Brief Report

Buchang Naoxintong Restrains Oxidative Stress in Rats with Focal Cerebral Ischemia-Reperfusion

Zhiyou Cai\textsuperscript{a,b,*}, Yonglong Wang\textsuperscript{b}

Abstract: Buchang Naoxintong (BT), a traditional Chinese herbal drug, is an effective neuroprotective agent that has been used in clinics. However, its pharmacological mechanisms have not been clearly elucidated. The purpose of this study was to determine whether upregulation of superoxide dismutase (SOD) and attenuation of lipid peroxidation, as measured by levels of malondialdehyde (MDA), are involved in BT’s neuroprotection in a rat model of focal cerebral ischemia reperfusion. Wistar rats were used and the focal cerebral artery occlusion was created by inserting an intraluminal filament into the left middle cerebral artery. Rats were randomly divided into sham-operation group (Sham), focal ischemia/reperfusion group (Ischemia), and BT treatment group (BT). BT was administered after ischemia but at the onset of reperfusion. Following 30 min ischemia and 12 hr or 24 hr reperfusion, infarct volume was determined by morphometric analysis after histochemical staining and levels of serum and brain SOD and MDA were measured by colorimetric assays. Results show that focal infarct volume after cerebral ischemia/reperfusion injury decreased in the BT group. Levels of SOD in the BT group were significantly higher than that in the Ischemia group. Moreover, levels of MDA in the BT groups were significantly lower than that in the Ischemia group. Additionally, both levels of SOD and MDA did not show difference between 12 hr and 24 hr reperfusion. It is concluded that BT plays a neuroprotective role in cerebral ischemia/reperfusion injury, at least, by increasing SOD levels and decreasing MDA levels in both serum and the brain, and thereby attenuating oxidative damage and hence ischemic injury.

Keywords: buchang naoxintong, cerebral ischemia reperfusion, malondialdehyde, superoxide dismutase

1. Introduction

Cerebral ischemic injury involves the action and interaction of many factors such as excitatory amino acids, calcium overloading, oxidative damage by reactive oxygen species (ROS), periphery depolarization of infarction, neuroinflammation, and apoptosis [1-3]. ROS are toxic byproducts produced by living organism and can induce lipid peroxidation. Malondialdehyde (MDA), a product of lipid peroxidation, is one of the major parameters used for evaluation of oxidative damage and reflects directly the extent of ROS-induced damage [4]. On the other hand, superoxide dismutase (SOD), an enzyme that catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen, can enhance clearance of ROS. Therefore, SOD plays an important role in neuroprotection against cerebral ischemic injury [5, 6]. As such, levels of SOD may be pharmacologically modulated during oxidative stress.

Buchang Naoxintong (BT), a traditional Chinese herbal drug, is an effective neuroprotective agent. Although it has been widely used clinically, its pharmacological mechanisms remain unclear. In the present study, using a rat model of cerebral ischemic injury, we measured serum levels of SOD and MDA following focal cerebral ischemia/reperfusion injury determined by infarct volume. BT was administered at the onset of reperfusion following 30 min occlusion of the middle cerebral artery. Our results show that BT could decrease the focal infarct volume, upregulate SOD activity, and attenuate MDA content after ischemic stroke, suggesting that BT enhances ROS removal during the ischemia/reperfusion process.

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2. Materials and Methods

2.1. Animal and groups

Wistar rats (female, 10 weeks old, 200-250 mg) were purchased from the Field Zoology Research Institute of the Third Military Medical University and were randomly divided into sham-operation group (Sham), ischemia/reperfusion group (Ischemia), BT treatment group (by douche via stomach). BT was offered by Buchang Medicine Limited Company (0.4 g/capsule). Each group had six animals. All animal experiments were performed according to international ethical standards and approved by the research ethics committee of Chongqing Medical University, Chongqing, China.

2.2. Surgery and drug administration

BT was diluted to 0.5 mg/ml by physiological saline and was administered through douche via stomach. Sham-operation group received no ischemia and received the same volume of saline. Focal cerebral ischemia was performed using an intraluminal filament inserted into the left middle cerebral artery as previously described [7, 8]. Ischemia-reperfusion group without BT treatment also received the same volume of saline through douche via stomach. BT treatment group was given BT through douche via stomach at a dosage of 0.45g/kg/d as previously described [9]. For all studies, BT or saline was administered at the onset of reperfusion after 30 min ischemia.

2.3. Morphometric Analysis of Infarct Volume

Infarct areas were measured by 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) staining and were calculated by an “indirect” morphometric analysis. This method of measuring infarct volumes correlated with a conventional histological method of hematoxylin and eosin stain. The infarct volume was calculated by summing the infarct areas measured in the component brain slices.

2.4. Measurement of brain and serum MDA and SOD levels

Following 30 min ischemia and either 12 hr or 24 hr reperfusion, rats were sacrificed. Blood was collected for serum preparation and brain was rapidly removed for homogenate preparation. Kits for quantitating MDA and SOD were purchased from Nanjing Jiancheng Biology and Technology Company. Basically, MDA content (nmol/mL) in serum and brain homogenate was measured by the method of sulfo-barbitone acid; SOD activity in serum and brain homogenate (U/mL) was measured by the xanthine oxidase method. All the experiment procedures were carried out according to the instruction’s manual. Spectrophotometer used was from FACS caliber (model 721, Becton Dickinson, USA).

2.5. Statistical analysis

All statistical data were analyzed with the SPSS software (11.0) for Windows (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (X ±s) and Student’s t test for inter-group variations was performed. P<0.05 was considered statistically significant.

3. Results

3.1. BT decreased focal infarct volume

Results in Table 1 show that focal infarct volume in the ischemia group was significantly higher than that in both the sham group and the BT group (P<0.01). Importantly, while the infarct volume in the BT group was significantly higher than that in the sham groups P<0.01, it was significantly lower than that in the ischemia group (P<0.01). Additionally, there was no difference in the infarct volume between 12 hr and 24 hr reperfusion. These results demonstrate that BT could decrease the focal infarct volume caused by cerebral ischemia/reperfusion injury.

Table 1: Focal infarct volume for all groups (mm³, mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>12h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>103.6±31.4</td>
<td>101.2±33.2</td>
</tr>
<tr>
<td>BT</td>
<td>126.3±35.6</td>
<td>122.8±47.6</td>
</tr>
<tr>
<td>Ischemia</td>
<td>156.5±40.4</td>
<td>152.1±34.6</td>
</tr>
</tbody>
</table>

Note: *P<0.01, compared with sham; **P<0.01, compared with ischemia.

3.2. BT increased SOD activities

Results in Table 2 indicate that BT could decrease the focal infarct volume induced by cerebral ischemia/reperfusion injury. As ROS are involved in brain injury after ischemic stroke, less injury may indicate a higher level of SOD activity as SOD can remove ROS. To test this possibility, we used the xanthine oxidase method to measure SOD activities in both serum and the brain homogenate. Indeed as predicted, SOD activity in the ischemia group was significantly higher than that in the sham group (P<0.01). Moreover, SOD activity in the BT group was significantly higher than that in both the sham group (P<0.01) and the ischemia group (P<0.01) (Table 2).

Table 2: Serum and brain SOD activities for all groups (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (U/mL)</th>
<th>Brain tissue(U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12h</td>
<td>24h</td>
</tr>
<tr>
<td>Sham</td>
<td>77.5±4.8</td>
<td>79.6±5.8</td>
</tr>
<tr>
<td>BT</td>
<td>137.6±6.6b</td>
<td>136.7±4.5b</td>
</tr>
<tr>
<td>Ischemia</td>
<td>110.9±4.8b</td>
<td>118.1±3.6b</td>
</tr>
</tbody>
</table>

Note: *P<0.01, compared with sham; **P<0.01, compared with ischemia.

Additionally, there was no significant difference in SOD activities between 12 hr and 24 hr reperfusion. These results...
demonstrate that BT could increase SOD activities in both serum and the brain during cerebral ischemic injury.

3.3. BT decreased MDA levels

Our results that SOD activity was higher in the BT group suggest that levels of ROS were low and there should be less lipid peroxidation that took place in the BT treated group. To test this possibility, we measured MDA levels also in serum and the brain homogenate. Results in Table 3 indicate that MDA levels in the ischemia group were significantly higher than that in the sham group (P<0.01). Moreover, while MDA levels in the BT group were significantly higher than that in the sham group (P<0.01), they were significantly lower than that in the ischemia group (P<0.01) (Table 3). Similar to that of SOD activities, there was also no significant difference in MDA levels between 12 hr and 24 hr reperfusion. These results show that BT could decrease the MDA levels during cerebral ischemia/reperfusion injury.

Table 3: Serum and brain MDA levels for all groups (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (U/mL)</th>
<th>Brain tissue (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12h</td>
<td>24h</td>
</tr>
<tr>
<td>Sham</td>
<td>1.7±0.40</td>
<td>2.0±0.32</td>
</tr>
<tr>
<td>BT</td>
<td>5.67±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.87±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ischemia</td>
<td>11.72±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.23±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: <sup>a</sup>P<0.01, compared with sham; <sup>b</sup>P<0.01, compared with ischemia.

4. Discussion

In the present study, we have demonstrated that BT is neuroprotective in ischemic stroke. Our results show that when administered at the onset of reperfusion, BT could upregulate both cellular and extracellular SOD activities (serum and brain, Table 2). Furthermore, this upregulation was in well agreement with lowered levels of MDA (Table 3) and a smaller infarct volume in the BT treated group (Table 1), suggesting that ROS levels are low after BT administration. Our results thus also agree with previous findings that ROS are involved in the pathogenesis of cerebral ischemic injury [10, 11] and that ROS generation is a common pathophysiological mechanism underlying brain ischemic damage [12-15]. It is likely that modulation of this ROS production process via upregulation of SOD by BT may be a central pathway for BT’s neurovascular protective properties. Indeed, our results suggest that BT plays a neuroprotective role in cerebral ischemia/reperfusion injury by enhancing the clearance of ROS.

In summary, BT can abrogate focal infarct volume by increasing SOD activities and decreasing MDA levels in both serum and the brain. Therefore, BT plays a neuroprotective role during cerebral ischemia/reperfusion injury by enhancing clearance of ROS. However, the very mechanism by which SOD is upregulated by BT needs to be further investigated.

Conflict of interest: None declared.

References: