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 μmol·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}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}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.

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Effect of histidine on myocardial mitochondria and platelet aggregation during thrombotic cerebral ischemia in rats[1]

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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 ± 0.5) Ω and (4.3 ± 0.5) Ω, respectively. Heart mitochondria volume (V), volume density (V_v), surface density (N_m), and surface density of outer membrane (S_{vl}) increased (8.2 ± 5.5, 0.59 ± 0.16, 0.11 ± 0.03, and 0.22 ± 0.05, respectively, $P < 0.01$), but numerical density (N_v), specific surface of inner membrane (δ_2) and of the cristae (δ_3) decreased (0.07 ± 0.02, 2.8 ± 0.8, and 2.4 ± 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 ± 1.08) Ω, $P < 0.01$], 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[1], we observed that cardiac systolic and diastolic dysfunctions recovered completely in 5 - 7 d. Histidine shortened the reversible injury, known as "stunning"[2] by improving the myocardial contractility[1], 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 ± 20) g under anesthesia with 2.5 % thiopental sodium (40 mg·kg^{-1}) ip. The rats were given rose bengal (10 mg·kg^{-1}) in 7.5 g·L^{-1} saline solution and the intact skall was irradiated with green light (560

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nm, bandwidth 60 nm) for 20 min[1]. The treated rats received iv 0.5 % histidine 5 mg·kg^{-1} 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^{-1} 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^{-1} 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)[3].

Myocardiac ultrastructure and mitochondria stereology The myocardium was fixed in 2.5 % glutaraldehyde at 4 ℃ overnight, and the trimmed pieces were placed in 2.5 % glutaraldehyde at 4 ℃ for 2 h, washed with phosphate buffer, fixed with 1 % OsO_4 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[4].

Statistical analysis Date 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_v), surface density (N_m) and surface density of outer mitochondrial membrane (S_{v1}) 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_2), specific surface of the cristae (δ_3), and numerical density (N_v) 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_m and S_{v1} 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^{-1} on platelet aggregation during cerebral thrombosis. $\bar{x} \pm s$, $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *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^c	0.18 ± 0.01^c	0.07 ± 0.01^a	5.1 ± 0.5^b
Histidine	10	0.32 ± 0.13^a	0.12 ± 0.05^a	0.05 ± 0.03^a	2.9 ± 1.1^c
Twenty-four hours after cerebral thrombosis					
Ischemia	7	0.35 ± 0.05^a	0.16 ± 0.03^a	0.10 ± 0.03^c	4.3 ± 0.5^a
Histidine	6	0.38 ± 0.15^a	0.07 ± 0.04^a	0.05 ± 0.02^a	3.0 ± 0.8^c

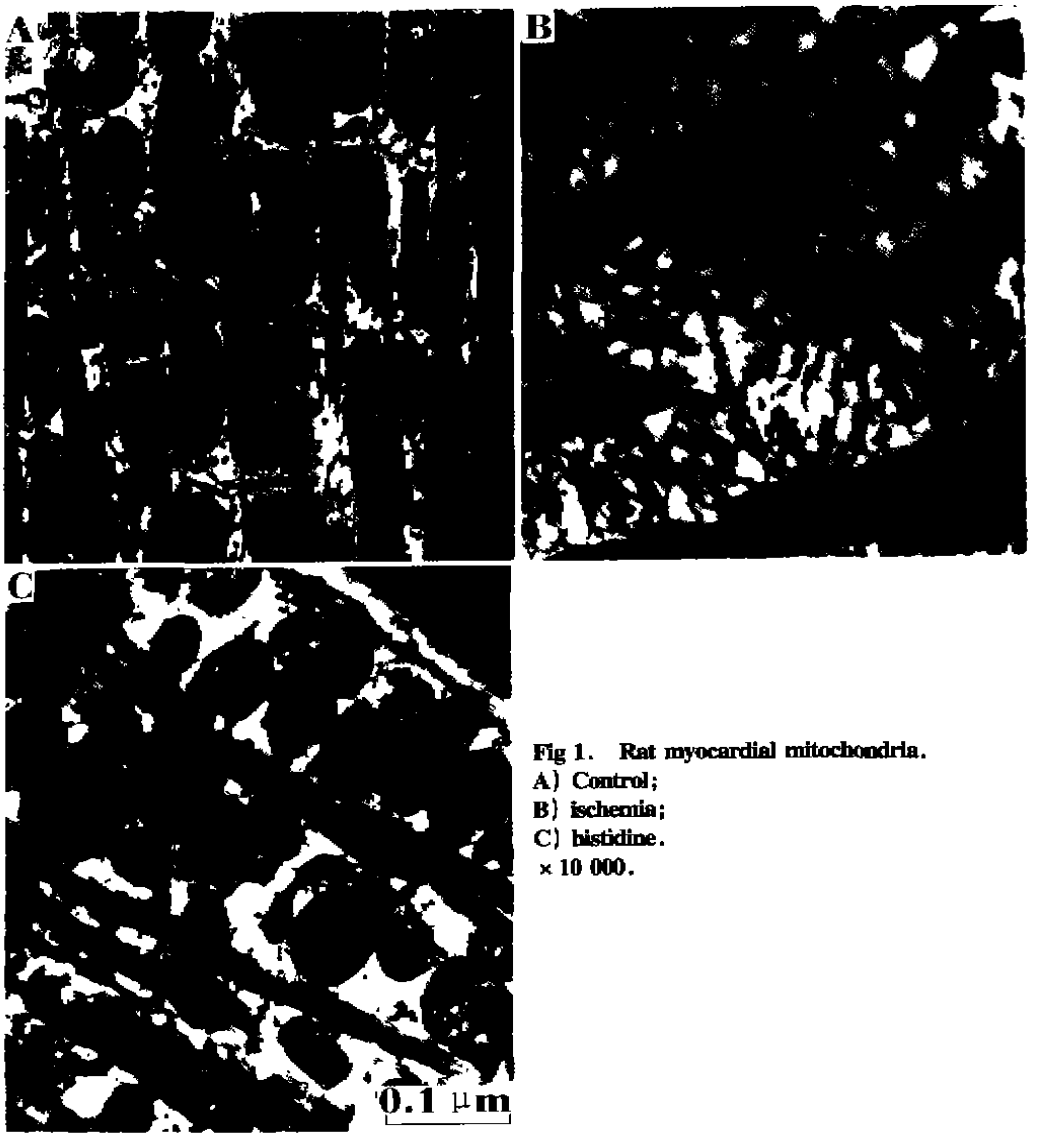

Fig 1. Rat myocardial mitochondria.
A) Control;
B) ischemia;
C) histidine.
× 10 000.

Tab 2. Effect of histidine on stereological parameters of myocardial mitochondria 4 h after cerebral thrombosis. n = 36 rats. $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline control.

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

is more sensitive than the optical method at the presence of small platelet aggregates which may deposit on the electrodes[4]. 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[5], 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[6], 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 myocardiac 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^{2+} overloading[2]. 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.

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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[1]

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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^{-1})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_v)、面密度(N_m)及外膜表面(S_{vl})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_v)、内膜比表面(δ_2)和嵴膜比表面(δ_3)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.