Antagonistic effects of shikimic acid against focal cerebral ischemia injury in rats subjected to middle cerebral artery thrombosis

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ABSTRACT

AIM: To study the effects of shikimic acid (SA) on focal cerebral ischemia injury after middle cerebral artery thrombosis (MCAT). METHODS: Thrombosis was induced by FeCl₃ in middle cerebral artery of rats. The influences of SA on neurologic deficit (ND), infarct size (IS), brain edema, and cerebral blood flow (CBF) in ischemic region were observed. RESULTS: SA 25 and 50 mg·kg⁻¹ ip for 3 d before MCAT ameliorated ND, and reduced IS by 51 % and 61 %, and decreased brain water content from 80.7 ± 0.2 % to 79.5 ± 0.2 % and 79.9 ± 0.2 %; and increased CBF after infusion from 50.2 % of the preischemic level to 73.5 % and 73.3 %, respectively. In pathologic examination, there was less infarcts in MCA territory with the pretreatment by SA 25 mg·kg⁻¹. The volume of brain ischemia was much less than that of control. CONCLUSIONS: SA reduced focal cerebral ischemia injury induced by middle cerebral artery thrombosis.

INTRODUCTION

Shikimic acid (SA), isolated from Fructus Amomi seeds and had analgesic effect(1). In previous studies, we found SA inhibited arterial thrombosis and venous thrombosis in rats. In this paper, the protective effects of SA against focal cerebral ischemic injury were studied.

MATERIALS AND METHODS

Rats Wagyu rats (n = 100), weighing 220–270 g, which were Grade II, and Certificate No. 013006, were obtained from Experimental Animal Center, Chinese Academy of Medical Sciences. Drugs and reagents SA (purity > 98 %), extracted by Department of Phytochemistry, Beijing University of Traditional Chinese Medicine, was dissolved in normal saline (NS) and its pH value was adjusted to 7.0 with NaOH. Lignostazine (1Lg) was bought from Beijing Institute of Pharmaceutical Industries. 2,3,5-Triphenyltetrazolium chloride (TTC) was from Beijing chemical Plant.

Neurologic model Rats were randomly divided into 6 groups: sham-operation, control, SA 12, 25, 50 mg·kg⁻¹, and lignostazine 25 mg·kg⁻¹ pretreatment groups. Anesthesia was induced with 12 % chloral hydrate 350 mg·kg⁻¹, ip. The right MCA (from the ophthalmic tract to the inferior cerebral vein) was exposed transcranially under a dissecting microscope. A small piece of quantitative filter paper with 50 % FeCl₃, 10 μl solution (dissolved in H2O 1 mol·L⁻¹) was
applied to the surface of the MCA, while the tissue 
around the MCA was covered by a piece of plastic film 
to avoid injury. About 30 min later the paper with 
FeCl₃ was taken out, and the wound was washed with 
NS. The skin incision was sutured. The room 
temperature was kept at 23 – 25 °C. Sham operation 
was performed without FeCl₃. Drugs were injected ip for 
3 d before MCAT. The same volume of NS was 
given to control and sham-operation groups.

Neurologic deficits (ND) At 6 and 24 h after 
surgery, the neurologic status of each rat was 
evaluated with blinded method. A scoring scale of 0 – 11 was 
used. Rats were observed for left forelimb flexion, 
shoulder adduction, and internal rotation when hung 
downwards by the tail. scored 1 – 4. By the degree of 
decreased resistance to lateral push toward the parietal 
side and decreased muscular tension of left forelimb, 
rats were scored 1 – 3, respectively. Rats that circled 
toward the parietal side consistently were scored 7.

Infarct size (IS) At 24 h after surgery, rats were 
decapitated and the brains were coronally sectioned 
into 5-mm sections which were immersed in 2 % TTC 
at 37 °C for 30 min. The stainless necrotic tissue 
was pooled and weighed as well as the nonnecrotic 
tissue. IS was expressed in the percentage of the 
necrotic tissue occupying in the entire cerebrum.

Brain water content At 24 h after surgery, 
rats were decapitated. The left (nonlesioned) and 
right (lesioned) cortices were weighted and dried to the 
constant weight at 105 °C for 24 h [10]. The water 
content = (wet weight – dry weight)/(wet weight) × 100 %.

Histopathology At 24 h after surgery, rats were 
decapitated. The brains were immersion in 10 % 
phosphate buffered formalin for at least 7 d. The 
coronal sections around optic chiasas was cut to be 
dehydrated, embedded in paraffin, sliced, and stained 
with hematoxylin and eosin (H&E). The histologic 
sections were reviewed by a neuropathologist who had 
knowledge of the experiment group to which the rats 
belonged under a light microscope.

Cerebral blood flow (CBF) By the method 
of hydrogen clearance [5], a small hole was drilled in 
the right skull 0.7 mm anterior and 4.0 mm lateral to 
the bregma [12]. A Teflon-coated platinum electrode 
(diameter 0.2 mm) was stereotaxically implanted in the 
right parietal cortex to a depth of 1.4 mm. A

Statistical analysis Unpaired t test was used 
to determine differences between groups.

RESULTS
ND All the rats with MCAT exhibited ND with 
the control rats suffered more at 6 and 24 h after 
MCAT. SA 25 and 50 mg·kg⁻¹ and ligustrazine 
improved the movement function of the rats with 
MCAT. Sham-operation rats did not exhibit observa-
tions (Tab 1).

IS At 24 h after surgery, all the rats developed 
obvious infarction in the right hemisphere except 
sham operation. SA 25 and 50 mg·kg⁻¹ and ligustrazine 
reduced IS by 51 %, 42 %, and 49 %, respectively 
(Tab 1). A close correlation was found between 
20 scores and IS (r = 0.989, P < 0.01).

Water content The water content of the right 
cortices of the rats with MCAT increased markedly 
compared to the left cortices. Pretreatment of SA 25 
and 50 mg·kg⁻¹ and ligustrazine alleviated the 
increased brain water content. There was no bias 
existed in the sham-operation rats. (Tab 1)

Histopathology In the right cerebral hemi-
sphere of control rats, the right MCA territory appears 
pale and the injured MCA appeared black. Under a 
light microscope, the vessel size showed that the MCA 
was blocked with mixed thrombus made up of platelet 
erthrocytes, fibrin, and leukocytes. But there was 
markedly less thrombosis formation in the MCA of rats 
pretreatment with SA 25 mg·kg⁻¹, though it 
endarteritis was injured by FeCl₃ (Fig 1).

Brain slice, degenerative changes, or 
necrosis (manifested as sparse neurones, vacuolar 
shrinkage, pyknosis of the nuclei, karyorhexis, or 
karyolysis) were seen in the right forebrain cortex 
control rats. SA 25 mg·kg⁻¹ reduced the brain 
damage (Fig 1).

CBF The CBF of the rats with MCAT decreased 
obviously 10 min after MCAT, while the control a
Effects of SA, Lig, and saline on neurologic deficits, infarct size, brain water content, and cCBF in rats subjected to middle cerebral artery thrombosis. 

- The CBF before MCAT was taken as the baseline.
- Data are presented as mean ± SD.
- Statistical significance was evaluated by one-way ANOVA followed by Duncan's post hoc test.

<table>
<thead>
<tr>
<th>mg·kg⁻¹</th>
<th>Neurologic deficit score</th>
<th>Infarct size/</th>
<th>Water content/</th>
<th>cCBF/ % after MCAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6.0 ± 1.9</td>
<td>5.8 ± 1.9</td>
<td>9 ± 3</td>
<td>78.67 ± 0.26</td>
</tr>
<tr>
<td>12</td>
<td>5.1 ± 1.7</td>
<td>4.8 ± 0.9</td>
<td>6 ± 2</td>
<td>73.7 ± 0.33</td>
</tr>
<tr>
<td>25</td>
<td>3.5 ± 1.5</td>
<td>4.4 ± 1.3</td>
<td>4.1 ± 2.1</td>
<td>73.30 ± 0.25</td>
</tr>
<tr>
<td>50</td>
<td>4.1 ± 1.4</td>
<td>3.9 ± 1.0</td>
<td>4.8 ± 2.4</td>
<td>73.75 ± 0.20</td>
</tr>
<tr>
<td>75</td>
<td>1.0 ± 1.0</td>
<td>3.6 ± 1.0</td>
<td>4.2 ± 2.2</td>
<td>73.80 ± 0.35</td>
</tr>
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</table>

DISCUSSION

MCAT is a newly developed focal cerebral ischemia model with good reproducibility. The
characteristics of cerebral infarction and neurologic deficits produced in the MCAT model were similar to those in the middle cerebral artery occlusion model built by Tanaka[2]. The difference was that the MCA was kept in MCAT model, and the change of the MCA after treatment with drug could be observed, which was more close to the clinical course. The cerebral infarctions with uniform size and location were also produced in the model of MCAT made here, which was consistent with the literature[5]. In order to observe neurological status of rats carefully, ND was scored in a 0–11 scale with the method of LIU Xing-Guang et al[15], which was modified based on Bredesen's[14]. The close correlation between ND scores and IS proved the rationality of this scoring system.

FeCl3 damaged the endothelia of MCA vessel which led to platelet aggregation, thrombus formation and eventually to the occlusion of the blood vessel. Thrombosis formation to block the MCA and vasococontractive substance such as platelet activating factor (PAF) released from platelet aggregation may cause CBF decreasing[10]. The energy metabolic depression and PAF also may play a major role in brain edema. Neuronal necrosis after MCAT may be secondary to the cerebral ischemia caused by thrombosis. In the present study, pretreatment with SA effectively reduced the IS, ND, and brain edema, and attenuated the reduction in CBF, which indicated that SA protected cerebral function in the rats with MCAT and this protective effect was related to SA attenuating the reduction in CBF. Considering together with the result of histopathology examination that there was just a very slight thrombosis formation in the MCA of the rats pretreated with SA, it is suggested that the key action may be that SA inhibited the thrombosis formation and platelet aggregation.

REFERENCES

参考文献