

核苷酸(ODN)对表皮生长因子(EGF)诱导的培养大鼠血管平滑肌细胞增生的抑制作用。方法:用脂质体将 p42-和 p44-MAPK ODN  $0.2 \mu\text{mol} \cdot \text{L}^{-1}$  转染入大鼠血管平滑肌细胞,设正义及随机 ODN 为对照,用 Western Blot 法结合 P-81 滤纸法以髓磷脂碱性蛋白为底物测定 MAPK 活性。 [ $^3\text{H}$ ]胸腺嘧啶核

苷酸掺入测定平滑肌细胞 DNA 合成。结果: MAPK ODN 能明显抑制 EGF 诱导的 MAPK 蛋白表达及 MAPK 活性,并明显抑制血管平滑肌细胞的 [ $^3\text{H}$ ]胸腺嘧啶核苷酸掺入。结论:针对 p42-和 p44-MAPK 起始部位设计的 17-mer ODN 能有效抑制 EGF 诱导的血管平滑肌细胞的增生。

## Effect of histidine on myocardial mitochondria and platelet aggregation during thrombotic cerebral ischemia in rats<sup>1</sup>

LI Shu-Qing<sup>2</sup>, ZHAO Gang, LI Jun, QIAN Wei<sup>3</sup> (Department of Pathophysiology; <sup>3</sup>Electron Microscope Laboratory, Kunming Medical College, Kunming 650031, China)

**KEY WORDS** photochemistry; thrombosis; cerebral ischemia; platelet aggregation; myocardium; heart mitochondria; histidine

**AIM:** To study the effect of histidine on cerebral thrombosis and possible mechanism.

**METHODS:** Cerebral-cardiac stroke was produced by photochemically induced thrombotic cerebral ischemia in rats. **RESULTS:** Platelet aggregation in whole blood increased markedly, peak heights at 4 and 24 h were  $(5.1 \pm 0.5) \Omega$  and  $(4.3 \pm 0.5) \Omega$ , respectively. Heart mitochondria volume ( $V$ ), volume density ( $V_v$ ), surface density ( $N_m$ ), and surface density of outer membrane ( $S_{v1}$ ) increased ( $8.2 \pm 5.5$ ,  $0.59 \pm 0.16$ ,  $0.11 \pm 0.03$ , and  $0.22 \pm 0.05$ , respectively,  $P < 0.01$ ), but numerical density ( $N_v$ ), specific surface of inner membrane ( $\delta_2$ ) and of the cristae ( $\delta_3$ ) decreased ( $0.07 \pm 0.02$ ,  $2.8 \pm 0.8$ , and  $2.4 \pm 0.7$ , respectively,  $P < 0.01$ ) after cerebral thrombosis. The myocardial histopathologic characteristics were different from those of ischemic necrosis and myocardial damage caused by ischemic reperfusion. In rat treated with histidine after photochemical reaction, platelet aggregation decreased markedly [ $(2.93 \pm 1.08)$

$\Omega$ ,  $P < 0.01$ ], reversible change often went with parameters related to the inner mitochondrial membrane but not the outer mitochondrial membrane. **CONCLUSION:** Histidine depressed platelet aggregation and reduced myocardial mitochondrial damage resulted from cerebral ischemia.

In "cerebral-cardiac stroke" induced by photochemical reaction<sup>(1)</sup>, we observed that cardiac systolic and diastolic dysfunctions recovered completely in 5-7 d. Histidine shortened the reversible injury, known as "stunning"<sup>(2)</sup> by improving the myocardial contractility<sup>(1)</sup>, suggesting that the functional heart depression but not morphologic changes occurred at cerebral thrombosis. To further elicit the nature of cardiac complication of stroke, we observed the changes of myocardium ultrastructure and whole blood aggregation after cerebral thrombosis, and it might be helpful to clarify the possible mechanism of histidine in improving myocardial stunning.

## MATERIALS AND METHODS

**Thrombotic cerebral ischemia** The cerebral thrombosis was produced in 45 ♂ Wistar rats (Grade Clean) weighing  $(280 \pm 20)$  g under anesthesia with 2.5 % thiopental sodium ( $40 \text{ mg} \cdot \text{kg}^{-1}$ ) ip. The rats were given rose bengal ( $10 \text{ mg} \cdot \text{kg}^{-1}$ ) in  $7.5 \text{ g} \cdot \text{L}^{-1}$  saline solution and the intact skull was irradiated with green light (560

<sup>1</sup> Project supported by the Science Foundation of Ministry of Public Health of China, No 941358.

<sup>2</sup> Correspondence to Prof LI Shu-Qing. Phn 86-871-533-8591.

Fax 86-871-533-2571.

Received 1997-03-27

Accepted 1998-02-10

nm, bandwidth 60 nm) for 20 min<sup>[1]</sup>. The treated rats received iv 0.5 % histidine 5 mg · kg<sup>-1</sup> 30 min after the irradiation, while ischemic rats received only saline after the irradiation.

#### Measurement of platelet aggregation

Sodium citrate 3.0 % containing heparin 2 kU · L<sup>-1</sup> was used. Platelet aggregation was measured using whole blood (WB) aggregator (QX-200, Shanghai Medical University) according to the impedance method. Using a linear recorder (XWT, Da Hua Instrument Co, Shanghai). A chart paper unit = 50 Ω. The duration from the addition of ADP 25 μmol · L<sup>-1</sup> to the start and maximal height of aggregation was expressed with 0 - 60 s aggregation slope (AS 0 - 60), 60 - 120 s aggregation slope (AS 60 - 120), 120 - 180 s aggregation slope (AS 120 - 180), and aggregation peak height (APH). The aggregation curve was in units of electric resistance (Ω)<sup>[3]</sup>.

**Myocardial ultrastructure and mitochondria stereology** The myocardium was fixed in 2.5 % glutaraldehyde at 4 °C overnight, and the trimmed pieces were placed in 2.5 % glutaraldehyde at 4 °C for 2 h, washed with phosphate buffer, fixed with 1 % OsO<sub>4</sub> for 2 h, dehydrated in graded ethanol, and embedded in Epon 618. The epoxy blocks were sliced on a LKB-V ultratome. The sections (0.5 μm) were stained with uranylacetate and lead citrate, and examined under JEM-100 CX transmission electron microscope. Mitochondria stereology was analyzed using multipurpose test system<sup>[4]</sup>.

**Statistical analysis** Data were presented as  $\bar{x} \pm s$  and analyzed with *t* test.

## RESULTS

### Platelet aggregation *ex vivo*

Infusion of

rose bengal for 30 min, 4 and 24 h in rats did not induce platelet aggregation. In contrast, ADP caused marked aggregation in WB after photochemical reaction. All the variables (AS 0 - 60, AS 60 - 120, AS 120 - 180, and APH) reflecting platelet aggregation after photochemical reaction were increased markedly, APH at 4 and 24 h were (5.1 ± 0.5) and (4.3 ± 0.5) Ω, respectively. It decreased markedly at 4 and 24 h (2.9 ± 1.1) and (3.0 ± 0.8) Ω, respectively; *P* < 0.01) after the treatment with histidine (Tab 1).

**Ultrastructure and stereological parameters of myocardial mitochondria** The mitochondrial ultrastructure was normal in control group (Fig 1).

The mitochondria swelling, the cristae disorganizing and amorphous matrix densities were seen in myocardium of the rats after cerebral thrombosis. Mitochondria volume (*V*), volume density (*V<sub>v</sub>*), surface density (*N<sub>m</sub>*) and surface density of outer mitochondrial membrane (*S<sub>v1</sub>*) showed an increase (8.2 ± 5.5, 0.59 ± 0.16, 0.11 ± 0.03, and 0.22 ± 0.05, *P* < 0.01) but specific surface of the inner mitochondrial membrane (*δ<sub>2</sub>*), specific surface of the cristae (*δ<sub>3</sub>*), and numerical density (*N<sub>v</sub>*) were decreased (2.8 ± 0.8, 2.4 ± 0.7, and 0.07 ± 0.02, *P* < 0.01, Tab 1). There was no significant difference in histidine-treated rats compared with control rats. But the *N<sub>m</sub>* and *S<sub>v1</sub>* of the outer mitochondrial membrane were increased more than those of the control (*P* < 0.01) (Tab 2).

## DISCUSSION

### Inhibitory action of histidine on WB platelet aggregation

The impedance technique

Tab 1. Effect of histidine 5 mg · kg<sup>-1</sup> on platelet aggregation during cerebral thrombosis.  $\bar{x} \pm s$ , <sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs saline control.

	Rats	AS 0 - 60 (Ω/s)	AS 60 - 120 (Ω/s)	AS 120 - 180 (Ω/s)	APH (Ω)
Saline control	12	0.44 ± 0.09	0.13 ± 0.03	0.06 ± 0.02	4.1 ± 0.6
Four hours after cerebral thrombosis					
Ischemia	8	0.58 ± 0.07 <sup>c</sup>	0.18 ± 0.01 <sup>c</sup>	0.07 ± 0.01 <sup>a</sup>	5.1 ± 0.5 <sup>b</sup>
Histidine	10	0.32 ± 0.13 <sup>a</sup>	0.12 ± 0.05 <sup>a</sup>	0.05 ± 0.03 <sup>a</sup>	2.9 ± 1.1 <sup>c</sup>
Twenty-four hours after cerebral thrombosis					
Ischemia	7	0.35 ± 0.05 <sup>a</sup>	0.16 ± 0.03 <sup>a</sup>	0.10 ± 0.03 <sup>a</sup>	4.3 ± 0.5 <sup>a</sup>
Histidine	6	0.38 ± 0.15 <sup>a</sup>	0.07 ± 0.04 <sup>a</sup>	0.05 ± 0.02 <sup>a</sup>	3.0 ± 0.8 <sup>c</sup>

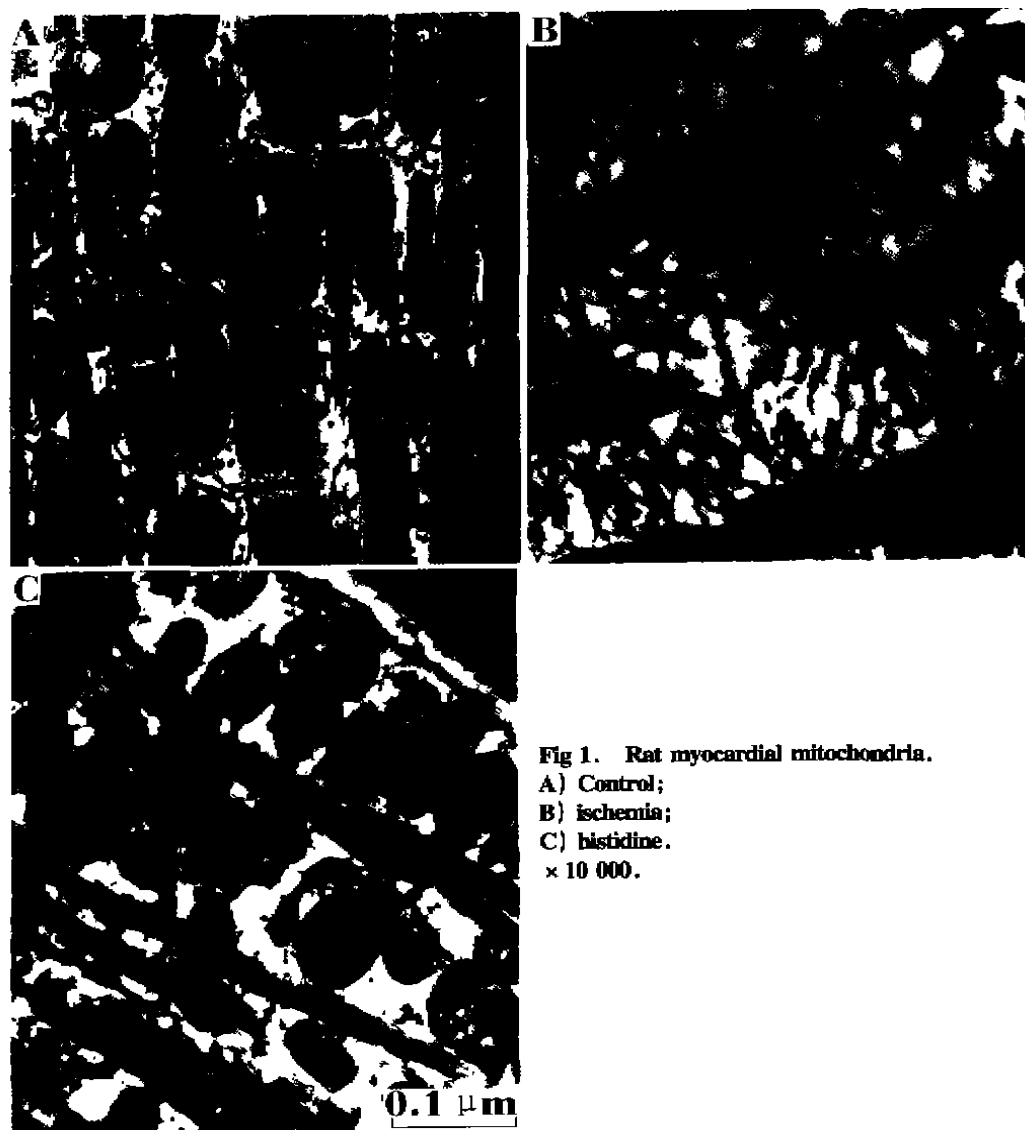


Fig 1. Rat myocardial mitochondria.  
A) Control;  
B) ischemia;  
C) histidine.  
× 10 000.

Tab 2. Effect of histidine on stereological parameters of myocardial mitochondria 4 h after cerebral thrombosis.  $n = 36$  rats.  $\bar{x} \pm s$ .  
<sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs saline control.

	Saline	During cerebral thrombosis	
		Ischemia	Histidine
$V_v/\%$	$0.47 \pm 0.12$	$0.59 \pm 0.16^c$	$0.46 \pm 0.15^a$
$S_{v1}/\mu\text{m}^{-1}$	$0.16 \pm 0.06$	$0.22 \pm 0.05^c$	$0.23 \pm 0.08^c$
$S_{v2}/\mu\text{m}^{-1}$	$1.6 \pm 0.5$	$1.5 \pm 0.4^a$	$1.6 \pm 0.6^a$
$S_{v3}/\mu\text{m}^{-1}$	$1.5 \pm 0.5$	$1.4 \pm 0.4^a$	$1.4 \pm 0.5^a$
$\delta_1/\mu\text{m}^{-1}$	$0.35 \pm 0.13$	$0.39 \pm 0.12^a$	$0.52 \pm 0.21^c$
$\delta_2/\mu\text{m}^{-1}$	$3.5 \pm 0.7$	$2.8 \pm 0.8^c$	$3.6 \pm 1.1^a$
$\delta_3/\mu\text{m}^{-1}$	$3.2 \pm 0.7$	$2.4 \pm 0.7^c$	$2.9 \pm 1.0^a$
$N_m/\mu\text{m}^{-2}$	$0.08 \pm 0.07$	$0.11 \pm 0.03^c$	$0.12 \pm 0.04^c$
$N_v/\mu\text{m}^{-3}$	$0.13 \pm 0.03$	$0.07 \pm 0.02^c$	$0.11 \pm 0.04^a$
$V/\mu\text{m}^3$	$3.7 \pm 1.9$	$8.2 \pm 5.5^a$	$4.1 \pm 3.1^a$

is more sensitive than the optical method at the presence of small platelet aggregates which may deposit on the electrodes<sup>[4]</sup>. Therefore it is possible, using the WB aggregometer, to reveal the change of platelet function following photochemical reaction and the relationship between the platelet activation and myocardial mitochondria damage. On the basis of the present experiment results<sup>[5]</sup>, we believe that photochemical reaction may injure endothelial cells and induce platelet aggregation. The increase of platelet aggregation is not only the cause of the secondary cerebral ischemia but also an important factor leading to the myocardial stunning. The decrease of the platelet aggregation values after iv histidine may be

associated with the radical scavenging activity of histidine<sup>[6]</sup>, but the exact mechanism of the latter action on the platelet is still to be determined.

**Protective effect of histidine on myocardial mitochondria** The results of mitochondria ultrastructure and stereology showed that after the cerebral thrombosis a significant change of myocardium occurred. Its histopathologic characteristics were different from those of ischemic necrosis and the myocardial damage caused by ischemic reperfusion. As indicated by the stereology, the reversible change often went with the parameters associated to the inner but not the outer mitochondrial membrane following iv histidine. This difference of the outer and inner mitochondrial membrane could be related with the difference of their biochemical and physiologic function. There are more amorphous matrix densities being showed in the mitochondria, which might be due to the platelet activation and mitochondrial Ca<sup>2+</sup> overloading<sup>[2]</sup>. The ultrastructural changes in these reversibly injured mitochondria were defined as "stunning", which began to return to the control level with the platelet aggregate decrease after the treatment with histidine, suggesting that the platelet might play a role in the myocardial mitochondria damage. It could help to explain the pathophysiological alteration of cardiac dysfunction after cerebral thrombosis. Histidine reduces the ultrastructure change of myocardial mitochondria, possibly, by altering membrane function and ion homeostasis.

REFERENCES

- 1 Li SQ, Han H, He J. Histidine ameliorated brain edema and cardiac dysfunction during local thrombotic cerebral ischemia in rats. *Acta Pharmacol Sin* 1995; 16: 156-9.
- 2 Atar D. Mechanisms of contractile dysfunction in stunned

myocardium. *Heart News Views* 1994; 2: 3-4.

- 3 Mannucci L, Redaelli R, Tremoli E. Effects of aggregating agents and of blood cells on the aggregation of whole blood by impedance technique. *Thromb Res* 1988; 52: 143-51.
- 4 Weile ER. Stereological principles for morphometry in electron microscopic cytology. *Int Rev Cytol* 1969; 26: 235-9.
- 5 Li SQ, Yang QY, Zhao WJ, Ye JM, Sun JY, Wu SF. Photochemically induced thrombotic cerebral ischemia and its metabolic alterations. *Chin J Pathophysiol* 1994; 10: 95-9.
- 6 Hartman PE, Hartman Z, Ault KT. Scavenging of singlet molecular oxygen by imidazole compounds: high and sustained activities of carboxy terminal histidine dipeptides and exceptional activity of imidazole-4-acetic acid. *Photochem Photobiol* 1990; 51: 59-66.

483-496  
 组氨酸对大鼠血栓性脑缺血时心肌线粒体及血小板聚集的影响<sup>1</sup>  
 R243.310.5  
 R977.4  
 李树清<sup>2</sup>, 赵刚<sup>1</sup>, 李俊, 钱伟<sup>3</sup> (昆明医学院病理生理教研室; <sup>3</sup>电镜室, 昆明 650031, 中国)

关键词 光化学; 血栓形成; 脑缺血; 血小板聚集; 心肌; 心脏线粒体; 组氨酸

目的: 研究组氨酸对脑血栓形成的影响及可能机制. 方法: 采用光化学诱导“脑—心卒中”模型并给组氨酸(iv 5 mg·kg<sup>-1</sup>)治疗. 结果: 脑血栓形成后 4 和 24 h 全血血小板聚集的峰值为(5.1 ± 0.5) Ω 和(4.3 ± 0.5) Ω. 心肌线粒体体积(V)、体密度(V<sub>v</sub>)、面密度(N<sub>m</sub>)及外膜表面(S<sub>v1</sub>)增加(8.2 ± 5.5, 0.59 ± 0.16, 0.11 ± 0.03 和 2.2 ± 0.05, P < 0.01), 但数密度(N<sub>v</sub>)、内膜比表面(δ<sub>2</sub>)和嵴膜比表面(δ<sub>3</sub>)减少(0.07 ± 0.02, 2.8 ± 0.8 和 2.4 ± 0.7, P < 0.01), 心肌病理学改变有别于缺血性坏死和缺血再灌所致的心脏损伤. 组氨酸治疗后, 全血血小板聚集降为(2.9 ± 1.1) Ω (P < 0.01), 与内膜有关的体视学参数可逆改变. 结论: 组氨酸可抑制全血血小板聚集, 并减轻由于脑缺血所致的心肌线粒体损伤.

欢迎订阅《中国药师》

《中国药师》由国家医药管理局培训中心和武汉医药(集团)股份有限公司主办, 已于1998年6月正式出版, 公开发行. 该刊设有“药师与法规”、“论著”、“执业药师”、“继续教育”、“药学进展”、“药物与临床”、“药师论坛”、“药苑精英”、“新药介绍”、“药师园地”等栏目. 该刊将成为药师的良师益友, 医师的用药指南. 双月刊, 大16开48页, 每期定价5元, 全年订费30.00元. 邮发代号38-325, 请及时经邮局订阅; 漏订者请汇款至湖北武汉兰陵路2号《中国药师》发行部, 芦德梅收, 邮编430014, 电话027-8283-5077.