aggregation and relieving microvascular spasm, and in the study, it is further confirmed the superimposition effect of butylphthalide with aspirin and other antiplatelet drugs. Thrombelastogram is the gold standard that reflects the patients’ overall blood coagulation function, the blood coagulation reaction time (R) represents the blood coagulation activity, MA represents the platelet aggregation function, both
clot formation rate (Angle) and clot formation time (K) reflect the function of fibrinogen, and CI represents the patients’ overall blood coagulation function. It was found in the study that R and K value of observation group were bigger while MA, Angle and CI value were smaller after treatment, indicating that butylphthalide combined with aspirin and clopidogrel antiplatelet therapy has more advantages in optimizing the patients’ overall blood coagulation function, and can reduce the hypercoagulable state in patients.

There is severe cerebral dysfunction in most patients with PCI, and the cerebral perfusion further declines in this kind of patients, which causes that the glutamic acid, glycine and other excitatory amino acids accumulate in ischemic area, stimulate N-methyl-D-aspartate receptor and start the cascade formed by oxygen free radicals. Massive formation of oxygen free radicals can lead to neuron calcium overload and mitochondrial dysfunction, which further leads to nerve cell injury in the ischemic area and the overall neural function damage in patients[10]. Middle cerebral artery is artery most common involved in PCI, its blood flow state greatly determines cerebral ischemia state in patients, and it was found in the study that the middle cerebral artery PSV, EDV, Vm and PI under color Doppler ultrasound of observation group were higher while RI was lower, indicating that the large artery blood flow increases after butylphthalide treatment, and this is mainly because that butylphthalide relieves microvascular spasm, increases the number of brain capillaries, improves collateral circulation establishment and microcirculation improvement, etc.

In the case of nerve cell damage in patients with PCI, several intracellular cytokines are released into the extracellular fluid, enter into the blood circulation through the damaged blood brain barrier and are detected. NSE, BDNF, MBP and S100B are typical nerve function-related indexes, and their sensitivity to nerve damage is high[11]. NSE, MBP and S100B are released into the blood in nerve cell damage, and their levels are positively associated with the degree of nerve dysfunction; BDNF can nourish the nerves and inhibit neuron damage[12,13]. H2S is a new type of gas signal molecule with neuroregulation function, and studies have found that H2S has neuroprotective effect and can avoid the neuron apoptosis. MMP-9 is a cytokine associated with brain injury discovered in recent years, astrocytes can synthesize MMP-9, and massive MMP-9 can cause negative impact on brain function[14,15]. In the study, the plasma content of above nerve function-related indexes of two groups were tested after treatment, and it was found that BDNF and H2S content in plasma of observation group were higher while NSE, MBP, S100B and MMP-9 content were lower, indicating that adding butylphthalide on the basis of conventional antiplatelet therapy can significantly optimize patients’ neural function, and it is specifically related to the inhibited release of excitatory amino acids by butylphthalide as well as the calcium overload in nerve cells caused by hypoxia and hypoglycemia.

To sum up, it is concluded as follows: butylphthalide combined with aspirin and clopidogrel antiplatelet therapy can effectively optimize the platelet and blood coagulation function in patients with progressive cerebral infarction, promote the middle cerebral artery blood flow recovery and exert positive neuroprotective effect, and it is worth popularization and application in clinical practice in the future.

References