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Protective effects of flunarizine on hemorrhagic shock in rats

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ABSTRACT Intravenous injections of flunarizine 0.25, 0.5, or 1.0 mg · kg^{-1} 10 min before hemorrhage increased the maximal bleeding volume from 4.3 ±1.1 to 5.5±1.1 ml. As the dose of flunarizine increased, the aurvival time in rats subjected to hemorrhage was prolonged in a dose-dependent manner. Five houra after the reinfusion, flunarizine 1 mg · kg^{-1} markedly improved the survival rate to 70% compared with nil in the abock group. Flunarizine reduced the increase of lactate in blood, ameliorated the depletion of ATP stores in tissues, and prevented the calcium accumulation in heart and liver. The results auggest that flunarizine may produce a protective effect on hemorrhagic abock, probably related to the decreaae of calcium accumulation in the ischemic tissues.

KEY WORDS hemorrhagic shock; flunarizine; blood gas analyais; calcium

Calcium channel blockers exerted a protective effect against hypoperfusion and reperfusion injury[1,2], indicating a potential role of these agents in treating hemorrhagic shock. Verapamil decreased the amount of hemorrhage in the bowel and prolong the survival time after shock[3]. Flunarizine (Flu), a diphenyalkyamine calcium channel blocker, reduced the level of free Ca^{2+} within the cell, thereby preventing an excessive calcium load induced by various pathologic stimuli[4]. Flu produced a protective effect on cerebral ischemia in rats[5], but its action on hemorrhagic shock has not been reported. The present investigation was undertaken to study the effects of Flu on hemorrhagic shock in rats.

MATERIALS AND METHODS

Hemorrhagic shock model A total of 70 Sprague-Dawley rats of either sex, weighing 245±*s* 37 g, was fasted overnight and anesthetized with urethane 850 mg · kg^{-1} and chloralose 50 mg · kg^{-1} ip. A catheter was played in a carotid artery for blood pressure measurement and another in a femoral artery for letting blood. The rats were bled at a constant rate leading to a mean arterial blood pressure of 4 kPa (30 mmHg) in 10 min[4]. Blood reletting or reinfusion was then instituted to maintain this pressure. Rats were taken to the point at which decompensation began as indicated by the need to reinfuse 50% of the maximal volume of the blood loss, and followed by reinfusion of shed blood.

Reagents and drugs Both lactic dehydrogenase (LDH) and adenosine triphosphate sodium (ATP · 2Na) were purchased from Sigma. Firefly luciferase was obtained from Shanghai Institute of Plant Physiology. Flu hydrochloride, provided by Janssen Pharmaceutical Co and dissolved in ethanol-glucose (2:8), 0.25, 0.5, or 1.0 mg · kg^{-1} was given as a bolus injection via tail vein 10 min hefore hemorrhage.

Biochemical assay Blood gases were measured within 30 min of collection using Radiometer Model ABL-3 (made in Copenhagen). Enzymatic methods were used to determine lactic acid in the arterial blood. Small pieces of cardic and hepatic tissues, taken by a pair of precooled stainless steel tong, were immersed in liquid nitrogen. The samples were then homogenized in a solution containing 10% trichloroacetic acid and centrifuged. The heart and liver specimens were dried at 140℃, and digested in nitric acid 16 mol · L^{-1} and perchloric acid 12 mol · L^{-1}(4:1) for 48 h. Total tissue calcium, magnesium, and zinc contents were determined by atomic absorption spectroscopy.

Data analysis The results were expressed as $\bar{x} \pm s$. Mortality rates were analyzed by chi-square test, the others were compared by *t* test and regression analysis.

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RESULTS

Flu 1.0 mg · kg^{-1} increased the maximal bleeding volume. As the dose of Flu was increased from 0.25 to 1.0 mg · kg^{-1}, the survival times in rats subjected to hemorrhage were prolonged in a dose-dependent manner ($r=0.9443$, $P<0.05$) (Tab 1). For the following studies on the effect of Flu, 1.0 mg · kg^{-1} was thus utilized. Flu 1.0 mg · kg^{-1} before hemorrhage prevented the decline of arterial pressure following reperfusion (Fig 1).

Flu 1.0 mg · kg^{-1} improved acidosis by blocking lactate production and preserving blood HCO_3^- levels (Tab 2).

Flu 1 mg · kg^{-1} markedly decreased the ATP consumption and prevented the increase of tissue calcium (Tab 2). Tissue Mg^{2+} and Zn^{2+} contents showed no significant changes. Tissue Mg^{2+}/Zn^{2+} ratios was significantly lowered in the shock group, while Flu pretreatment prevented the ischemic tissue

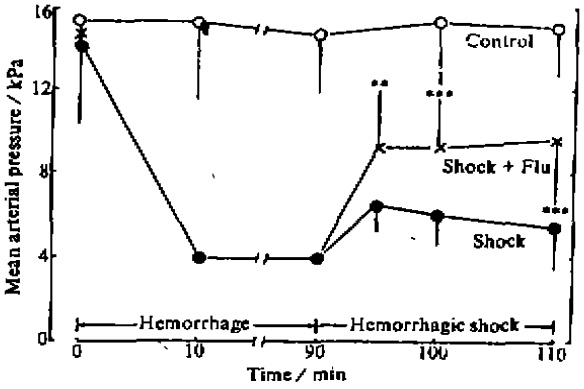

Fig 1. Effects of flunarizine 1 mg · kg^{-1} on mean arterial pressure in rats subjected to hemorrhagic shock. $n=10$, $\bar{x}\pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* shock.

Tab 1. Effects of flunarizine on bleeding and survival in rats subjected to hemorrhagic shock. $n=10$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* 0 mg·kg^{-1}.

Flunarizine/mg·kg^{-1}	0	0.25	0.50	1.00
Bleeding volume (BV_{10})/ml	2.2±0.5	2.1±0.7*	1.9±0.6*	2.1±0.5*
Bleeding volume (BV_{max})/ml	4.3±1.1	3.8±1.1*	4.8±1.4*	5.5±1.1**
Compensatory time/min	139±60	112±51*	139±63*	176±64*
Survival time/min	69±76	105±55*	210±89***	246±90***
Survival rate in 5 h/%	0	0*	40*	70**

Tab 2. Effects of flunarizine 1 mg·kg^{-1} on arterial blood gases and lactate contents; ATP, Ca^{2+} contents in liver and heart in rats subjected to hemorrhagic shock. $n=10$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. $^{\dagger}P>0.05$, $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* shock. $^{\S}P>0.05$, $^{\S\S}P<0.05$, $^{\S\S\S}P<0.01$ *vs* control.

		Control	Shock	Flunarizine
pH		7.38±0.07	6.90±0.13***	7.14±0.13††† §§§
pO_2/kPa		13.9±1.3	12±6*	15±4† §
pCO_2/kPa		3.8±0.5	5.8±2.0***	4.6±0.8† §§
HCO_3/mol·L^{-1}		16.5±2.0	8.0±2.3***	12±3** §§§
Lactate/mmol·L^{-1}		2.7±0.5	11.3±1.8***	7.8±1.7††† §§§
Liver	ATP/μmol·g wet weight	1.48±0.14	1.11±0.13***	1.40±0.21††† §
	Ca^{2+}/μmol·g dry weight	39±6	53±10***	42±8†† §
Heart	ATP/μmol·g wet weight	2.15±0.13	1.45±0.25***	1.69±0.18†† §§§
	Ca^{2+}/μmol·g dry weight	41±10	54±13**	40±10†† §

against this decline. In the shock group, hepatic cell hyaline change and fatty degeneration were severer than in the Flu group. Following reperfusion, hepatic cells subjected to hemorrhage were irreversibly injured and, in some areas, necrosis of hepatic cells was evident. In addition, hemorrhage was seen in the myocardium. Flu group showed little or no hemorrhage. Based on histological examination, pretreatment of rats with Flu prevented the cell necrosis and provided a protective effect against ischemic injury.

DISCUSSION

Calcium channel blockers may exert beneficial actions in shock[8,9]. It was proposed that the beneficial effects of verapamil on hemorrhagic shock were due to an increase in the myocardial blood flow and a decrease in the myocardial oxygen utilization[10]. In this study, Flu was found to alleviate the postischemic acidosis, reduce the tissue ATP consumption, and improve the myocardial function. Thus, the survival rate of rats was increased.

An increase in total tissue calcium content occurred with reperfusion after ischemia, which was accompanied by mitochondrial calcium accumulation[11]. In the present study, measurement of total tissue calcium showed that ischemia produced calcium increase in liver and heart, while Flu administration prevented tissue calcium accumulation. In addition, the results indicated that Flu might protect the ischemic tissue against the drop of Mg^{2+}/Ca^{2+} ratios. Unfortunately, we did not detect the free calcium contents in the cytosol of cell. Geerts[4], using the digital image processing, showed that Flu could reduce intracellular free calcium levels in tissue under pathologic conditions. Our data suggested that it was important to maintain cellular calcation homeostasis in the prevention of ischemic cell injury. The beneficial effects of Flu against hemorrhagic shock may be related to the delay in the injury-induced tissue calcium accumulation.

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氟桂利嗪对大鼠失血性休克的保护作用

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摘要　失血前 iv 氟桂利嗪0.25或1.0 mg·kg^{-1}，大鼠的最大失血量从4.3±1.1增加到5.5±1.1 ml，存活时间从69±76延长到246±90 min，5 h 存活率提高40%－70%．失血再灌后，大鼠的酸中毒减轻，ATP 的消耗量减少；心、肝组织学损伤得到保护．组织总 Ca^{2+} 含量提示，氟桂利嗪的抗失血性休克作用可能与其减轻缺血组织 Ca^{2+} 聚积有关．

关键词　失血性休克；氟桂利嗪；血气分析；钙

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Effects of glaucocalyxin A on aggregation and cAMP levels of rabbit platelets *in vitro*

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ABSTRACT　Glaucocalyxin A (Gla A) is a new diterpenoid isolated from ethereal extract of the leaves of *Rabdosia japonica* (Burm f) Hara var *glaucocalyx* (Maxim) Hara (Labiatae) collected in Northeastern China. When incubated with washed rabbit platelets, Gla A inhibited ADP-, AA-, and PAF-induced aggregation of rabbit platelets with IC_{50} values of 3.44, 13.32, and 7.74 μmol·L^{-1}, respectively. Gla A 10 and 100 μmol·L^{-1} increased the cAMP levels in platelets. In combination with imazodan hydrochloride, Gla A (1－100 μmol·L^{-1}) caused a marked increase of platelet cAMP levels, while no effect with PGE_1.

KEY WORDS　glaucocalyxin A; platelet activating factor; adenosine cyclic monophosphate; imazodan; platelet aggregation; radioimmunoassay

Glaucocalyxin A (Gla A) is a new diterpenoid isolated from the Chinese herb, *Rabdosia japonica* (Burm f) Hara var *glaucocalyx* (Maxim) Hara (Labiatae) which is generally used for relieving inflammation and improving circulation[1,2]. Gla A iv increased the myocardial uptake of ^{86}Rb[3]. Our preliminary studies showed that Gla A inhibited rat and rabbit platelet aggregation induced by ADP and AA *in vitro*.

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This study was to examine the effect of Gla A on aggregations induced by ADP, AA, and PAF by using washed rabbit platelets. Effects on platelet cAMP levels was investigated by RIA.

MATERIALS AND METHODS

Reagents　Fatty acid-free bovine serum albumin (BSA, Shanghai Institute of Biochemistry, Chinese Academy of Sciences); adenosine 5'-diphosphate (ADP, Sigma) was dissolved in 0.1 mmol·L^{-1} phosphate buffered saline (pH 7.4); arachidonic acid (AA, Sigma), platelet activating factor (PAF, 1-*O*-octadecyl-2-acetly-*sn*-glyceryl-3-phosphocholine, gifted from Dr P Hadvary, F Hoffmann-La Roche & Co, Basle, Switzerland) were dissolved in absolute ethanol; PGE_1 (Norman Bethune University of Medical Sciences) was dissolved in Na_2CO_3 100 mmol·L^{-1}; acid-citric dextrose (ACD), composed of citric acid (0.8%), trisodic citrate (2.2%) and glucose (2.45%); [^{3}H]cAMP RIA kit (Shanghai Second Medical University).

Buffers　Tyrode's gelatin no calcium (TG no Ca): KCl 195 mg, $MgCl_2$·$6H_2O$ 212.5 mg, NaCl 8 g, $NaHCO_3$ 1.015 g, glucose 1 g, ethylene-glycol-bis-(β-amino-ethylether)-*N*, *N*'-tetraacetic acid (EGTA) 76.07 mg, gelatin 2.5 g, vol to 1 L, pH adjusted to 6.5 with HCl 1 mol·L^{-1}. Tris-Tyrode's BSA (TT-BSA): KCl 195 mg, $MgCl_2$·$6H_2O$ 212.5 mg, NaCl 8