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

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

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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

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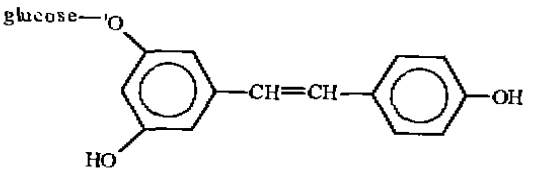
Polydatin[1] (Pol), a colorless crystal, was extracted from the root and stem of *Polygonum cuspidatum* Sieb et Zucc. by Department of Chemistry of our University in our country 11 years later than Japanese (Zhong Cao Yao Tong Xun 1974; **2**: 6—10).



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-

ue 0.08, by thin-layer chromatography and the content 98.5 %, by HPLC) was supplied by the Department of Chemistry of our University. Aspirin (Asp) was made by Huabai Pharmaceutical Factory, China. TXA_2-receptor brocker, vapiprost hydrochloride (Vap), from Glaxo Group Research Ltd and was presented by Dr BM Bain as a gift. All the drugs were dissolved in saline. Trypsin, arachidonic acid (AA) and Iscove's modified Dulbecco medium (IMDM) cultured base were Sigma products. ^{125}I-thromboxane B_2 (^{125}I-TXB_2, radioactivity 1.48 TBq·g^{-1}) and ^{125}I-6-keto-prostaglandin $F_{1\alpha}$ (^{125}I-6-keto-$PGF_{1\alpha}$, radioactivity 14.8 TBq·g^{-1}) RIA kits were purchased from Thrombosis and Hemostasis Research Unit, Suzhou Medical College. Baby umbilical cords were supplied by the Delivery Room of Nanfang Hospital, First Affiliated Hospital of Zhoushan Medical University, № 157 Hospital in Guangzhou Military Command, Guangdong Provincial Hospital, Maternity and Child Health Institutes of Guangzhou, of Yuxue District and Dongshan District in Guangzhou.

Thrombus model[4] and anti-thrombosis actions of iv Pol Rabbits of either sex weighing 2.2±s 0.2 kg, were anesthetized with iv urethane (1.0 g·kg^{-1}). The right carotid artery of 25 mm in length was isolated and sealed by 2 arterial clamps. Two needles (№ 8) connecting with the inflow and the outflow tubes of SJB-J infusion pump were inserted into the cavity of the sealed section of carotid artery and were fixed with suture to protect the liquid from leakage. This section of artery was perfused by 1 % trypsin 0.6 mL·min^{-1} for 15 min to induce injury of endothelial cells and was washed by saline at the same speed and duration. The needles were drawn out and the poles of needles were stitched up with suture. The injured carotid artery was reperfused by blood for 1 h after the clamps being removed. Finally, the thrombus adherent to the wall was weighed.

The rabbits were divided into 5 groups: (1) Pol 5 mg·kg^{-1}; (2) Pol 10 mg·kg^{-1}; (3) Asp 5 mg·kg^{-1}; (4) Vap 5 mg·kg^{-1} and (5) saline in same volume. The drugs were injected into the marginal ear vein before surgery.

Platelet aggregation in rabbits with thrombus Blood (3 mL) was collected from rabbits by cardiac puncture before and 1.5 h after injecting the drug (1 h after blood reperfusion in the damaged carotid artery) and the platelet suspension was prepared[6] and the number of platelets was adjusted to 4×10^{11}/L with Tyrode-HEPES solution. AA 0.2 mmol·L^{-1} was used as an inducer for aggregating platelets. The aggregation was measured at 37 ℃ in 0.5 mL of platelet suspension by the turbidity method[7] in an SPA-4 model autobalanced platelet aggregometer (Keda Measuring Instrument Factory, Shanghai). Results were exprssed as aggregation rate (%) = aggregating value after drug adiministration/aggregating value before drug administration × 100 %.

PGI_2 generation stimulated by AA or thrombin in vascular endothelial cells The primary cultured endothelial cells of human umbilical vein were prepared[8,9] and randomly divided into 5 groups with 15 samples in each group: (1) Pol 0.07 mmol·L^{-1}; (2) Pol 0.30 mmol·L^{-1}; (3) Pol 1.15 mmol·L^{-1}; (4) Asp 0.72 mmol·L^{-1} and (5) saline. After drug administration, the samples were incubated at 37 ℃ for 15 min. Then, each was further divided into 3 subgroups with 5 samples each, into which AA 0.8 μL (0.2 mmol·L^{-1}), thrombin 5 μL (1000 IU·L^{-1}) and saline 0.8 μL were added respectively. The mixture was incubated for another 10 min and 0.4 mL of culture solution were centrifuged at 670×g for 8 min. The supernatants were collected immediately to detect 6-keto-$PGF_{1\alpha}$ content by RIA[10].

TXA_2 generation stimulated by AA or thrombin in platelets The platelet suspension was prepared[6] and the number of platelets was adjusted to 10^{11}/L. Grouping of the samples was the same as in test of vascular endothelial cells, 15 min after the drugs being added into the suspension. AA, thrombin and saline were added into each subgroup (5 samples each) and the mixture was incubated for another 10 min. The platelet suspension were centrifuged 670×g for 8 min and TXB_2 content in supernatants was determined by RIA[11].

Data were analyzed statistically by t test.

RESULTS

Inhibition of of carotid thrombus formation and the platelet aggregation by Pol The moist weight of the thrombus was 6.6±1.8 mg 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, $P<0.05$, $P<0.01$). Meanwhile, in Pol 5 and 10 mg·kg^{-1} groups, a dose-dependent inhibition of platelet aggregation caused by AA was observed 1.5 h after iv Pol. The inhibitory effects of thrombosis and platelet aggregation of Pol 5 mg·kg^{-1} were not different from that of Asp 5 mg·kg^{-1} group and the effect of Pol 10 mg·kg^{-1} was consistent with that of Vap 5 mg·kg^{-1} group (Tab 1).

Tab 1. Inhibitory effect of polydatin (Pol) iv on rabbit arterial thrombosis induced by endothelial cell damage *in vivo* and platelet aggregation *in vitro*. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline.

Drug	Dose /mg·kg^{-1}	Thrombus /mg	Platelet aggregation /%
Saline		10.9±1.9	100±30
Pol	5	6.6±1.8^b	62±27^b
	10	4.8±1.6^c	38±22^c
Vap	5	3.9±1.5^c	32±26^c
Asp	5	5.3±1.3^c	50±30^b

These results showed that Pol had a simultaneous inhibitory effect on platelet aggregation and thrombus formation.

Effect of Pol on the generation of PGI_2 and TXA_2 caused by AA or thrombin The amount of 6-keto-$PGF_{1\alpha}$ in endothelial cells was 0.71±0.12 and 0.18±0.14 μg·L^{-1} in AA 0.2 mmol·L^{-1} and thrombin 1000 IU·L^{-1} groups, respectively, which was higher than that in the control (0.09−0.02 μg·L^{-1}). Pol 0.07−1.15 mmol·L^{-1} did not change the production while Asp 0.72 mmol·L^{-1} lowered the production of PGI_2 induced by AA or thrombin ($P<0.05$, $P<0.05$) (Tab 2).

AA 0.2 mmol·L^{-1} and thrombin 1000 IU·L^{-1} increased the TXB_2 production in platelet ($P<0.01$, $P<0.01$). Pol 0.30−1.15 and Asp 0.72 mmol·L^{-1} abated the generation of TXB_2 induced by AA or thrombin in a dose-dependent manner (Tab 2).

DISCUSSION

The damage of vascular endothelial cells is one of the important initiating factors of pathologic thrombosis. The anti-thrombus action of Pol was studied using the Imura's endothelial cell damage model[5]. The results showed that Pol obviously provented the carotid thrombosis from the endothelial damage *in vivo* and the antithrombosis action of Pol was closely related to its anti-platelet aggregation action. So were Vap and Asp. Because Vap is a TXA_2 receptor blocker and Asp is a cycloxygenase inhibitor, it is evident that the anti-thrombosis mechanism of Vap and Asp was associated with the inhibition of TXA_2 inducing platelet aggregation and playing an important role in thrombus formation,

Tab 2. Effect of polydatin (Pol) on arachidonic acid (AA) and thrombin-induced 6-keto-$PGF_{1\alpha}$ synthesis in cultured human umbilical vein endothelial cells and TXB_2 synthesis in platelets. $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline, $^dP>0.05$, $^eP<0.05$, $^fP<0.01$, *vs* no stimulus group.

Drug	mmol·L^{-1}	6-keto-$PGF_{1\alpha}$/μg·L^{-1}			TXB_2/μg·L^{-1}		
		No stimulus	AA	Thrombin	No stimulus	AA	Thrombin
Saline		0.09±0.02	0.71±0.12^f	0.18±0.04^e	1.1±0.4	20.5±2.9^f	24±7^f
Pol	0.07	0.13±0.05^e	0.77±0.12^e	0.21±0.07^e	0.8±0.4^e	14±6^e	17±9^e
	0.30	0.08±0.04^d	0.68±0.09^e	0.18±0.04^e	0.39±0.23^e	14±4^e	12±6^b
	1.15	0.08±0.04^d	0.62±0.07^e	0.175±0.016^e	0.59±0.12^e	12±4^b	6±5^c
Asp	0.72	0.082±0.019^d	0.33±0.08^b	0.09±0.03^b	0.65±0.14^e	4.5±1.7^c	0.8±0.3^c

which was consistent with Imura's observation. Hence, it was suggested that antithrombosis and anti-platelet effects of Pol were also associated with inhibition of TXA_2. This idea has been demonstrated by our further experiment in which Pol greatly decreased the TXA_2 generation and did not decrease the formation of PGI_2 that markedly inhibited platelet aggregation and greatly relaxed blood vessels. In contrast, Asp abated PGI_2 formation. It was concluded that Pol, as a new antithrombosis drug, works on a better pharmacologic basis than Asp did.

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3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷减轻动脉内皮损伤性血栓形成的作用

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目的：3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷(Polydatin，Pol)对兔颈动脉内皮损伤性血栓形成的影响. **方法**：采用胰蛋白酶损伤兔颈动脉内皮诱导血栓形成模型. **结果**：Pol iv 5或10 mg·kg^{-1}均可显著减少血栓湿重($P<0.05$，$P<0.01$)并抑制血小板聚集($P<0.05$，$P<0.01$). Pol 0.30—1.15 mmol·L^{-1}抑制血小板TXA_2生成($P<0.05$，$P<0.01$)，不影响人脐静脉内皮细胞生成PGI_2. **结论**：Pol具有抗血栓作用；不减少PGI_2，选择性抑制TXA_2是其重要机制之一.

关键词 3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷；血管内皮；血栓；培养的细胞；血小板聚集；血栓素A_2；依前列醇；虎杖苷

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Effects of N^6-cyclopentyladenosine on afterdepolarizations and triggered activity induced by isoproterenol in guinea pig papillary muscle[1]

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AIM: To investigate the effects of N^6-cyclopentyladenosine (CPA, selective adenosine A_1 receptor agonist) on afterdepolarizations and triggered activity induced by isoproterenol (Iso) in guinea pig papillary muscle. **METHODS:** The stable and reproducible early afterdepolarization (EAD) and delayed afterdepolarizaiton (DAD) of guinea pig papillary muslce were induced by Iso 50 nmol·L^{-1}. The parameters of EAD and DAD were recorded using intracellular microelectrodes. RESULTS: CPA markedly attenuated the development of EAD, DAD, and triggered activity (TA) induced by Iso in guinea pig papillary muscle. The inhibitory effects of CPA on Iso-induced EAD and DAD were antagonized by 8-phenyltheophylline (8-PT) and glibenclamide (Gli). **CONCLUSION:** ATP-sensitive K^+ channels were involved in Iso-induced EAD and DAD, and in the inhibitory effects of CPA on EAD and DAD.

KEY WORDS adenosine; catecholamines; theophylline; glyburide; papillary muscles; electrophysiology

Triggered activity (TA) caused by either early afterdepolarizations (EAD) or delayed afterdepolarizations (DAD) has been emphasized as an important cellular mechanism for the genesis of arrhythmias in human[1] and dog[2]. DAD have been well characterized and attributed to an oscillatory membrane current occurring near the very end of repolarization or after full repolarization[3,4]. EAD is a depolarizing after-potential that occurs during phase 2 or phase 3 of repolarization and has been induced in isolated cardiac tissues under a variety of conditions[5].

TA can be induced in isolated ventricular myocytes exposed to catecholamines[6,7]. Adenosine effectively terminates isoproterenol (Iso)-induced ventricular tachycardias in patients with heart disease[8]. We hypothesized that effects of adenosine on Iso-induced ventricular tachycardias were mediated by the inhibitory effects of adenosine on TA caused by either EAD and DAD. The purpose of this study was to observe the effects of N^6-cyclopentyladenosine (CPA, selective A_1 adenosine receptor agonist) on afterdepolarizations and TA induced by Iso.

MATERIALS AND METHODS

Papillary muscle Guinea pigs of either sex weighing 0.38 ± *s* 0.05 kg were decapitated and the hearts were superfused with cold Tyrode's solution. Isolated papillary muscle of right ventricle was mounted on a perforated silicon rubber block in a tissue bath and perfused at a rate of 8 mL·min^{-1} with Tyrode's solution (NaCl 130, KCl 4.5, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 1.8, $NaHCO_3$ 18, glucose 5.5 mmol·L^{-1}) gassed with 100 % O_2 was maintained at 35±1 ℃.

The preparation was stimulated through a bipolar electrode at a control basic cycle length (BCL) of 500 ms (5 ms rectangular pulse and two times threshold intensity) from the stimulator (SEN-3201). Transmembrane potentials were led to the microelectrode amplifier (MEZ-8201) by a standard intracellular glass

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