Effect of clopidogrel and atorvastatin on hemorheology, inflammatory reaction and blood coagulation in patients with cerebral infarction

Wei Yang, Jie-Qing Zhao, Xiao-Ying Ding

Pharmacy Department, Taikang Xianlin Drum Tower Hospital, Nanjing 210046, Jiangsu, China

ARTICLE INFO

Objective: To observe the effect of clopidogrel combined with atorvastatin on hemorheology, inflammation and coagulation in patients with cerebral infarction. Method: A total of 100 patients with cerebral infarction treated in our hospital from October 2015 to September 2017 were selected and divided into clopidogrel group and combination group according to random number table, each with 50 cases. The clopidogrel group received clopidogrel treatment, and the combination group received atorvastatin treatment on the basis of clopidogrel group. The changes of hemorheology, inflammatory factors and coagulation were compared between the two groups. Results: There was no significant difference in the levels of hemorheology, inflammatory cytokines and coagulation function between the two groups before treatment. After treatment, the levels of hs-CRP, TNF-\(\alpha\), MMP-9 in the combination group were significantly lower than those before treatment; while only HBV, PV, EAI and HTC in the clopidogrel group decreased in both groups, and the five indexes in the combination group were significantly lower than those before treatment; while only HBV, PV, EAI and HTC in the clopidogrel group were significantly lower than those before treatment. After treatment, the levels of HBV and PV in the combination group were significantly lower than those in the clopidogrel group. After treatment, the levels of hs-CRP, TNF-\(\alpha\), MMP-9 in the two groups were significantly lower than those before treatment, and the levels of hs-CRP (14.94±6.21) mg/L, TNF-\(\alpha\) (0.91±0.54) μg/L, and MMP-9 (1.93±0.29) μg/L in the combination group were significantly lower than those of clopidogrel group. After treatment, the levels of APTT and PT in the two groups had no significant changes, while the levels of Fib and DD were significantly lower than those before treatment. After treatment, the levels of APTT and PT in the combination group were higher than those in the clopidogrel group, but the difference was not significant. Fib level (3.02±1.06) g/L, DD level (0.27±0.08) mg/L of the combination group was significantly lower than those in the clopidogrel group. Conclusions: Clopidogrel combined with atorvastatin treatment of cerebral infarction can improve the patient’s hemorheology level, and significantly reduce the body’s inflammatory response, which plays a certain role in promoting the coagulation function. Thus it is worthy of clinical promotion.

1. Introduction

Cerebral infarction is a common ischemic cerebrovascular disease. Cerebral atherosclerosis, verteobasilar artery or carotid artery insufficient blood supply, and blood hypercoagulability state can lead to cerebral vascular stenosis or occlusion, then resulting in acute cerebral insufficiency and local brain ischemic necrosis[1-2]. In clinic, clopidogrel and atorvastatin are commonly used for treatment of thromboembolic disease and hypercholesterolemia[3]. Studies have shown that[4] clopidogrel combined with atorvastatin for the treatment of cerebral infarction can significantly reduce the plaque area as well as improve coagulation. In this study, atorvastatin combined with clopidogrel was adopted to treat cerebral infarction, in order to explore its effect on inflammatory response, hemorheology and coagulation function.
2. Materials and methods

2.1. General data

A total of 100 patients with cerebral infarction admitted in Taikang Xianlin Drum Tower Hospital from October 2015 to September 2017 were enrolled and divided into clopidogrel group and combination group according to random number table. Among them, clopidogrel group included 31 males and 19 females, aged form 48-76 years old, with 12-46 h course of disease. Combined group included 29 males and 21 females, aged from 50-78 years old, with 12-47 h course of disease. There was no significant difference in general data between the two groups (P>0.05). The study was approved by Medical Ethics Committee of our hospital the patients were informed and signed informed consent.

Inclusion criteria: (1) According to the classification and diagnostic criteria of cerebrovascular diseases revised at the Sixth National Conference on Cerebrovascular Disease[5], all the selected cases met the related criteria of cerebral infarction and were diagnosed by brain CT or MRI. (2) The first time attacked by the disease. (3) Strong compliance and able to complete all inspection items cooperately. (4) Not taking other drugs recently. (5) The time from onset to admission less than 48 h. Exclusion criteria: (1) Accompanied by other heart disease, liver and kidney dysfunction. (2) With history of stroke. (3) Coagulation disorders or bleeding tendency. (4) Allergic reaction or intolerance with the drugs used in this study.

2.2. Treatment method

After admission, both groups were given conventional treatment: brain protection, dehydration and decrease of intracranial pressure, anti-platelet aggregation, scavenging oxygen free radicals, nourishing nerve treatment, and improving circulation and anti-infection. On the basis of conventional treatment, clopidogrel group was given oral clopidogrel (Hangzhou Sanofi Pharmaceutical Co., Ltd., lot number 20150605,20170510), 75 mg/time, once a day. On the basis of clopidogrel group, combination group was given atorvastatin for oral treatment additionally (Pfizer Ireland Drug Company, lot number 20150427), 20 mg/time, once a day before going to bed. Two groups were treated for one month.

2.3. Indicators detection

2.3.1. Hemorheology indicators detection

Hemorheology indicators were detected before treatment and after 1 month of treatment, respectively. A total of 10 mL venous blood was collected from each patient on an empty stomach in two groups and the supernatant was obtained after centrifuged at 3000 r/min for 10 min. Five mL of supernatant was taken and whole blood viscosity (WBV), high-shear viscosity (HBV), low-shear viscosity (LBV), plasma viscosity (PV), erythrocyte aggregation index (EAI) and hematocrit (HCT) were measured by the hemorheology analyzer (LBY-N6B analyzer, Beijing Pulisheng Company).

2.3.2. Detection of inflammatory cytokines

Five mL of supernatant was taken and ELISA (Elisa) was adopted for detection of high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α) and matrix metalloproteinase-9 (MMP-9) (kit purchased from Shanghai Bohu Biological Co., Ltd., lot number 20150605,20170510).

2.3.3. Detection of coagulation index

Coagulation index was detected before treatment and after 1 month of treatment respectively. About 5 mL of anterior elbow venous blood was collected from patients in two groups who have been fasted for more than 12 h. Sodium citrate negative pressure anticoagulation tube (1:9) has been given anticoagulant treatment and plasma was seperated within 2 h. The activated partial thromboplastin time (APTT), plasma prothrombin time (PT), fibrinogen (Fib) and D-dimer (DD) levels were measured by the Japanese Sysmex CA-1500 automatic coagulation analyzer. The kits were products of the United States Dade Behring Company.

2.4. Statistical analysis

In this study, hemorheology indicators, inflammatory factors hs-CRP, TNF-α, MMP-9 and coagulation function indicators were subjected to normal distribution, expressed as Mean ± SD. t test were used for intragroup and intergroup comparison before and after treatment group. Besides, statistical software SPSS 18.0 was used as the data analysis tool. P<0.05 indicated significant difference.

3. Results

3.1. Comparison of hemorheology level of patients in two groups before and after treatment

There was no significant difference in HBV, LBV, PV, EAI and HTC between the two groups before treatment (P>0.05). After treatment, the levels of HBV, LBV, PV, EAI and HTC all decreased in both groups, and the five indexes in combination group were significantly decreased than those before treatment (P<0.05), in clopidogrel group only four indicators HBV, LBV, PV, EAI and HTC significantly reduced than those before treatment (P<0.05). After treatment, the levels of HBV and PV in the combination group were significantly lower than those in the clopidogrel group (P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>WBV (m.pas)</th>
<th>LBV (m.pas)</th>
<th>PV (m.pas)</th>
<th>EAI (%)</th>
<th>HCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>Before treatment</td>
<td>7.05±1.61</td>
<td>11.42±4.43</td>
<td>3.04±0.27</td>
<td>7.53±0.41</td>
<td>48.72±10.14</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>4.34±1.41*</td>
<td>10.13±3.34*</td>
<td>1.93±0.34*</td>
<td>4.73±0.59*</td>
<td>42.58±7.19*</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Before treatment</td>
<td>7.12±1.79</td>
<td>11.61±4.57</td>
<td>3.12±0.33</td>
<td>7.64±0.47</td>
<td>49.35±9.67</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>5.13±1.55*</td>
<td>11.47±2.28</td>
<td>2.41±0.41*</td>
<td>4.76±0.62*</td>
<td>40.89±8.21*</td>
</tr>
</tbody>
</table>

*p<0.05, compared with those before treatment in this group; *p<0.05, compared with clopidogrel group at the same time.
3.2. Comparison of inflammatory factors in two groups

There was no significant difference in hs-CRP, TNF-α and MMP-9 levels between the two groups before treatment ($P>0.05$). The levels of hs-CRP, TNF-α and MMP-9 in the two groups after treatment significantly decreased than those before treatment ($P<0.05$), and the levels of hs-CRP (14.94 ± 6.21) mg/L, TNF-α (0.91 ± 0.54) μg/L and MMP-9 (1.93 ± 0.29) μg/L in the combination group were significantly lower than those in clopidogrel group ($P<0.05$)(Table 2).

Table 2.
Comparison of inflammatory factors in two groups ($n=50$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>hs-CRP (mg/L)</th>
<th>TNF-α (μg/L)</th>
<th>MMP-9 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>Before</td>
<td>26.21±13.54</td>
<td>2.85±0.82</td>
<td>3.68±1.41</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>14.94±6.21*</td>
<td>0.91±0.54*</td>
<td>1.93±0.29*</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Before</td>
<td>26.14±11.49</td>
<td>2.92±0.79</td>
<td>3.73±1.66</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>19.29±7.19*</td>
<td>1.73±0.65*</td>
<td>2.47±0.32*</td>
</tr>
</tbody>
</table>

* $P<0.05$, compared with those before treatment in this group; $^{*}$ $P<0.05$ compared with clopidogrel group at the same time.

3.3. Comparison of coagulation levels in two groups before and after treatment

There was no significant difference in APTT, Fib, PT, DD levels between the two groups before treatment ($P>0.05$). After treatment, the levels of APTT and PT in the two groups had no significant changes ($P>0.05$), while the levels of Fib and DD significantly decreased than those before treatment ($P<0.05$). After treatment, the levels of APTT and PT in the combination group were higher than those in the clopidogrel group, but the difference was not significant ($P>0.05$); the Fib level (3.02±1.06) g/L and the DD level (0.27±0.08) mg/L were obviously lower than those in clopidogrel group, the difference was statistically significant ($P<0.05$)(Table 3).

Table 3.
Comparison of coagulation levels in two groups before and after treatment ($n=50$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>APTT (s)</th>
<th>PT (s)</th>
<th>Fib (g/L)</th>
<th>DD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>Before</td>
<td>31.75±9.51</td>
<td>11.30±2.11</td>
<td>4.11±1.57</td>
<td>1.57±0.84</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>32.67±8.23</td>
<td>11.82±2.51</td>
<td>3.02±1.06*</td>
<td>0.27±0.08*</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Before</td>
<td>31.42±9.39</td>
<td>11.42±2.28</td>
<td>3.97±1.49</td>
<td>1.54±0.79</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>30.86±8.61</td>
<td>10.21±2.41</td>
<td>3.38±1.02*</td>
<td>0.51±0.14*</td>
</tr>
</tbody>
</table>

$^*P<0.05$, compared with those before treatment in this group; $^{*}P<0.05$, compared with clopidogrel group at the same time.

4. Discussion

Cerebral infarction is a common ischemic cerebrovascular disease, with high mortality, caused by metabolic disorders in the body. The main cause of this disease is the plaques off formed by atherosclerosis, the formation is related closely to elevated blood lipids, blood viscosity and inflammation in vivo[6-7]. Currently clopidogrel and statin drugs are clinically use in the treatment of cerebral infarction[8]. Clopidogrel[9,10] is an adenosine diphosphate (ADP) receptor antagonist that selectively binds to ADP receptors and acts to inhibit platelet aggregation and reduce blood viscosity, thus effectively inhibiting thrombus formation and achieving the purpose of inhibiting atherosclerosis and preventing cerebral infarction. Its mechanism[11] has mainly the following aspects: (1) has an antagonism effect on platelet membrane ADP receptor and inhibit thromboxane formation and release of active factors in platelet; (2) activates platelet adenyly cyclase and inhibits platelet aggregation; (3) blocks binding between ADP receptor and platelet receptor, thereby inhibiting platelet aggregation. Statins drugs have significant effects on anti-oxidant, anti-inflammatory and lipid-lowering and are widely used in the treatment of cerebrovascular diseases. Atorvastatin can selectively inhibit hydroxymethyl glutaryl coenzyme A reductase, inhibit extremely low density protein cholesterol synthesis and reduce plasma fibrinogen content, thereby regulating blood lipid balance, preventing atherosclerosis and protecting blood vessels[12,13]. Its mechanism[14] is mainly through the inhibition of hydroxymethyl glutaryl coenzyme A reductase activity to reduce the body’s cholesterol content, inhibit atherosclerotic plaque formation, reduce the inflammatory reaction at the atherosclerotic plaque, as well as lower plasma viscosity and Fib content; atorvastatin also inhibits the proliferation of vascular smooth muscle cells and stabilizes plaque through changing the atherosclerotic plaque cell structure.

Studies have shown that[15,16] the process of cerebral infarction is closely related to abnormal blood rheology levels. In the pathogenesis of cerebral infarction, increase of blood viscosity and cerebrovascular ischemia play key roles. In addition, the increase of levels such as plasma specific viscosity, fibrinogen and red blood cells will cause increased blood viscosity, which can easily lead to cerebral ischemia. In this study, after treatment, the levels of HBV, LBV, PV, EAI and HTC all decreased in both groups, and the five indexes in the combination group were significantly lower than those before treatment ($P<0.05$), HBV, PV, EAI, HTC four indicators in clopidogrel group significantly decreased than those before treatment ($P<0.05$). After treatment, the levels of HBV and PV in the combination group were significantly lower than those in
the clopidogrel group (P<0.05). The results showed that the levels of hemorheology in the two groups were significantly improved after treatment, and the efficacy of combination group was significantly better than the clopidogrel group, suggesting that the combination of the two drugs may help to improve the cerebrovascular function in patients, the reason might be that clopidogrel can selectively bind ADP receptors, inhibit platelet aggregation and reduce blood viscosity, thereby reducing thrombosis formation; atorvastatin sustained lipid-lowering effect and effective inhibition of total cholesterol synthesis. Two drugs can alleviate high blood viscosity, high aggregation and hypercoagulable state at some degree.

The main cause of cerebral infarction is atherosclerosis, and the inflammatory response promotes the formation of atherosclerosis. The vascular endothelial dysfunction can activate inflammatory chemokines, and the aggravation of inflammation can easily lead to plaque into vulnerable plaque, its rupture can activate platelet coagulation, fibrinolytic system activity and platelet function, the level of inflammatory cytokines, and the aggravation of inflammation can easily lead to plaque into vulnerable plaque, its rupture can activate platelet coagulation, fibrinolytic system activity and platelet function. hs-CRP is an acute phase reaction protein, and closely related to coagulation, fibrinolytic system activity and platelet function, the high level will damage vascular endothelial cells, mediate monocyte-macrophage to adhere to vascular endothelial cells, enter the arterial wall, and promote plaque rupture and thrombosis formation[19]. TNF-α is mainly produced by brain tissue neurons, stellate cells and oligodendrocytes, and is closely related to cerebrovascular disease. TNF-α can promote the inflammatory cells aggregation and infiltration, cause inflammatory and immune responses and induce the toxic effect of sensitive cells, easily leading to cytotoxic brain edema[20]. MMP-9 usually plays a role in the process of extracellular matrix degradation, which is an ion-dependent protease and promote plaque instability and plaque rupture[21]. In this study, after treatment, the levels of hs-CRP, TNF-α and MMP-9 in the two groups significantly decreased (P<0.05), and the levels of hs-CRP, TNF-α and MMP-9 in the combination group were significantly lower than those in the clopidogrel group (P<0.05). It is indicated that clopidogrel and atorvastatin can effectively control the inflammatory response, and the combination of the two drugs has more significant effect. It may be due to that clopidogrel, as an antagonist of ADP receptors, inhibits the induction of macrophages by the release of active factors from platelets and thus reduces the level of inflammatory cytokines, whereas atorvastatin has an effect of reducing the inflammatory response at atherosclerotic plaques. Both pharmacological effects are similar and have strong anti-inflammatory activity, which can reduce the accumulation of inflammatory cells and thus reduce the inflammatory response. Combined application for the treatment can play a synergistic effect and further enhance the anti-inflammatory effect.

Coagulation index APTT, as an endogenous coagulation system test indicator, can reflect the lack of prothrombin and Fib; PT can reflect the abnormalities of quality and quantity of coagulation system factors as exogenous coagulation system test indicators; Fib is coagulation enzyme substrate which reflects thrombus formation and coagulation status. DD has a high sensitivity and specificity for thrombotic diseases. Its increased levels suggested that blood fibrinolysis is activated and the coagulation system is hypercoagulable[22]. The results of this study showed that, after treatment, APTT and PT levels did not change significantly (P>0.05), while Fib and DD levels significantly decreased than those before treatment (P<0.05). After treatment, the levels of APTT and PT in the combination group were higher than those in the clopidogrel group, but the difference was not significant (P>0.05). The levels of Fib and DD in the combined group were significantly lower than those in the clopidogrel group (P<0.05). It suggested that coagulation and fibrinolysis system function improved significantly, and the combination group was better than clopidogrel group. It may be because clopidogrel can antagonize the platelet membrane ADP receptor, inhibit thromboxane formation and platelet aggregation, which can effectively reduce Fib and DD levels, prevent thrombosis, as well as regulate the balance of coagulation system state. At the same time, combination with atorvastatin in patients can more effectively improve coagulation, fibrinolysis system, probably due to atorvastatin can inhibit extreme low density protein cholesterol synthesis and reduce plasma fibrinogen content, which plays a role in reducing plasma viscosity and regulating blood lipid balance, thus helping to improve the patient’s coagulation status.

In summary, clopidogrel combined with atorvastatin treatment of cerebral infarction can improve the patient’s blood rheology, significantly reduce the body’s inflammatory response and promote coagulation in patients. Thus it is worthy of clinical promotion.

References


