西安710032，中国）　梅其炳，陶静仪　（第四军医大学药理教研室，西安710032，中国）

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

BIBLID：ISSN 0253-9756　中国药理学报　*Acta Pharmacologica Sinica*　1993 Jul；14 (4)：347—350

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*.

Received 1990-03-10　Accepted 1993-02-15

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

g, $CaCl_2 \cdot 2H_2O$ 191 mg, Trihydroxymethylaminomethane (Tris) 1.21 g, glucose 1 g, BSA 2.5 g, vol to 1 L, pH adjusted to 7.4 with HCl 1 mol·L^{-1}. Tris-HCl: Tris 6.06 g, NaCl 7.80 g, glucose 1 g, vol to 1 L, pH adjusted to 7.4 with HCl 1 mol·L^{-1}.

Drugs Gla A is a colorless needle-like crystal (mp 220 — 222℃) provided by Hospital 202, Shengyang, China. It was dissolved in ethanol : polyethyleneglycol (1 : 1, vol/vol) and diluted with saline. Imazodan (4, 5-dihydro-6-(*p*-imidazol-1-ylphenyl)-3 (2*H*)-pyridazinone monohydrochloride, a potent inhibitor of phosphodiesterase III) was synthesized by Pharmacochemistry Department of our school and dissolved in double distilled water.

Preparation of washed rabbit platelets Blood was collected by cardiac puncture from conscious rabbits (New Zealand rabbits, ♂ or ♀, 2.9±*s* 0.5 kg) into ACD. Washed platelets were prepared according to the method of Benveniste *et al*[4] modified after Lalau Keraly *et al*[5]. The final platelet suspension was kept at 25℃ till use.

Platelet aggregation Aggregation was measured at 37℃ in 200 μl of platelet suspension by the absorbance method in a PPP-autobalanced platelet aggregometor. The platelets (3×10^8 cells·ml^{-1}) were preincubated for 10 min without stirring in the presence of inhibitors or their solvent before addition of the appropriate agonist. Prior to inhibitory experiments, the threshold concentration of each agonist was determined: (a) on untreated platelets, for aggregation induced by ADP and AA, (b) on platelets treated with ADP and ASA 0.1 mmol·L^{-1} during the washed procedure (the platelets were refractory to both ADP and AA) for PAF-induced aggregation. The maximal amplitude was measured from each aggregation obtained in the presence of the inhibitor, and the inhibition was calculated in comparison with the maximal amplitude from the control aggregation. An inhibition-concentration curve was drawn in each series of the experiments. This curve was used to determine the concentration-induced 50% inhibition (IC_{50}). The $\bar{x}\pm s$ of the % of inhibition were obtained from at least 3 experiments[8].

Measurement of cAMP Washed rabbit platelets were prepared as described above (without preincubation with ASA and ADP) and resuspended at 5×10^8 cells·ml^{-1} in Tris-HCl buffer (50 mmol·L^{-1}, pH 7.4). Platelet suspensions 0.5 ml were preincubated with Tris-HCl or tested drugs for 15 min at 37℃. Some platelet suspensions were incubated with test drugs for 14 min, then PGE_1 (50 nmol·L^{-1}) was added and incubated for further 1 min. All the reactions were stopped by adding HAc-ethanol 3.3% 0.1 ml and heated at 93℃ for 5 min. The pellet was discarded and the supernatant, measured by RIA for cAMP after centrifugation at $1400 \times g$ for 15 min[7]. The results were expressed as pmol in 5×10^8 platelets.

RESULTS

Effect of Gla A on rabbit platelet aggregation induced by ADP and AA Gla A inhibited rabbit platelets aggregation triggered by the threshold concentration of ADP and AA. The inhibition was proportional to Gla A concentration with IC_{50} of 3.44 (95% confidence limits 1.21 — 9.74) and 13.32 (95% confidence limits 6.55 — 27.02) μmol·L^{-1}, respectively (Fig 1) (n=3).

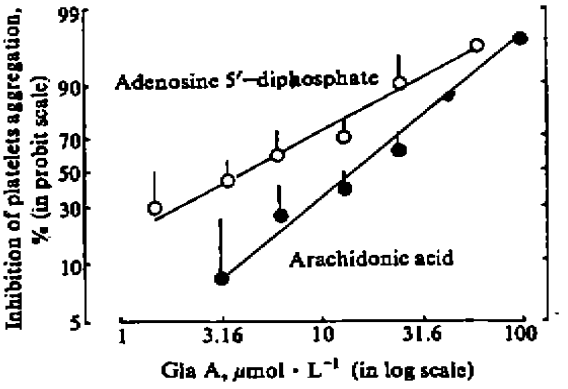

Fig 1. Effect of Gla A on washed rabbit platelet aggregation, which was triggered by threshold ADP or AA to induced maximum (light transmission>60%) in the presence of solvent. n=3, $\bar{x}\pm s$.

Effect of Gla A, Ro 19-3704 on rabbit platelet aggregation induced by PAF Gla A (1 — 100 μmol·L^{-1}) inhibited PAF-induced rabbit platelet aggregation which was refractory to ADP and AA. The inhibition was concentration-dependent with IC_{50} of 7.74 (95% confidence limits 1.93—31.09) μmol·L^{-1}(n=

6) while PAF antagonist Ro 19-3704 inhibited platelet aggregation with IC_{50} of 43.50 (95% confidence limits 11.64 — 162.18) nmol · L^{-1} (Fig 2).

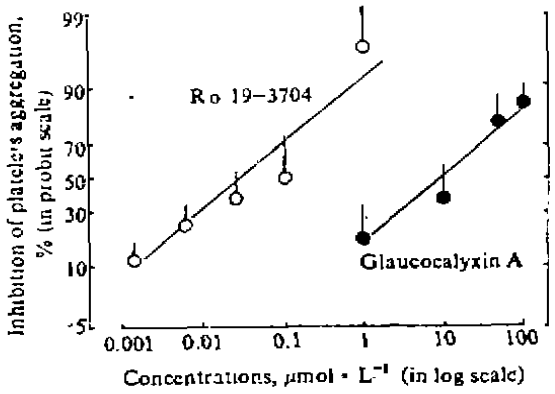

Fig 2. Effect of Gla A and Ro 19-3704 on ASA- or ADP-pretreated washed rabbit platelet aggregation induced by threshold concentration of PAF. $n = 6$, $\bar{x} \pm s$.

Effect of Gla A on cAMP levels in rabbit platelets In Tris-HCl group, the basic level of cAMP was 5.5 pmol in 5×10^8 platelets. While Gla A was used alone at 1, 10, 100 μmol · L^{-1}, the increased percentage of cAMP were 13.8%, 47.4%, 23.9%, respectively ($P < 0.05$), when Gla A (1, 10, 100 μmol · L^{-1}) was used with imazodan (10 μmol · L^{-1}), it increased cAMP levels significantly compared with Tris-HCl ($P < 0.01$) or with Gla A alone ($P < 0.05$). The growth rates were 66.4%, 70.3%, 63.5%, respectively *vs* control. Yet Gla A did not change the basic cAMP levels used with PGE_1 (50 nmol · L^{-1}) (Tab 1).

Tab 1. Effect of Gla A, Gla A + imazodan, Gla A + PGE_1 on cAMP levels in rabbit platelets. $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control. $^{\dagger}P > 0.05$, $^{\dagger\dagger}P < 0.05$ *vs* Gla A alone.

Drugs	n	cAMP, pmol/5×10^8 platelets
Tris-HCl	6	6.6±0.5
Gla A (alone)		
1 μmol · L^{-1}	4	8.1±0.4*
10 μmol · L^{-1}	4	9.7±0.8**
100 μmol · L^{-1}	4	8.5±0.2**
Gla A + imazodan		
1 μmol · L^{-1}	3	10.8±1.2***††
10 μmol · L^{-1}	4	10.9±0.8***†
100 μmol · L^{-1}	4	10.7±6.4**††
Gla A + PGE_1		
1 μmol · L^{-1}	4	7.6±1.0**
10 μmol · L^{-1}	4	8.7±0.7**†
100 μmol · L^{-1}	4	8.2±0.5*†

DISCUSSION

The present study demonstrated that Gla A inhibited PAF induced rabbit platelet aggregation which was refractory to ADP and AA. This suggested that the anti-PAF effect of Gla A was not mediated through a mechanism relevant to the inhibition of the ADP and prostaglandin pathways. Yet its anti-PAF effect is weaker than that of PAF antagonist Ro 19-3704[8]. Our further study showed that Gla A increased cAMP levels in platelets in a concentration similar to that used in anti-aggregation. And its effects on cAMP levels were enhanced while being used with imazodan. But Gla A did not change the cAMP levels when used with an adenylate activator, PGE_1. There was no sufficient evidence to ascertain Gla A's target enzyme in our study. Anyhow it is an effective compound in anti-platelet activation and its mechanism is partly via its effect of elevating the cAMP levels in platelets, Gla A may be of some practical significance in the prevention of thrombosis.

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蓝萼甲素对兔血小板聚集及 cAMP 含量的影响

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R 965.2

摘要 蓝萼甲素(Glaucocalyxin A)能显著抑制 ADP，AA，PAF 等诱导的兔血小板聚集，其 IC_{50}分别为 3.44，13.32，7.74 $\mu mol \cdot L^{-1}$。蓝萼甲素10，100 $\mu mol \cdot L^{-1}$显著升高血小板内 cAMP 水平($P<0.05$)，与 imazodan (10 $\mu mol \cdot L^{-1}$)合用时蓝萼甲素在1—100 $\mu mol \cdot L^{-1}$显著升高细胞内 cAMP 水平($P<0.01$)，而与 PGE_1(50 $nmol \cdot L^{-1}$)合用时，对细胞内 cAMP 水平无明显影响。

关键词 蓝萼甲素；血小板活化因子；腺苷环一磷酸；伊马唑旦；血小板聚集；放射免疫测定

350-353 (18)

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Jul; 14 (4) : 350—353

四种药物对冠脉闭塞和再灌注所致心肌损伤作用的图象分析[1]

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Image analysis of effects of 4 drugs on coronary occlusion and myocardial reperfusion injury

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ABSTRACT Myocardial infarction induced by coronary occlusion (O model) and reperfusion (R model) and the effects of nitroglycerin (Nit), propranolol (Pro), lidocaine (Lid), nifedipine (Nif), and saline were studied in rats by image analysis and weighing methods. The results showed that the myocardial infarct size (MIS) in R and O models were 27±8% and 39±6%, respectively ($P<0.01$). The roundness and the distances from the border of the infarct zone (IZ) to endocardium or epicardium in R model were greater than those in O model, while the gray level difference between normal and infarct myocardium ($\triangle G$) in the R model was less than that in O model. MIS of Nit group in O and R models were 23±12% and 16±7%, respectively, which were significantly less than those in Lid, Nif groups, and saline. The reduction of MIS was also found in Pro group in both models. The results suggested that early restoration of blood flow resulted in the salvage of injured myocardium and Nit and Pro were found to have beneficial effects in both models.

Received 1991-05-27 Accepted 1992-09-22
[1] Project supported by the Fund of Chinese New Drug Research, № New 89—14.

KEY WORDS myocardial infarction; myocardial reperfusion injury; computer-assisted image processing; nitroglycerin; propranolol; lidocaine; nifedipine