BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Mar ; 16 (2): 156—159

# Histidine ameliorated brain edema and cardiac dysfunction during local thrombotic cerebral ischemia in rats[1]

LI Shu-Qing, HAN Hong, HE Jie
(*Department of Pathophysiology, Kunming Medical College, Kunming 650031, China*)

**AIM:** To observe the effects of histidine (iv 5 mg · kg$^{-1}$) on brain edema and cardiac disturbance after cerebral thrombosis. **METHODS:** Regional cerebral thrombosis was induced by photochemical method in rats. **RESULTS:** It was showed that the brain water content increased markedly (85.6 ±3.8 %, $P<0.01$); the left ventricular systolic pressure (LVSP, 17.9±1.5 kPa) and the maximal left ventricular contractility decreased (+ $dp/dt_{max}$ 645 ± 110 kPa and $-dp/dt_{max}$ 473±106 kPa, $P<0.05$). In rats treated with histidine after photochemical reaction, the brain water content decreased (81.5±2.0 %) while LVSP (21.2±1.1 kPa) and left ventricular $+dp/dt_{max}$ and $-dp/dt_{max}$ increased markedly (777 ± 144 kPa and 604 ± 157 kPa, respectively). **CONCLUSION:** Histidine has protective effects on the brain and cardiac function during cerebral thrombosis.

**KEY WORDS** photochemistry; thrombosis; cerebral ischemia; brain edema; myocardial contraction; histidine

Histidine showed antioxidant activity by quenching of singlet oxgyen, known as singlet oxygen scavenger[1] and protected $Ca^{2+}$-ATPase activity of cardiac sarcoplasmic reticulum against rose bengal-derived singlet oxygen due to *in vitro* photochemical reaction[2]. In this paper, a photochemical method was employed to cause cerebral thrombosis by forming singlet oxygen and damaging vascular epithelium. One of the advantages of this model is that it is a closed cranium and less invasive approach than other methods of causing focal cerebral ischemia and the protective effect of histidine on cardica-cerebral functions was studied.

[1]Project supported by the Science Foundation of the Ministry of Public Health of China, № 941358.
Received 1993—10—05 Accepted 1994—10—24

## MATERIALS AND METHODS

**Regional cerebral thrombosis**[3] The regional cerebral thrombosis was performed on 65 ♀ Wistar rats weighing 280±*s* 25 g. On the day of the experiment, anesthesia was induced with 2.5 % thiopentalum natricum (40 mg · kg$^{-1}$) ip. A sagittal incision was made and the right side of the skull was exposed. An aliquot of 7.5 g · L$^{-1}$ saline solution of rose bengal (1.33 mL · kg$^{-1}$) was injected vis the tail vein 5 min before the irradiation. An intense green light (λ 560 nm △λ 60 nm, 5×10$^4$ W/m$^2$) was passed through an interference filter and a heat filter on to the parietal bone (5 mm×6 mm) for 20 min[4].

**Measurement of brain water content** The rats were decapitated before and at 4 and 24 h after the irradiation. The left (nonlesioned) and right (lesioned) cortices were weighted and dried to the constant weight in 120 C for 48 h[5].

**Measruements of heart functions** A catheter (regional heparinized) was secured in the left ventricle via left common carotid artery. The left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP) were continuously recorded with a RM-6000 recorder. The maximal rates of rise ($+dp/dt_{max}$) and fall ($-dp/dt_{max}$) of developing pressure were graphically evaluated with a EQ-600G pressure processor. The mean arterial pressure (MAP) and heart rate (HR) were recorded and the index of myocardial oxygen consumption (IMV $O_2$) was calculated as MAP×HR[6].

**Group division** The 21 treated rats received iv 0.5 % histidine 5 mg · kg$^{-1}$ 30 min after the irradiation, while 28 ischemic rats received only saline after the irradiation. The control rats (sham operation) underwent the same surgical procedure and were either irradiated for 20 min following the injection of saline or injected with rose bengal but not irradiated.

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

## RESULTS

**Brain edema** The water content of the left and right cortices were 81.6±1.6 % and 81.0±1.0 %, respectively ($P>0.05$), before the irradiation. Brain water content within the irradiated zone increased slightly ($P>0.05$) by 4 h. At 24 h after the irradiation the brain water content increased to 85.8±3.8 % from the 81.0±2.0 % ($P<0.01$). In the treated rats the water content was 81.5±2.0 % 24 h after the irradiation (Tab 1).

**Heart functions** All indices did not change by only irradiation or iv rose bengal or histidine except the irradiation in the presence of rose bengal during a period of 60 min. LVSP decreased and the $+dp/dt_{max}$ and $-dp/dt_{max}$ decreased markedly 4 h after the irradiation. HR tended to decrease ($P>0.05$). A depression of the heart functions caused by cerebral ischemia was also seen *vs* control values 24 h after the irradiation. In rats treated with histidine, the MAP increased, $+dp/dt_{max}$ and $-dp/dt_{max}$ recovered to the control level ($P>0.05$) and the change of the latter was more than that of the former at 4 h after the irradiation. MAP, SAP, LVSP, and IMV $O_2$ increased after treatment with histidine ($P<0.05$) besides the recovery of the maximal left ventricular contractility at 24 h after the irradiation (Tab 2).

**Tab 1. Effect of histidine (5 mg · kg$^{-1}$ iv) on brain water content in cortex after cerebral thrombosis.** $\bar{x}\pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs* **left.**

| | | $n$ | Brain cortex Left | Right |
|---|---|---|---|---|
| Pre | | 5 | 81.6±1.6 | 81.0±0.9[a] |
| Post-ischemia | 4 h | 7 | 81.6±2.4 | 82.0±3.4[a] |
| | 24 h | 7 | 81.6±4.5 | 85.6±3.8[c] |
| Pre | | 5 | 79.4±0.6 | 79.6±0.7[a] |
| Histidine | 4 h | 5 | 80.3±1.6 | 80.2±1.1[a] |
| | 24 h | 5 | 82.3±0.8 | 81.5±2.0[a] |

## DISCUSSION

**Action of histidine on improving ischemic brain edema** Photochemically induced thrombosis is based on that rose bengal under

**Tab 2. Protective effect of histidine (5 mg·kg$^{-1}$ iv) on cardiac function after cerebral thrombosis.** $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$ *vs* **control**, $^{d}P<0.05$, $^{f}P<0.01$ *vs* **ischemic group.**

| | Control | After cerebral thrombosis 4 h Ischemia | 4 h Histidine | 24 h Ischemia | 24 h Histidine |
|---|---|---|---|---|---|
| Rats | 6 | 8 | 5 | 6 | 6 |
| HR, beat·min$^{-1}$ | 365±44[a] | 342±49[a] | 340±56[a] | 370±42[a] | 400±33[a] |
| MAP, kPa | 13.6±1.9[a] | 13.9±1.7[a] | 16.3±2.4[d] | 13.7±2.3[a] | 15.4±1.0[b] |
| SAP, kPa | 16.6±1.8[a] | 17.3±1.5[a] | 18.5±2.5[a] | 16.3±1.7[a] | 18.1±0.9[f] |
| DAP, kPa | 11.6±2.2[a] | 12.1±1.4[a] | 14.3±2.7[a] | 11.8±2.3[a] | 13.6±1.0[a] |
| IMV $O_2\times10^1$ | 4.9±1.2[a] | 4.8±0.8[a] | 5.6±1.4[a] | 5.2±0.6[a] | 6.2±0.5[b] |
| LVSP, kPa | 19.1±1.4[a] | 17.9±1.5[a] | 20.3±2.5[a] | 17.6±1.6[a] | 21.2±1.1[f] |
| $+dp/dt_{max}$, kPa | 840±136[a] | 645±110[b] | 797±127[a] | 684±66[b] | 777±144[a] |
| $-dp/dt_{max}$, kPa | 639±146[a] | 473±106[b] | 645±201[a] | 478±66[b] | 604±157[a] |
| LVEDP, kPa | 1.0±0.4[a] | 0.7±0.3[a] | 0.7±0.3[a] | 0.80±0.20[a] | 0.70±0.10[a] |

a specific light condition produces singlet oxygen which damages the endothelia of cerebral blood vessels and leads to platelet aggregation, thrombus formation and eventually to the occlusion of the blood vessels[3]. Neuronal necrosis after photochemical reaction may be secondary to the cerebral ischemia caused by thrombosis[4]. The energy metabolic depression and platelet activating factor (PAF) released from platelet aggregation may play a major role in brain edema. However, the brain water content decreased markedly following iv histidine, which may be associated with the radical scavenging activity of histidine, leading to the quenching of singlet oxygen via C-terminal histidine dipeptide reacting with singlet oxygen[1]. This might be the key action of histidine on reducing brain edema and protecting cerebral functions.

**Protective effect of histidine on cardiac function during cerebral ischemia** Cerebral dysfunction follows heart disease, but the changes of heart functions after cerebral ischemia[7], and the mechanisms and the causal relationship between cerebral and cardiac abnormalities under pathological state are not yet fully understood. Our results presented here suggest that the depression of heart functions following cerebral thrombosis is in accordance with the published concept of "cerebral-cardiac stroke[8]". The depressed myocardial contractility after the cerebral thrombosis (Tab 2) may be associated with cerebral metabolic deprivation, brain edema formation and the negative inotropic effect of PAF on myocardium. Our studies suggest that heart functions are reduced with the increase of platelet aggregation after the cerebral thrombosis[9]. The beneficial effect of histidine scavenging singlet oxygen during photochemical reaction is possibly attributable to protecting vascular endothelia, inhibiting platelet activation and improving cardiac and cerebral functions. The increase of MAP, SAP, LVSP and IMV $O_2$ in animals at 24 h after iv histidine may be related with the increased myocardial contractility and cardiac output (CO). These data suggest that histidine has protective effects on the brain and cardiac function during cerebral thrombosis.

## REFERENCES


1 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.

2 Kukreja RC, Jesse RL, Hess ML. Singlet oxygen: a potential culprit in myocardial injury? Mol Cell Biochem 1992; **111**: 17—24.

3 Watson BD, Prado R, Dietrich WD, Ginsberg MD, Green BA. Photochemically induced spinal cord injury in the rat. Brain Res 1986; **367**: 296—300.

4 Li SQ, Zhao WJ, Yang QY. The effects of photochemically induced regional cerebral thrombosis on cardiac hemodynamics. Chin J Pathophysiol 1993; **9**: 494—7.

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 Gerold M, Eigenmann R, Haeusler G. Cardiovascular effects of tiapamil (Ro 11-1781), a new calcium-entry blocker. J Cardiovasc Pharmacol 1982; **4**: 419—29.

7 Perez-Trepichio AD, Williams JL, Block CH, Jones SC. Cardiovascular changes during focal cerebral ischemia in rats. Stroke 1993; **24**: 691—6.

8 Yang ZL. Acute cerebrovascular disease and myocardial infarction. Chin J Neurol Psychiatry 1991; **24**: 135—7.

9 Li SQ, Zhao WJ, Yang QY. The studies on the mechanism of cardiac insufficiency in cerebral thrombosis. J Mol Cell Cardiol 1993; **25** Suppl 3: S25.


## 组氨酸改善大鼠血栓形成性局部脑缺血时脑水肿及心功能障碍

李树清，魏 红，何 洁

(昆明医学院病理生理教研室，昆明650031，中国)

目的：观察组氨酸对脑血栓形成后脑水肿及心

功能障碍的影响. **方法**：用光化学法诱导大鼠血栓形成性局部脑缺血. **结果**：大鼠脑血栓形成后脑水份明显增加($P<0.01$)，左室收缩压(LVSP)峰值及左室内压变化速率($dp/dt_{max}$)明显降低($P<0.05$). **结论**：iv 组氨酸5 mg·kg$^{-1}$可明显改善脑缺血所致脑水肿及心功能障碍而具有保护脑功能效应.

**关键词** 光化学；血栓形成；脑缺血；脑水肿；心肌收缩；组氨酸

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Mar; 16 (2): 159—162

# Reducing effect of 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside on arterial thrombosis induced by vascular endothelial injury

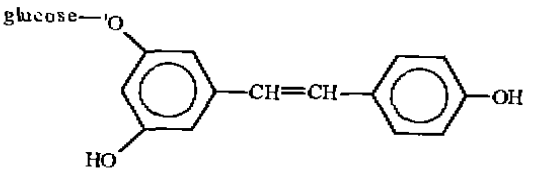
WANG Yao-Zhong, LUO Su-Fang, ZHANG Pei-Wen, YU Chuan-Lin
*(Department of Pharmacology, First Military Medical University, Guangzhou 510515, China)*

**AIM**: To study the effect of 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside (Polydatin, Pol) on rabbit arterial thrombosis. **METHODS**: Rabbit arterial thrombosis was induced by vascular endothelial damage with trypsin. **RESULTS**: It was showed that the moist weights of the thrombus were 6.6±1.8 and 4.8±1.6 mg in Pol 5 and 10 mg·kg$^{-1}$ groups, respectively, which was lighter than that in control (10.9±1.9 mg, $P<0.05$, $P<0.01$); the platelet aggregation was inhibited simultaneously. *In vitro*, Pol 0.30—1.15 mmol·L$^{-1}$ reduced $TXA_2$ produced in platelets. It did not affect the production of $PGI_2$ in cultured human umbilical vein endothelial cells. **CONCLUSION**: Thrombosis was abated by Pol. The selective inhibition of production of $TXA_2$ rather than $PGI_2$, is one of the mechanisms involved.

**KEY WORDS** 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside; vascular endothelium; thrombosis; cultured cells; platelet aggregation; thromboxane $A_2$; epoprostenol; polydatin

Received 1993-05-18 Accepted 1994-12-17

Polydatin[1] (Pol), a colorless crystal, was extracted from the root and stem of *Polygonum cuspidatum* Sieb et Zucc. by Department of Chemistry of our University in our country 11 years later than Japanese (Zhong Cao Yao Tong Xun 1974; **2**: 6—10).




3,4′,5-Trihydroxystibene-3-β-mono-*D*-glucoside

Pol inhibited the rabbit platelet aggregation and release of thromboxane $A_2$ ($TXA_2$) both *in vivo* and *in vitro*[2-4]. In this experiment, the arterial thrombosis model of rabbits was established by damaging the vascular endothelium with trypsin. This study was aimed to identify whether Pol could simultaneously inhibit the thrombosis and platelet aggregation and to determine the effect of the drug on the production of exogenous or endogenous arachidonic acid metabolites $TXA_2$ in rabbit platelet and prostacyclin (epoprostenol, $PGI_2$) in cultured endothelial cells from human umbilical vein.

## MATERIALS AND METHODS

Pol (double mp 144—6 C and 235—7 C, $R_f$ val-