11 Corvazier E, Maclouf J. Interference of some flavonoids and non-steroidal anti-inflammatory drugs with oxidative metabolism of arachidonic acid by human platelets and neurrophils. Biochim Biophys Acta 1985; 835:315-21.

槲皮素对血小板聚集和胞浆游离钙的影响

肖 东，顾振纶（苏州医学院药理学教研室，苏州 215007，中国） 白建平（大同医学专科学校药理学教研室，大同037008，中国） 汪 钟（中国医学科学院药理学教研室，北京100005，中国）

目的：研究槲皮素对凝血酶诱导的血小板聚集和胞浆游离钙浓度的影响及钙对槲皮素的血小板聚集抑制效应的作用。**方法**：用荧光钙离子指示剂观察槲皮素对血小板胞浆游离钙的影响。**结果**：槲皮素明显抑制凝血酶诱导的血小板聚集和游离钙的升高。IC_{50}和95 %可信区间分别为146.2（92.4－231.3）和78.5（49.5－124.4）$\mu mol \cdot L^{-1}$。槲皮素对血小板的抑制作用可被钙翻转。槲皮素对凝血酶诱导的钙释放无影响。**结论**：抑制钙内流是槲皮素抑制血小板聚集和$[Ca^{2+}]_i$升高的机制。

关键词 槲皮素；凝血酶；血小板聚集；钙

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 226－230

Effects of propylene glycol mannate sulfate on thrombosis in abdominal aorta in rabbits

CHEN Xiao-Ming, PAN Jia-Qi, ZHANG Zhi-Nan
(*Division of Hematology, Peking Union Medical College Hospital, Beijing 100730, China*)

AIM: To study the effects of propylene glycol mannate sulfate (PGMS) on platelet adhesion, aggregation and thrombosis in abdominal sorta in rabbits. **METHODS**: Platelet adhesion assay was performed with a platelet-adhesion-meter. The platelet concentration was determined with an electronic particle counter, and the total radio-activity was determined wiht Clinigamma-1272. **RESULTS**: PGMS inhibited washed platelet aggregation induced by thrombin *in vitro*. The value of IC_{50} was 0.9 $mg \cdot L^{-1}$. (95 % confidence limit = 4.1－13.7 $mg \cdot L^{-1}$). At 10, 30, and 60 min after iv PGMS 75 $mg \cdot kg^{-1}$, the inhibition rates of platelet adhesion were 90.4 %, 41.8 %, and 26.3 % and the inhibition rates of platelet aggregation induced by thrombin were 99 %, 122 %, and 110 %, respectively. It did not exhibit any noticeable effect on platelet aggregation induced by ADP and collagen at this dosage. After 1.5 h of iv PGMS 75 $mg \cdot kg^{-1}$ and total autologous ^{111}In-platelets 3.3×10^9 the radioactivity and dry weight of the injured and uninjured segments were determined. The PGMS group showed a significantly lower radioactivity (1.1 ± 0.6 MBq), ^{111}In-platelets (8.3 ± *s* 3.3×10^8) as welll as the radioactivity deposited $\cdot g^{-1}$ out of the total radioactivity infused % (0.24 ± 0.21) compared with the control group (3.7 ± 0.5 MBq, 24.6 ± 3.5×10^8, and 0.86 ± 0.25, respectively). **CONCLUSION**: PGMS prevented the platelet adhesion and aggregation at the injured arterial wall.

Received 1991-10-22 Accepted 1994-07-15

KEY WORDS propanediols; platelet aggre-

gation; indium radioisotopes; vascular endothelium; thrombosis; polysaccharides; abdominal aorta

Propylene glycol mannate sulfate (PGMS) was first extracted from *Sargassum pallidum* (Turn) in China. Chemically, PGMS is a sulfated polysaccharide of mannuronic acid. PGMS *po* decreased the total serum cholersterol and increased the ratio of plasma PGI_2 in mice, while its iv administration showed an anticoagulation effect in rabbits[1-3]. On the basis of these findings, effects of PGMS on blood platelet adhesion, aggregation and thrombus formation in abdominal aorta in rabbits were studied in our laboratory.

MATERIALS

PGMS (11 IU/mg) was provided by Qindao Marine University; $^{111}InCl_3$ (74 GBq·L^{-1}) was supplied by the Institute of Applied Chemistry, Beijing Normal University. Heparin, 125 IU/mg (Shanghai Biochemical and Pharmaceutical Laboratories); Oxine (8-hydroxyquinoline, AR, Beijing Chemical Plant); adenosine diphosphate (ADP) (Helena Lab), collagen (Chrono-Log Corp); thrombin (Raritan NJ, USA).

METHODS AND RESULTS

Effect on washed platelet aggregation *in vitro* Platelet-rich plasma (PRP) was collected from the peripheral blood of 6 ♀ adult New Zealand rabbits weighing 2.7±*s* 0.4 kg, anticoagulated with edetic acid-Na_2 (2 %, 1∶10) and centrifuged at 220×*g* for 10 min. The washed platelet was prepared by being sedimented form the PRP (1000×*g* for 10 min) and resuspended in Hank's solution after washing twice with TEN solution (Tris 20 mol·L^{-1}, edetic acid-Na_2 0.6 mol·L^{-1}, NaCl 148 mol·L^{-1}, glucose 5 mol·L^{-1}, pH 7.4). Each platelet suspension containd platelets 45×10^{10}·L^{-1}. Platelet aggregation was measured with turtbimetric method[4]. Platelet suspension 0.45 mL was placed in a cuvette and stirred with the drug tested or control solution 50 μL at 37 ℃ for 5 min, then thrombin 5 μL was added (final concentration 1×10^3 IU·L^{-1}). Platelet aggregation was monitored with a Lumin-aggregometer (Chrono-Log corp. USA) and compared by determining the maximal change in light transmission within 3 min. PGMS caused concentration-dependent inhibition of platelet aggregation. The values of IC_{50} were 8.9 mg·L^{-1} compared to 2.1 mg·L^{-1} of heparin. The 95 % confidence limit was 4.1 — 13.7 and 0.96 — 3.20 mg·L^{-1}, respectively.

Effect on platelet adhesion and aggregation *in vivo* Blood was drawn from femoral vein at 0, 10, 30, and 60 min after iv PGMS 75 g·L^{-1} in 5 ♀ adult New Zealand rabbits weighing 2.78±*s* 0.2 kg. Platelet adhesion assay was performed with a platelet-adhesionmeter (Institute of Hematology, Chinese Academy of Medical Sciences). Blood mixed with sodium citrate (3.8 %, 1∶10) and centrifuged for 10 min at 220×*g* to obtain PRP. The remaining sample was further centrifuged at 1000×*g* for 10 min to prepare the platelet-poor-platelet (PPP). The PRP was diluted with homologous PPP to yield a final platelet count of 4×10^{11} L^{-1}. PRP 0.5 mL was preincubated in the aggregometer for 5 min before stimulus was applied (final concentration: ADP 2 μmol·L^{-1}, collagen 20 mg·L^{-1} and thrombin 1×10^3 IU·L^{-1}). the inhibition rate was calculated by the formula (A−B)/A×100 % (A: the aggreation rate % before and B: medication after). Paired *t* test was used for comparison between A and B. PGMS inhibited the platelet adhension and aggregaion induced by thrombin at 10, 30, and 60 min after medication. The inhibition rates of the platelet aggregation induced by thrombin were

99 %, 122 %, and 110 %, respectively, but it did not show noticeable effect on platelet aggregation induced by ADP and collagen (Tab 1).

Tab 1. Effects of propylene glycol mannate sulfate 75 mg·kg^{-1} iv on rabbit platelet adhesion and adhesion and aggregation induced by thrombin, collagen, and ADP. $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Adhesion /%	Platelet aggregation/% Thrombin	Collagen	ADP
Control	43±11	52±7	45±5	33±2
10 min	4±1^c	1±3^c	53±6^a	40±9^a
30 min	25±3^b	−11±0^c	51±2^a	40±11^a
60 min	32±8^a	−5±5^c	44±13^a	36±7^a

Effect on thrombosis in abdominal aorta ^{111}In-oxine complex was prepared by an absolute ethanal method[5,6]. Rabbit platelets were labeled by ^{111}In-oxine method[7,8]. Labeling procedures were carried out under sterile condition and plastic equipments were used throughout. Blood was collected from femoral vein, 20 mL were mixed with 4 mL ACD solution and 9 mL with 1 mL of sodium citrate solution. The samples were centrifuged at 220 ×*g* for 10 min. In order to remove the erythrocytes completely, the supernatant of PRP was transferred into another tube and centrifuged at 100×*g* for 5 min. The upper two-third or ACD-PRP and citrate-PRP were further centrifuged at 1000×*g* for 10 min to obtain ACD-PPP and citrate-PPP. The pellet of ACD-PPP was washed twice with 10 mL ACD saline solution and then incubated with ^{111}In-oxine complex at 22 C for 20 min. The mixture was centrifuged and the platelet pellet washed once with 8 mL ACD-PPP to remove the residual unbounded ^{111}In. The pellet was suspended in 4 mL citrate-PPP, the platelet concentration was determined with an electronic particle counter (Seqoia-Turner), and the total radioactivity was determined with Clinigamma-1272 (Pharmacia LKB) controlled by a personal computer (M240 Olivetti Co, Italy). ^{111}In labeled platelets showed a normal platelet aggregation response to collagen (final concentration 40 mg·L^{-1}) and the initial recovery rate % was 61±14.

Thirteen New Zealand rabbits weighing 3.1±0.3 kg were anesthetized by sodium pentobarbital iv (30 mg·kg^{-1}) and randomized into sham, control (normal saline) and PGMS (75 mg·kg^{-1} iv) groups. De-endothelialization of abdominal aorta was induced with a balloon catheter[9]. The endothelium of abdominal aorta was denuded by passing a 5 F Fogarty catheter (Edwards Lab, USA) into the aorta via femoral artery. ^{111}In-platelets and PGMS or normal saline were iv via seperate marginal veins. Surgical procedures were performed by the same personnel. After 1.5 h, 1000 IU of heparin and 0.5 % Evans blue 5 mL were given iv to prevent postmortem clotting and the rabbit was killed by an overdose of barbiturate. The aorta was dissected out and the attached fibroadipose tissue gently stripped. The lower 10 cm of the injured segment was dyed with Evans blue and the upper 5 cm of the uninjured segment was gently rinsed twice with normal saline to remove any blood from the lumen of the vessels. After radioactivity being measured the segments were placed in a desiccator (37 C) for 48 h and weighed. The radioactivity · g^{-1}, the number of ^{111}In-platelets deposited · g^{-1} and the radioactivity doposited · g^{-1} among the total radioactivity infused (%) were calculated. Each value was expressed as $\bar{x}\pm s$. Data were analyzed with *t* test and the analysis of variance with the Procedures of Medical Statistics. In the control group, the radioactivity per gram dry weight of the injured segments was 10 times as much as that of the uninjured and 20 times as the

corresponding one of the sham group. ^{111}In-platelet was also much higher. As compared with the control group, the PGMS group showed a lower radioactivity. ^{111}In-platelet levels as well as the radioactivity deposited $\cdot g^{-1}$ out of the total radioactivity infused (%) ($P<0.01$, Tab 2).

DISCUSSION

The present study confirmed that PGMS mainly inhibited the platelet aggregation induced by thrombin both *in vitro* and *in vivo*. It was less potent than heparin in inhibiting the thrombin-induced washed platelet aggregation, which probably had only a weak effect on platelet function in contrast to heparin. PGMS *po* has been reported to inhibit the rat platelel aggregation induced by ADP[10]. Our results, however, showed that PGMS did not have such an effect. The reason might be that the dose of PGMS used in earlier studies was higher than that in this paper. PGMS 100 mg $\cdot kg^{-1}$ *po* inhibited the platinum wire-induced arterial thrombosis[11] and came to a maximal effect of decreasing the plasma lipids and increase the plasma PGI_2 in mice. This, PGMS 75 mg $\cdot$ kg^{-1} was used according to the bioavailability and a dose convertion method among the animals. In this study, the ^{111}In-platelet labeling technique was used to investigate the interaction between platelet and subendothelial tissue and to evaluate the effects of the drug on the platelet thrombus formation in living experimental animals. The mean initial recovery rate of the labeling platelet was similar to that of the rabbit normal value[17]. The radioactivity $\cdot g^{-1}$ and the number of ^{111}In-platelets deposited $\cdot g^{-1}$ in the injured segments showed a significant difference, while those of the uninjured segments did not show significant difference between the sham and the control group, indicating that this kind of radiometric method was reliable in quantitating the platlet accumulated at the damaged vessel wall. Normal endothelium presents a nonreactive surface to the circulating blood. When the vascular endothelium was de-endothelialized by the baloon catheter, platelets come into contact with the subendothlial components. Thrombin, a strong stimulus of platelet activation and aggregation, was formed in both intrinsic and extrinsic hemostasis pathways. These resulted in the activation of platelet membrane phospholi-

Tab 2. Effects of PGMS 75 mg·kg^{-1} iv on radioactivity and number of ^{111}In-platelets deposited at injured and uninjured segments of rabbits aorta. $\bar{x}\pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs* sham; $^{d}P>0.05$, $^{f}P<0.01$ *vs* control.

	Sham $n=3$	Control $n=5$	PGMS $n=5$
Injured segments			
Radioactivity/(MBq)	0.3 ± 0.1	3.7 ± 0.5^{c}	1.1 ± 0.5^{cf}
^{111}In-platelets ($\times10^{-6}$)	1.9 ± 0.6	24.6 ± 3.5^{c}	8.3 ± 3.3^{cf}
$\frac{\text{Radioactivity deposited}\cdot g^{-1}}{\text{Total radioactivity infused}}\times100$ %	0.05 ± 0.02	0.86 ± 0.25^{c}	0.24 ± 0.21^{cf}
Unjured segments			
Radioactivity/(MBq)	0.3 ± 0.1	0.3 ± 0.1^{a}	0.4 ± 0.1^{ad}
^{111}In-platelets ($\times10^{-6}$)	2.7 ± 0.7	2.3 ± 0.4^{a}	2.7 ± 0.4^{ad}
$\frac{\text{Radioactivity deposited}\cdot g^{-1}}{\text{Total radioactivity infused}}\times100$ %	0.06 ± 0.02	0.08 ± 0.03^{a}	0.08 ± 0.01^{ad}
Injured/uninjured	0.09 ± 0.01	10.9 ± 1.1^{c}	3.0 ± 1.1^{cf}

pases and the liberation of arachidonic acid, which was further connverted into thromboxane A_2 (TXA_2). TXA_2 promoted the platelet release reaction and aggregate formation. As the intima was damaged and the prostacyclin biosynthesis compromised, this left the aggregating effect of TXA_2 unopposed and led to platelet thrombus formation. PGMS an anionic substance with functional sulfate groups, could strongly interact with the vascular wall, which might result in both biochemical and pharmacologic modulations, such as an increase of PGI_2/TXA_2 ratio in plasma[12], but the mechanism of antithrombotic effect remains to be further clarified.

REFERENCES

1 Chen XM, Yuan W. Effects of propylene glycol mannate sulfate on serum cholesterol, triglyceride and lipid peroxides in rats. J Biochem Pharm 1991; (2): 65—9.

2 Chen XM, Yuan W. Effect of Gan Tang Zhi on plasma PGI_2/TXA_2 in mice.
Chin J Marine Drugs 1992; **11** (2): 12—6.

3 Jiang GH, Zhang SL, Yuan W, Zhu YY, Yang CX. The influence of Gan Tang Zhi on rabbit coagulation and fibrinolytic function. Chin Pharmacol Bull 1991; **3**: 201—4.

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

5 Thakur ML, Welch MJ, Joist JH, Coleman RE. Indium-111-labeled platelets; studies on preparation and evaluation of *in vitro* and *in vivo* functions.
Thromb Res 1976; **9**: 345—57.

6 Sinzinger H, Virgolini I, Vinazzer H. Autologous platelet-labeling in thrombocytopenia.
Thromb Res 1990; **60**: 223—30.

7 Hearon WA, Davis HH, Welch MJ, Mathias CJ, Joist JH, Sherman LA, *et al*. Indium-111: a new radionuclide label for studying human platelet kinetics.
Br J Haematol 1979; **42**: 613—22.

8 Peters AM. Review of platelet labelling and kinetics.
Nucl Med Commun 1988; **9**: 803—8.

9 Spaet TH, Stemerman MB, Verth FJ, Lejnieks I. Intimal injury and regrowth in the rabbit aorta.
Circ Res 1975; **36**: 58—70.

10 Chen SM, Li LH, He ZJ, Fu DH. The effects of propylene glycol mannitase sulfate (PGMS) on platelet aggregation and thrombosis in rats and rabbits.
Chin J Marine Drugs 1990; **9** (4): 4—6.

11 Chen XM, Yuan W, Zhang SL, Shang XY, Lu FY, Jiao B, *et al*. Antithrombotic effect of MS_{871} in rats.
Chin J Marine Drugs 1991; **10** (1): 4—5.

226-259 8

硫酸甘糖酯对兔实验性腹主动脉血栓形成的影响

R973.2 R965.2

陈献明, 潘家绮, 张之南
(北京协和医院内科血液组, 北京100730, 中国)

目的: 研究硫酸甘糖酯(PGMS)对兔实验性腹主动脉血栓形成的影响. **方法**: 用电粒子计数器测血小板浓度, Clinigamma-1272测放射活性. **结果**: PGMS 抑制凝血酶诱导的兔洗涤血小板聚集的 IC_{50} 为8.91 mg·L^{-1}; 其75 mg·kg^{-1} iv 后10, 30, 60 min, 血小板粘附抑制率分别为90.4 %, 41.8 %和26.3 %, 对凝血酶诱导的血小板聚集抑制率分别为99 %, 122 %及110 %. 用气囊损伤兔腹主动脉内膜后1.5 h, 每克干重动脉段放射活性, ^{111}铟-血小板数与生理盐水对照组比较具有明显差异. **结论**: PGMS 抑制受伤兔腹主动脉血小板粘附及聚集.

关键词 丙二醇类; 血小板聚集; 铟放射性同位素类; 血管内皮; 血栓形成; 多糖类; 腹主动脉

硫酸甘糖酯