Protective effect of matrine against cerebral ischemia reperfusion injury in rats

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ABSTRACT

Objective: To investigate the effects of matrine on expression of SOD and NO in rats with global cerebral ischemia reperfusion injury and provide the evidences for further study. Methods. The models were made by transient bilateral occlusion of the common carotid artery (CCA) and hypotension lasting for 10 min according to the method of Smith. Forty male Sprague-Dawley (SD) rats weighing 260-300g were randomly divided into the sham-operation group, the model group, the matrine group (20, 40, 60 mg/kg). Matrine was immediately administered in matrine group by intraperitoneal injection after ischemia-reperfusion. Saline water was administered in the other two groups by the same procedures. 24 hours later, the rats were sacrificed and brain tissues were isolated for the test of SOD, NO factors by color matching method. Results: The levels of SOD, NO in model group were higher than those in sham-operation group (P <0.05). But compared with model group, the levels of SOD were increased and NO in matrine group were decreased (P<0.05). Conclusion: Matrine can defend the brain ischemia reperfusion injury by alleviating the oxidative stress reaction. © 2013 Trade Science Inc. - INDIA

INTRODUCTION

Cerebrovascular disease is harmful to human life and health; it is a common diseases in which ischemia-reperfusion induced brain injury play an important role[1]. Ischemia-reperfusion resulted in substantial release of oxygen free radicals and macrophage activation induced secretion of tumor necrosis factor, which in turn mediated inflammatory response, is one of the mechanisms causing injury[2]. Matrine had the protective effect on multiple organ damage through anti-oxidation and reducing inflammation[3-5]. This study was designed to observe the matrine on focal cerebral ischemia-reperfusion injury, and explore matrine on cerebral ischemia-reperfusion injury in rats and its mechanism.

MATERIALS AND METHODS

1) Experimental animals SD rats 40, male, weighing 260-300g, supported by the Experimental Animal Center of Wuhan University.
2) Drugs and reagents: matrine (Ang Sheng Shaanxi
Biomedical Technology Co., content 98%), the experiment in order to prepare the required concentration with saline.

3) Animal grouping and administration: Rats were randomly divided into five groups, each eight were sham group, cerebral ischemia-reperfusion model group, and matrine 20,40,60 mg / kg dose group. Sham group, cerebral ischemia and reperfusion model group were given ip saline gavage dose matrine group according to the above 1 / d administered for 7 days after the last administration was prepared lh middle cerebral artery occlusion in rats transient focal ischemia model.

4) Cerebral ischemia / reperfusion model was prepared according to the literature[6]. Using the improved method of Longa. Rats with 3% chloral hydrate (300 mg • kg-1, ip) anesthesia on the operating table back recumbent stationary, blunt dissection of the neck muscle tissue, blunt dissection along the medial edge of the sternocleidomastoid muscle on the right common carotid artery (CCA) and the accompanying vagus nerve, and then separating the right external carotid artery (ECA), internal carotid artery (ICA) and the occipital artery, occipital artery electric condenser will burn off. Along the internal carotid artery was isolated wing Hubei (PPA). From the external carotid artery bifurcation of the common carotid artery ligature 0.8cm, combined with carotid artery clip to clamp the internal carotid artery, the external carotid artery from the common carotid artery bifurcation O.4cm place with ophthalmic scissors cut a “V” small mouth, nylon fishing line into the small mouth, and the pre-release of the external carotid artery root of the suture tension, loosen the sum of the internal carotid artery carotid artery clip, fishing line through the carotid artery bifurcation into the internal carotid artery taking care to avoid the fishing line into the PPA, will feel a slight resistance nylon fishing line slightly pulled one o’clock and fixed, from internal and external carotid artery bifurcation counting so far about 1.85cm. Plug After 1.5 h, gently pull out the nylon fishing line back to the head end of the ECA office can restore the artery recanalization, brain tissue to achieve reperfusion. Sham-operated rats were anesthetized and blood vessels only dissection.

5) Neurological scores and infarct size determination: after reperfusion 24h, the brains were removed before using the neurological deficit score five rats were neurological score. Rating criteria are as follows: 0, no neurological deficit symptoms; 1 minute, can not be extended contralateral forepaw; 2 minutes, walking around in the hemiplegic side; 3 minutes, walking to the hemiplegic side dumping; 4 points, not spontaneously walk, awareness lost. Determination of infarct area after 24 h reperfusion taken anesthetized rats, the brains were removed quickly, do coronal slices of brain tissue, quickly placed TTC staining solution, placed in 37°C constant temperature bath box stained 5 min, fixed with 10% formaldehyde, radiography, were measured by image analysis software brain slices infarct size and the total area of infarct size calculated the percentage of the total area of the brain.

6) Colorimetric assay SOD, NO: kit was purchased from Nanjing Jiancheng Institute of Biology, in strict accordance with instructions.

7) The data were expressed as the mean ± SEM and analyzed using SPSS 12.0 statistical software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Matrine on neurological scores and infarct size effects

cerebral ischemia and reperfusion model and matrine Rats administered anesthesia conscious there are varying degrees of mental state malaise, contralateral forelimb can not be extended and walk to the contralateral rotation, or dumping and other symptoms, but compared with the model group, each group matrine neurological behavior scores were significantly lower, with statistical significance (P <0.05); compared with model group, matrine group can be significantly reduced cerebral ischemia and reperfusion in rats with cerebral infarction area (P <0.05), and a dose-dependent manner (TABLE 1).

Brain tissue SOD, NO detection

comparison with the sham group, model group SOD, NO levels were significantly increased expres-
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F. Full Paper

Recent studies have found that the recovery of blood flow after ischemia in some cases can lead to further tissue damage and dysfunction that is caused by ischemia-reperfusion injury[7]. Therefore, inhibition of cerebral ischemia-reperfusion injury has become the treatment of ischemic stroke in the key. In this study, rats with focal cerebral ischemia-reperfusion model confirmed that matrine can reduce cerebral ischemia-reperfusion-induced neurological behavior change; reduction of infarct size on cerebral ischemia-reperfusion induced brain damage has a protective effect.

Ischemia-reperfusion, due to mitochondrial leakage, respiratory burst of phagocytic cells, xanthine oxidase activation, resulting in a large number of free radicals, causing oxidative damage. Common forms of free radicals: superoxide anion (•O₂⁻), hydroxyl radical (OH ⋅), hydrogen peroxide (H₂O₂), singlet molecular oxygen (¹O₂), lipid peroxides (ROO ⋅) and so on. Studies have shown that in the ischemia-reperfusion within 2 ~ 3 h (not yet appeared neuronal cell death), you can produce a large number of reactive oxygen species. The superoxide dismutase (SOD) under the action of superoxide anion (•O₂⁻) and hydrogen ions (H⁺) produced by the reaction H₂O₂ and O₂, is the body of the SOD enzyme clear •O₂⁻, which the level of activity reflects the body’s ability to scavenge free radicals. SOD contains three subtypes of CuZn-SOD, Mn-SOD and EC-SOD, respectively, mainly in the cytoplasm, mitochondria play an antioxidant and cell membrane[8]. The study group was observed after matrine treatment, oxygen free radicals in rat brain SOD increased significantly, probably in the protective effect of brain damage plays a role.

In recent years, NO on cerebral ischemia-reperfusion injury and effect change aroused widespread concern. NO in the central nervous system has a dual role, on the one hand with a neurotransmitter role in transmitting information and vasodilatory effects, on the other hand have neurotoxic effects[9]. NO in vivo from L-arginine and molecular oxygen in the role of NOS synthesis. Low concentrations of NO can vasodilation, inhibition of platelet aggregation and adhesion, regulation of glutamate ion channel down to prevent intracellular calcium overload, which has a protective effect on cells, but at high concentrations, NO due to lack of substrate L - arginine, NO and O₂ reaction to form primarily a strong oxidizing peroxide nitrosyl ion (ONOO⁻), the mitochondria Mn-SOD inactivation mitochondria not eliminate O₂, cascade induced damage. Studies have reported that cerebral ischemia, massive release of excitatory amino acids, excessive excitement NMDA receptor, causing the receptor-gated Ca²⁺ channels lot of openness, increased intracellular Ca²⁺, Ca²⁺ and calcium binding proteins can activate neurons NOS, so NO synthesis, NO through with some iron enzymes form complexes, nitrosation DNA and generate peroxy nitrite anion and other ways to play its neurotoxicity[10]. This study observed in rat forebrain lack 20min reperfusion can cause brain tissue NO significantly increased, which may occur subsequent brain damage of important reasons. And matrine can reduce ischemia-reperfusion brain tissue NO levels, thereby reducing its neurotoxic effects, which may be anti-cerebral ischemic injury matrine important mechanism.

### TABLE 1: Matrine on cerebral ischemia reperfusion neurological scores and infarct area (X ± s, n=8)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Neurological scores</th>
<th>Infarct area /%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>-</td>
<td>2.9±0.4</td>
<td>0.53±0.14</td>
</tr>
<tr>
<td>Matrine group</td>
<td>20</td>
<td>2.5±0.6</td>
<td>0.61±0.11</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.4±0.5</td>
<td>0.48±0.19</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.3±0.4*</td>
<td>0.34±0.12*</td>
</tr>
</tbody>
</table>

vs Model group: *P<0.05

### TABLE 2: SOD and NO level of the brain tissue (X ± s, n=8)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>SOD (U/mgprot)</th>
<th>NO (µmol/mgprot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham group</td>
<td>-</td>
<td>0.138±0.026</td>
<td>0.953±0.236</td>
</tr>
<tr>
<td>Model group</td>
<td>-</td>
<td>0.627±0.134*</td>
<td>4.293±1.152</td>
</tr>
<tr>
<td>Matrine group</td>
<td>20</td>
<td>0.813±0.125</td>
<td>3.386±1.013</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.135±0.113</td>
<td>3.024±0.974</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.358±0.226★</td>
<td>2.564±0.745★</td>
</tr>
</tbody>
</table>

vs Sham group: *P<0.05; vs Model group: ★P<0.05

### DISCUSS

Recent studies have found that the recovery of blood flow after ischemia in some cases can lead to further tissue damage and dysfunction that is caused by ischemia-reperfusion injury[7]. Therefore, inhibition of cerebral ischemia-reperfusion injury has become the treatment of ischemic stroke in the key. In this study, rats with focal cerebral ischemia-reperfusion model confirmed that matrine can reduce cerebral ischemia-reperfusion-induced neurological behavior change; reduction of infarct size on cerebral ischemia-reperfusion induced brain damage has a protective effect.
In conclusion, matrine had a obvious protective effect on cerebral ischemia-reperfusion induced neuronal damage. Matrine reduced tissue damage caused by free radicals by reducing ischemia-reperfusion brain tissue NO levels and increased SOD content, which may be the important mechanism of this drug against cerebral ischemia-reperfusion injury.

REFERENCES


