之间，但 CsCl 作用的有效浓度不同（心室肌为3 mmol·L^{-1}；心房肌为5 mmol·L^{-1}），且心房肌的 EAD 多表现为触发发放型（6/9）并具频率依赖性，心室肌的 EAD 多表现为平台突起型(14/17)而无频率依赖性. 结论：低浓度 CsCl 在小鼠心室肌较心房肌更易诱发 EAD 且二者表现形式不同，提示小鼠心房肌和心室肌的钾通道可能不同.

关键词 铯；动作电位；心肌 小鼠

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 265－268

Influence of 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside on vascular endothelial epoprostenol and platelet aggregation

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

AIM: To study the relationship between the inhibiting effect on platelet aggregation and the enhancing effect on epoprostenol (PGI_2) released from vascular endothelium with 3,4′,5 - trihydroxystibene - 3 - β - mono - *D* - glucoside (polydatin, Pol). **METHODS**: After having been incubated with Pol, the incubating medium was withdrawn from the bottles with newborn umbilical vein endothelial cells (VEC group, trypsin digesting method) and added to the platelets (washing method). The medium withdrawn from the bottles without VEC was designated as control group. Reduction of platelet aggregation rates (PAR, turbidity method) and changes of 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) (radioimmunoassay method) in the supernatant of the aggregated platelets induced by thrombin were scrutinized. **RESULTS**: PAR in the control group showed no reduction, whereas PAR reduction (－10 ± 10) and 6-keto-$PGF_{1\alpha}$ increase (108 ± 30 ng·L^{-1}) in the VEC group treated 10 min with Pol 0.41 mmol·L^{-1} (*vs* that of distilled water, ie, 2 ± 12 and 54 ± 20 ng·L^{-1}) occurred. **CONCLUSION**: Increase of PGI_2 from VEC by Pol was involved in its (Pol's) inhibition effect of platelet aggregation.

KEY WORDS 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside; polydatin; umbilical veins; vascular endothelium; platelet aggregation; thromboxane A_2; thromboxane B_2; epoprostenol; 6-ketoprostaglandin $F_{1\,alpha}$

Polydatin (Pol), a crystal extracted from the root and stem of *Polygonum cuspidatam* Sieb *et* Zucc[1], inhibited rabbit platelet aggregation *in vitro* and *in vivo*[2]. Pol 5 or 10 mg·kg^{-1} iv could inhibit rabbit arterial thrombosis induced by endothlial damage[3] (to be published). It was desirable to investigate whether the influence of Pol on the function of platelet in the microenvironment where arterial thrombosis took place depended on the release of epoprostenol (prostacyclin, PGI_2) from vascular endothelial cells (VEC).

Received 1993-11-05 Accepted 1994-10-25

MATERIALS AND METHODS

Pol (Department of Chemistry of our University) was dissolved in distilled water. Aspirin powder (Asp, Hua Ze Pharmaceutical Factory), frozen dried human thrombin (Hua Shan Hospital, Shanghai Medical University), culture medium Iscove's modified Dulbecco's medium (IMDM, Sigma), calf serum (Wei Wu Guang Ming Biological Products Factory, Shen Zhen), ^{125}I-thromboxane B_2 (^{125}I-TXB_2) and 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) radioimmunoassay kits (Institute of Thrombosis and Hemostasis, Suzhou Medical College).

Twenty Rabbits (2.0±s 0.3 kg) were from Animal Center of our University.

Newborn baby umbilical cords were obtained from various hospitals around Guangzhou.

Preparation of human umbilical vein endothelial cells and drug administration Newborn baby umbilical vein EC were derived from the 0.25 % trypsin digesting method[4]. These cells were sown to the IMDM at 37 C for 3—4 d. A monolayer of primary VEC fused together and attached to the wall of the bottles. These VEC were appraised morphologically by identification of Factor VIII with a fluoromicroscope. Pol or thrombin[5] was added in order to promote the release of PGI_2 from VEC. These bottles were divided into 6 groups designated as the VEC group: (1)—(3) Pol groups, 0.05, 0.14 and 0.41 mmol·L^{-1}, respectively; (4) thrombin group, 1000 IU·L^{-1}; (5) Asp group, 0.69 mmol·L^{-1}; (6) water group, triple distilled water 30 μL (same volume as others), with 8—12 bottles in each group. Before and 10, 30, 60 min after drug administration, from each bottle 50 μL of the medium were added to 150 μL of platelet suspension for observing the PAR and measuring the PGI_2 and thromboxane A_2 (TXA_2) in the supernatant after aggregation induced by thrombin. Since the half life of PGI_2 or TXA_2 was very short, their metabolites 6-keto-$PGF_{1\alpha}$ and TXB_2 were measured instead. Five other groups of 4—6 bottles each containing only the IMDM and without VEC served as control group. Pol (0.05, 0.14 and 0.41 mmol·L^{-1}), thrombin (1000 IU·L^{-1}) and distilled water were added, incubated. The overlying IMDM 50 μL were transferred to 150 μL platelet suspension. Platelet aggregation, 6-keto-$PGF_{1\alpha}$, TXB_2 were observed.

Washed platelet suspension and aggregation Washed rabbit platelets were prepared from fresh blood by cardiac puncture, using ACD solution as anticoagulant (9:1). The platelet-rich plasma was obtained by centrifuging at 107 × g for 12 min. Platelet suspension derived from centrifuging (672 × g for 6 min) and washing method[6]. The number of platelets was adjusted to 5×10^9·L^{-1}. The platelet suspension 150 μL and IMDM 50 μL treated with different agents were coincubated and stirred at 37 C for 1 min using a model SPA-4 autobalanced platelet aggregometer (Kodak Measuring Instrument Factory, Shanghai). Platelet aggregation induced by thrombin 140 IU·L^{-1} was shown as an increase in light transmission (turbidity)[7]. Curves and PAR at 1 and 5 min (PAR(1) and PAR(5)) were autoprinted by the aggrecoder.

Determination of 6-keto-$PGF_{1\alpha}$ and TXB_2 After PAR(1) and PAR(5) were recorded, the samples were centrifuged in the tubes pretreated with indometacin at 4 C, 672 × g for 8 min. Supernatants were frozen at −20 C. TXB_2 and 6-keto-$PGF_{1\alpha}$ in the supernatants were measured with ^{125}I radioimmunoassay[8] using a γ-counter.

RESULTS

VEC in inhibitory effect of Pol on platelet aggregation Comparison of PAR reduction between agents and water groups were carried out at 10, 30 and 60 min. Data were evaluated by t test.

In the presence of VEC, Pol and Asp yielded a decreasing tendency on PAR, especially PAR(5) with Pol 0.41 mmol·L^{-1} for 10 min, with Asp 0.69 mmol·L^{-1} for 60 min, and PAR (1) with thrombin 1000 IU·L^{-1} for 30 and 60 min. These decreases were more marked than those in the water group. (Tab 1)

No inhibitory effect on PAR were observed if no VEC had coexisted with Pol and thrombin.

PGI_2 from VEC in inhibitory effect of Pol on platelet aggregation 6-Keto-$PGF_{1\alpha}$ increased with Pol 0.41 mmol·L^{-1} for 10 or 30

Tab 1. Effects of polydatin (Pol), thrombin, and aspirin (Asp) on reduction of rabbit PAR (platelet aggregation rates) induced by thrombin 140 IU·L^{-1} in vascular endothelial cells group *in vitro*. $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* water.

Agents in medium		*n*	PAR(1)	Reduction of PAR(1) After agent administration/min 10	30	60	PAR(5)	Reduction of PAR(5) After agent administration/min 10	30	60
Water		9	37±15	3±10	3±9	0±12	63±17	2±12	−2±11	−7±24
Pol/ mmol·L^{-1}	0.05	12	43±21^a	0±11^a	−4±9^a	−8±10^a	69±19^a	2±8^a	−2±12^a	−8±14^a
	0.14	8	39±17^a	−2±8^a	−8±13^a	−9±14^a	64±17^a	−6±9^a	−12±20^a	−14±19^a
	0.41	9−10	43±22^a	−3±16^a	−3±17^a	−12±22^a	70±27^a	−10±10^b	−10±14^a	−18±22^a
Thrombin/ 1000 IU·L^{-1}		10	47±16^a	−4±11^a	−10±8^c	−12±11^b	66±21^a	0±14^a	−7±17^a	−2±21^a
Water		5	24±9	−2±10	−5±4	−9±7	61±5	−2±7	−2±8	6±5
Asp/ mmol·L^{-1}	0.69	5	19±8^a	0±6^a	−6±8^a	−6±6^a	62±11^a	−3±7^a	−6±9^a	−7±6^c

min ($P<0.05$, $P<0.01$), and with thrombin 1000 IU·L^{-1} for 30 or 60 min ($P<0.01$, $P<0.01$). TXB_2 decreased with Asp 0.69 mmol·L^{-1} for 60 min ($P<0.01$). (Tab 2)

The increase of 6-keto-$PGF_{1\alpha}$ (Tab 2) and reduction of PAR(5) (Tab 1) were found in the same sample with Pol 0.41 mmol·L^{-1} for 10 min. The reduction of TXB_2 (Tab 2) and PAR(5) (Tab 1) were found in the same sample with Asp 0.69 mmol·L^{-1} for 60 min.

DISCUSSION

In our research thrombin, as a positive agent, promoted VEC to release PGI_2. PAR decreased as these VEC media with thrombin and PGI_2 were transferred into the platelets. This result was consistent with that of Weksler[5] and suggested that PGI_2 formation from VEC was involved in the inhibitory effect of platelet aggregation in this test system. The inhibitory effect of Pol 0.41 mmol·L^{-1} on platelet aggregation was only available after its incubation with VEC. Additionally, 6-keto-$PGF_{1\alpha}$ was increased in the same Pol 0.41 mmol·L^{-1} sample. It was believed that (1) there was a dependent and inhibitory effect on

Tab 2. 6-keto-$PGF_{1\alpha}$ and TXB_2 with Pol and Asp from supernatant after rabbit platelet aggregation induced by thrombin 140 IU·L^{-1} in vascular endothelial group *in vitro*. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* water.

Agents in medium		*n*	6-keto-$PGF_{1\alpha}$/ng·L^{-1} Before	After agent administration/min 10	30	60	*n*	TXB_2/ng·L^{-1} Before	After agent administration/min 10	30	60
Water		4	51±20	54±20	65±11	61±24	6	58±12	66±24	41±22	45±25
Pol/ mmol·L^{-1}	0.05	4	80±30^a	54±40^a	65±11^a	83±24^a	6	58±13^a	48±24^a	65±11^a	52±12^a
	0.14	6	59±22^a	85±17^a	73±13^a	61±14^a	6	56±5^a	52±18^a	58±17^a	62±13^a
	0.41	4	65±27^a	108±30^b	105±18^c	98±30^a	6	55±16^a	59±17^a	61±17^a	46±28^a
Thrombin/ 1000 IU·L^{-1}		4	58.9±2.6^a	81±14^a	120±50^c	123±11^c	6	56±22^a	56±28^a	40±40^a	43±35^a
Water		5	19±19	20±10	26±14	24±18	10	21±18	10±4	6.3±1.5	8.4±1.7
Asp/ mmol·L^{-1}	0.69	5	17±5^a	16±7^a	23±7^a	20±8^a	10	17±10^a	11±5^a	8±5^a	4.2±2.5^c

platelet aggregation with Pol 0.41 mmol·L^{-1}. It depended on the VEC because it could not be found in the control group (without VEC); (2) PGI_2 production from VEC promoted by Pol was involved in this effect.

Pol not only decreased platelet TXA_2[11] but also enhanced VEC PGI_2 and therefore led to the reduction of platelet aggregation. These results indicated that Pol was different from Asp in treating thrombosis and occlusive deseases, especially in those thrombosis related to the decrease of PGI_2.

It was necessary to explain that 6-keto-$PGF_{1\alpha}$ increased significantly with Pol 0.41 mmol·L^{-1} for 30 min (Tab 2), however, the inhibitory effect of platelet aggregation was not found in the same sample (Tab 1). The reason was that 6-keto-$PGF_{1\alpha}$ measured was the metabolite of PGI_2. The half life of PGI_2 was only 2 — 3 min. Since the values of 6-keto-$PGF_{1\alpha}$ at 10 and 30 min were at the same high level, so there was not enough PGI_2 activity to inhibit the platelet aggregation at 30 min.

REFERENCES

1 Shan CW, Yang SQ, He HD, Shao SL, Zhang PW. Influences of 3,4,5-trihydroxystibene-3-β-mono-*D*-glucoside on rabbits' platelet aggregation and thromboxane B_2 production *in vitro*. Acta Pharmacol Sin 1990; **11**: 527 — 30.

2 Shan CW. Effects of polydatin on platelet aggregation of rabbits. Acta Pharm Sin 1988; **23**: 394 — 6.

3 Imura Y, Terashita Z, Nishikawa K. The role of thromboxane (TX) A_2 in rabbit arterial thrombosis induced by endothelial damage. Thromb Res 1990; **59**: 195 — 205.

4 Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins-identification by morphologic and immunologic criteria. J Clin Invest 1973; **52**: 2745 — 56.

5 Weksler BB, Ley CW, Jaffe EA. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin and the ionophore A 23187. J Clin Invest 1978; **62**: 923 — 30.

6 Ardlie NG, Packham MA, Mustard JF. Adenosine diphosphate-induced platelet aggregation in suspensions of washed rabbit platelets. Br J Haematol 1970; **19**: 7 — 17.

7 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; **194**: 927 — 9.

8 Wang ZY, Chen DC, He Y, Ruan CG. Radioimmunoassay of ^{125}I-labeled thromboxane. Acta Acad Med Suzhou 1986; **6**: 13 — 6.

3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷对血管内皮前列环素的影响与血小板聚集的关系

张佩文，余传林，王耀忠，骆苏芳，孙莉莎，李锐松

（第一军医大学药理教研室，广州 510515，中国）

目的：探讨3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷(polydatin, Pol)抑制血小板聚集与其使血管内皮释放前列环素作用间的关系。**方法**：Pol 与培养的人脐静脉内皮细胞(VEC，酶消化法)孵育为内皮组，无 VEC 为对照组；移细胞培养液至兔血小板(洗涤法)，测凝血酶诱聚后血小板聚集率(PAR，比浊法)及上清液6-酮-前列环素 $F_{1\,alpha}$(6-keto-$PGF_{1\alpha}$)，血栓烷 B_2(TXB_2)含量(放免法)。**结果**：无 VEC 组 Pol 不减少 PAR；VEC 组0.41 mmol·L^{-1} 10 min PAR 减少(−10±10)，6-keto-$PGF_{1\alpha}$增加(108±30 ng·L^{-1})(各与蒸馏水组之2±12，54±20 ng·L^{-1}比)。**结论**：Pol 抑制血小板聚集与其增加 VEC 前列环素释放有关。

关键词 3,4′,5-三羟基芪-3-β-单-*D*-葡萄糖苷；白藜芦醇苷；脐静脉；血管内皮；血小板聚集；血栓素 A_2；血栓素 B_2；前列环素；6-酮前列环素 $F_{1\,alpha}$