

## Antagonistic effects of shikimic acid against focal cerebral ischemia injury in rats subjected to middle cerebral artery thrombosis<sup>1</sup>

MA Yi<sup>2</sup>, XU Qiu-Ping<sup>3</sup>, SUN Jian-Ning, BAI Li-Min, GUO Ya-Jian, NIU Jian-Zhao

Department of Pharmacology, Beijing University of Traditional Chinese Medicine, Beijing 100029, China)

**KEY WORDS** shikimic acid; cerebral arteries; thrombosis; cerebral ischemia; cerebral infarction; brain edema; ligustrazine

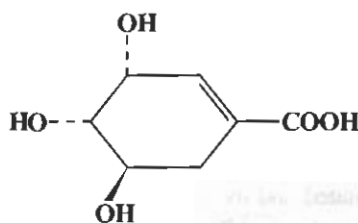
### ABSTRACT

**AIM:** To study the effects of shikimic acid (SA) on focal cerebral ischemic injury after middle cerebral artery thrombosis (MCAT). **METHODS:** Thrombosis was induced by FeCl<sub>3</sub> 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<sup>-1</sup> ip for 3 d before MCAT attenuated ND, and reduced IS by 51 % and 42 %; and decreased brain water content from 80.7 % to 79.8 % and 79.9 %; and increased CBF after ischemia from 50.2 % of the preischemic level to 75.5 % and 73.3 %, respectively. In pathologic examination, there was much less thrombosis in MCA in the rat with the pretreatment by SA 25 mg·kg<sup>-1</sup>. The extent of brain ischemia was much less than that of control. **CONCLUSIONS:** SA reduced focal cerebral ischemic injury induced by middle cerebral artery thrombosis.

### INTRODUCTION

Shikimic acid (SA), isolated from *Fructus Anisi stellati*, had analgesic effect<sup>[1]</sup>. 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.



Shikimic acid

### MATERIALS AND METHODS

**Rats** Wistar rats ( $n = 180$ , ♂, weighing 220 – 270 g, which were Grade II, and Certificate No 013008), 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. Ligustrazine (Lig) 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<sup>-1</sup>, and ligustrazine 25 mg·kg<sup>-1</sup> pretreatment groups. Anesthesia was induced with 12 % chloral hydrate 350 mg·kg<sup>-1</sup>, ip. The right MCA (from the olfactory tract to the inferior cerebral vein) was exposed transcranially under a dissecting microscope<sup>[2]</sup>. A small piece of quantitative filter paper with 50 % FeCl<sub>3</sub> 10 μL solution (dissolved in HCl 1 mol·L<sup>-1</sup>) was

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<sup>2</sup> Now in Department of Pharmacology, Capital University of Medical Science, Beijing 100054, China.

<sup>3</sup> Correspondence to Prof XU Qiu-Ping.

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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  $\text{FeCl}_3$  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  $\text{FeCl}_3$ . 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 blind method. A scoring scale of 0–11 was used<sup>(3)</sup>. 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 paretic side and decreased muscular tension of left forelimb, rats were scored 1–3, respectively. Rats that circled toward the paretic side consistently were scored 1.

**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<sup>(4)</sup>. 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 48 h<sup>(5)</sup>. The water content = (wet weight – dry weight)/(wet weight) × 100 %.

**Histopathology** At 24 h after surgery, rats were decapitated. The brains were immersed in 10 % phosphate buffered formalin for at least 7 d. The coronal sections around optic chiasm 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 no knowledge of the experiment group to which the rats belonged under a light microscope.

**Cerebral blood flow (CBF)** By the method of hydrogen clearance<sup>(6)</sup>, a small hole was drilled in the right skull 0.7 mm anterior and 4.0 mm lateral to the bregma<sup>(7)</sup>. 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

reference electrode of Ag/AgCl for CBF was placed on the nucha. CBF ( $\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ) in the cortex supplied by the right MCA of rats was measured before MCAT, and 10 min, 30 min, 120 min after MCAT. To exclude individual difference, relative CBF was recorded: (CBF after MCAT/CBF before MCAT) × 100 %.

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

## RESULTS

**ND** All the rats with MCAT exhibited ND while the control rats suffered more at 6 and 24 h after MCAT. SA 25 and 50  $\text{mg} \cdot \text{kg}^{-1}$  and ligustrazine improved the movement function of the rats with MCAT. Sham-operation rats did not exhibit abnormalities (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  $\text{mg} \cdot \text{kg}^{-1}$  and ligustrazine reduced IS by 51 %, 42 %, and 49 %, respectively (Tab 1). A close correlation was found between ND 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 as compared to the left cortices. Pretreatment of SA 25 and 50  $\text{mg} \cdot \text{kg}^{-1}$  and ligustrazine alleviated the increased brain water content. There was no brain edema in the sham-operation rats. (Tab 1)

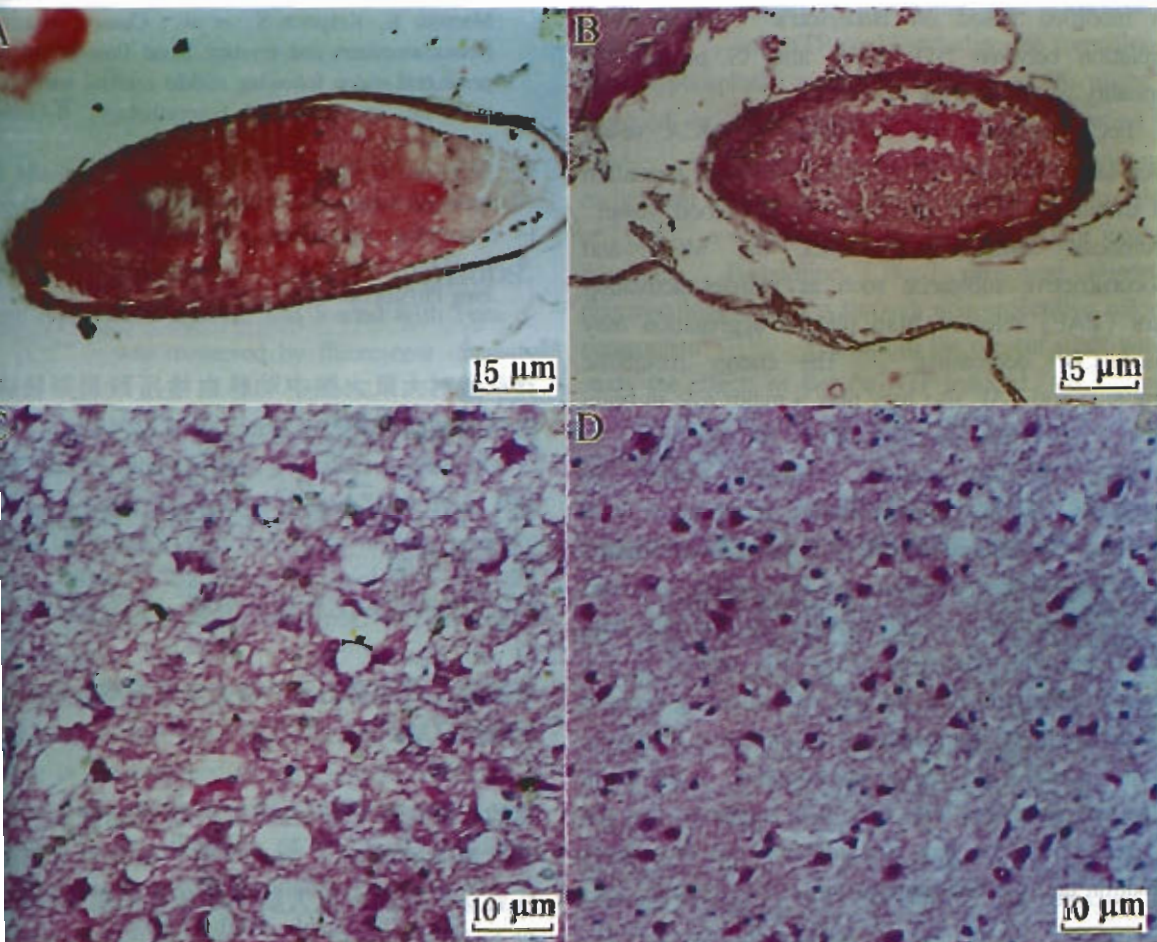
**Histopathology** In the right cerebral hemisphere of control rats, the right MCA territory appeared pale and the injured MCA appeared black. Under light microscope, the vessel slice showed that the MCA was blocked with mixed thrombus made up of platelets, erythrocytes, fibrin, and leukocytes. But there was much less thrombosis formation in the MCA of rats pretreatment with SA 25  $\text{mg} \cdot \text{kg}^{-1}$ , though the endangium was injured by  $\text{FeCl}_3$  (Fig 1).

From brain slice, degenerative changes, and necrosis (manifested as sparse neurons, vacuolation, shrinkage, pyknosis of the nuclei, karyorrhexis, and karyolysis) were seen in the right forebrain cortex of control rats. SA 25  $\text{mg} \cdot \text{kg}^{-1}$  reduced the brain damage (Fig 1).

**CBF** The CBF of the rats with MCAT declined obviously 10 min after MCAT, while the control rats

Effects of SA, Lig, and saline on neurologic deficits, infarct size, brain water content, and rCBF in rats subjected to middle cerebral artery thrombosis.  $n = 8 - 12$  rats.  $\bar{x} \pm s$ . The CBF before MCAT was taken as 100%. <sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs saline. <sup>P</sup> $P < 0.05$ , <sup>l</sup> $P < 0.01$  vs sham. <sup>l</sup> $P < 0.01$  vs left cortex.

mg · kg <sup>-1</sup> x 3 d	Neurologic deficit scores		Infarct size/ %	Water content/%		rCBF/% after MCAT		
	6 h	24 h		left cortex	right cortex	10 min	60 min	120 min
Sham				78.67 ± 0.28 <sup>a</sup>	78.62 ± 0.22 <sup>c</sup>	101 ± 9 <sup>c</sup>	102 ± 10 <sup>c</sup>	101 ± 11 <sup>c</sup>
Saline	6.0 ± 1.9	5.8 ± 1.9	8 ± 3	78.7 ± 0.3	80.7 ± 0.7 <sup>l</sup>	50 ± 11	52 ± 15	56 ± 16
SA 12	5.1 ± 1.2 <sup>a</sup>	4.8 ± 0.9 <sup>a</sup>	6.5 ± 2.0 <sup>a</sup>	78.75 ± 0.20 <sup>a</sup>	80.7 ± 0.5 <sup>l</sup>	60 ± 15 <sup>f</sup>	64 ± 14 <sup>f</sup>	65 ± 17 <sup>f</sup>
25	3.5 ± 1.5 <sup>c</sup>	3.4 ± 1.5 <sup>c</sup>	4.1 ± 2.1 <sup>c</sup>	78.80 ± 0.25 <sup>a</sup>	79.8 ± 0.6 <sup>ci</sup>	76 ± 21 <sup>cf</sup>	77 ± 15 <sup>cf</sup>	79 ± 12 <sup>cf</sup>
50	4.1 ± 1.2 <sup>b</sup>	3.9 ± 1.6 <sup>b</sup>	4.8 ± 2.4 <sup>c</sup>	78.76 ± 0.21 <sup>a</sup>	79.9 ± 0.6 <sup>ci</sup>	73 ± 18 <sup>cf</sup>	74 ± 13 <sup>cf</sup>	76 ± 10 <sup>cf</sup>
Lig 25	4.0 ± 1.6 <sup>b</sup>	3.6 ± 1.8 <sup>b</sup>	4.2 ± 2.1 <sup>c</sup>	78.89 ± 0.18 <sup>a</sup>	79.7 ± 0.5 <sup>ci</sup>	76 ± 22 <sup>cf</sup>	77 ± 14 <sup>cf</sup>	87 ± 18 <sup>ce</sup>



1. Effects of SA and saline on middle cerebral artery thrombosis and neuronal damage in forebrain cortex after chemical injury of rat MCA. (HE stain × 250). A - B) middle cerebral artery thrombosis; C - D) brain cortex; A, C) saline-treated; B, D) SA-treated (25 mg · kg<sup>-1</sup>, ip).

and more, almost to 50 % of the preischemic level. 5 and 50 mg · kg<sup>-1</sup> and ligustrazine attenuated the reduction in CBF. At 60 min and 120 min, CBF did not show a significant increase in each group (Tab 1).

## DISCUSSION

MCAT is a newly developed focal cerebral ischemia model with good reproduction. The

characteristics of cerebral infarction and neurologic deficits produced in the MCAT model<sup>[3]</sup> were similar to those in the middle cerebral artery occlusion model built by Tamura<sup>[2]</sup>. 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<sup>[3]</sup>. In order to observe neurological status of rats carefully, ND was scored in a 0-11 scale with the method of LIU Xiao-Guang *et al*<sup>[3]</sup>, which was modified based on Bederson's<sup>[8]</sup>. The close correlation between ND scores and IS proved the rationality of this scoring system.

FeCl<sub>3</sub> 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 vasoconstrictive substance such as platelet activating factor (PAF) released from platelet aggregation may caused CBF decreasing<sup>[9]</sup>. The energy metabolic depression and PAF also may play a major role in brain edema. Neuroal 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 little 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

- 1 Fang YZ, Song JY, Zeng WL, Xie DS. Study on analgesic effect of shikimic acid. *J Guizhou Coll Trad Chin Med* 1989; 1: 59-62.
- 2 Tamura A, Graham DI, McCulloch J, Teasdale GM. Focal cerebral ischemia in the rat. 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 1981; 1: 53-60.
- 3 Liu XG, Xu LN. A rat middle cerebral artery thrombosis

model for evaluation of thrombolytic and and antithrombotic agents. *Acta Pharm Sin* 1995; 30: 662-7.

- 4 Bederson JB, Pitts LH, Germano SM, Nishimura M, Davis RL, Bartkowski HM. Evaluation of 2, 3, 5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rat. *Stroke* 1986; 17: 1304-8.
- 5 Hallenbeck JM, Leitch DR, Dutka AJ, Greenbaum LJ. The amount of circumscribed brain edema and the degree of post-ischemic neuronal recovery do not correlate well. *Stroke* 1982; 13: 797-804.
- 6 Wang YL, He RR. Protective effect of dipfluzine on experimental brain edema in rats. *Acta Pharmacol Sin* 1994; 15: 201-5.
- 7 Takagi K, Ginsberg MD, Globus MYT, Dietrich WD, Martinez E, Kraydieh S, *et al*. Changes in amino acid Neurotransmitters and cerebral blood flow in the ischemic penumbra region following middle cerebral artery occlusion in the rat: correlation with histopathology. *J Cereb Blood Flow Metab* 1993; 13: 575-85.
- 8 Bederson JB, Pitts LH, Tsuji M. Rat middle cerebral artery occlusion; evaluation of the model and development of a neurologic examination. *Stroke* 1986; 17: 472-6.
- 9 Li SQ. Platelet activating factor and cerebral ischemia. *Prog Physiol Sci* 1991; 22: 165-8.

## 莽草酸对大鼠大脑中动脉血栓所致局部脑缺血损伤的拮抗作用<sup>1</sup>

马 怡<sup>2</sup>, 徐秋萍<sup>3</sup>, 孙建宁, 白丽敏, 郭建, 牛建昭

(北京中医药大学药理教研室, 北京 100029, 中国)

**关键词** 莽草酸; 脑动脉; 血栓形成; 脑缺血; 脑梗死; 脑水肿; 川芎嗪

**目的:** 研究莽草酸(SA)对大鼠中动脉血栓所致局部脑缺血的影响。 **方法:** 用三氯化铁局部涂伤血管形成的大鼠大脑中动脉血栓模型(MCAT)观察 SA 对行为障碍程度、脑梗塞范围、脑水肿和缺血区脑血流的影响。 **结果:** SA 25、50 mg·kg<sup>-1</sup> 于 MCAT 前连续给药 3 d 可分别减小脑梗塞率 51%、42%, 使脑含水量由 80.7% 降至 79.8%、79.9%, 并可改善行为障碍程度和缺血区脑血流的降低, 病理检查显示, SA 25 mg·kg<sup>-1</sup> 组大鼠 MCA 内血栓形成极少, 脑组织缺血病变较轻。 **论:** SA 可减轻大鼠中动脉血栓所致局部脑缺血损伤。 (责任编辑 李)