REFERENCES

1 Morley J, Page CP, Sanjar S. PAF and airway hyperreactivity. *Lancet* 1985; 2 : 451

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

3 Yue TL, Varma DR, Powell WS. Effects of protein deficiency on the metabolism of arachidonic acid by rat pleural polymorphonuclear leukocytes. *Biochim Biophys Acta* 1983; 751 : 332

4 Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; 37 : 911

5 Pinckard RN, Farr RS, Hanahan DJ. Physiochemical and functional identity of rabbit platelet-activating factor (PAF) released *in vivo* during IgE anaphylaxis with PAF released *in vitro* from IgE sensitized basophils. *J Immunol* 1979; 123 : 1847

6 Benveniste J, Le Couedic JP, Polonsky J, Tence M. Structural analysis of purified platelet-activating factor by lipases. *Nature* 1977; 269 : 170

7 Mazzoni L, Morley J, Page CP, Sanjar S. Induction of airway hyper-reactivity by platelet activating factor in the guinea pig. *J Physiol* (Lond) 1985; 365 : 107P

8 Lellouch-Tubiana A, Lefort J, Simon M, Pfister A, Vargaftig BB. Eosinophil recruitment into guinea-pig lungs after PAF-acether and allergen administration: modulation by prostacyclin, platelet depletion and selective antagonists. *Am Rev Respir Dis* 1988; 137 : 948

9 Joly F, Bessou G, Benveniste J, Ninio E. Ketotifen inhibits Paf-acether biosynthesis and β-hexosaminidase release in mouse mast cells stimulated with antigen. *Eur J Pharmacol* 1987; 144 : 133

10 Thompson PJ, Hanson JM, Bilani H, Turner-Warwick M, Morley J. Platelets, platelet activating factor and asthma. *Am Rev Respir Dis* 1984; 129 : A3

11 Nakamura T, Morita Y, Kuriyama M, Ishihara K, Ito K, Miyamoto T. Platelet-activating factor in late asthmatic response. *Int Arch Allergy Appl Immunol* 1987; 82 : 57

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3,4,5-三羟基芪-3-β-单-*D*-葡萄糖苷在体外对兔血小板聚集和产生血栓素 B_2 的影响[1]

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Influences of 3,4,5-trihydroxystibene-3-β-mono-*D*-glucoside on rabbits' platelet aggregation and thromboxane B_2 production *in vitro*

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ABSTRACT Platelet aggregation and thromboxane B_2 (TXB_2) production induced by arachidonic acid (AA) or Adenosine diphosphate (ADP) were studied by turbidimetry and radioimmunoassay in rabbits. 3,4,5-tri-hydroxystibene-3-β-mono-*D*-glucoside (PD) 6.7-107.2 μmol/L inhibited platelet aggregation and the production of TXB_2 as well. The inhibitions by PD were dose-dependent : 48-90 % (AA-induced) and 43-69 % (ADP-induced) for platelet aggregation; 50-87 % (AA-induced) and 43-68 % (ADP-induced) for TXB_2. There were positive correlations between the inhibition of platelet aggregation and production of TXB_2.

KEY WORDS glucosides; adenosine diphosphate; arachidonic acids; thromboxane B_2; platelet aggregation; nephelometry and turbidimetry; radioimmunoassay

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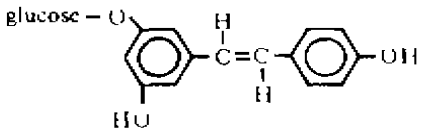
提要 用比浊法和放射免疫分析法测定了 PD 对兔血小板聚集和血栓素 B_2(TXB_2)产生的影响. PD 6.7–107.2 μmol／L 明显抑制 AA 和 ADP 诱导的兔血小板聚集和 TXB_2 的产生. 血小板聚集的抑制率分别为 48–90 %和 43–69%; TXB_2 产生的抑制率分别为 50–87%和 43–68%;血小板聚集的抑制率和 TXB_2 产生的抑制率间呈显著的正相关.

关键词 葡糖苷类; 腺苷二磷酸; 花生四烯酸类; 血栓素 B_2; 血小板聚集; 散射测浊法和比浊法; 放射免疫测定

虎杖是蓼科植物，学名 *Polygonum cuspidatum* Sieb et Zucc. 药用根茎. 本校化学教研室从虎杖根茎中提取出 3,4,5–三羟基芪–3–β–单–*D*–葡萄糖苷 (3,4,5–trihydroxy–stibene–3–β–mono–*D*–glucoside, Picid)又称虎杖晶Ⅳ号(Polydatin, PD), 为白色针状结晶, M_r 390, 结构式为:



3,4,5–Trihydroxystibene–3–β–mono–*D*–glucoside

PD 有改善微循环和兴奋心肌细胞的作用[1,2], 作者曾观察到 PD 在体内外都有抑制 ADP 和花生四烯酸诱导的兔血小板聚集作用[3], 所以本实验进一步观察 PD 对兔血小板聚集和产生 TXB_2 的影响, 以探讨 PD 抑制血小板聚集的机理.

MATERIALS

PD 由本校化学教研室提取; 吲哚美辛(indomethacin, IMC)由广州侨光制药厂生产; 氨茶碱(aminophyllinum)由重庆制药六厂生产; 二磷酸腺苷(adenosine diphosphate, ADP)由中国科学院上海生物化学研究所生产;花生四烯酸(arachidonic acid, AA)由 Fluka 产;乙酸乙酯(ethyl acetate)系浙江杭州双林化工试剂厂产;闪烁液 (scintillation liquid): PPO 4 g + POPOP 20 mg + 萘 (naphthalene) 60 g, 溶于 1000 ml 甲苯(toluene)中放置过夜后, 加无水乙醇 570 ml; 血栓素 B_2(thromboxane B_2, TXB_2)放射免疫分析药盒由中国人民解放军总医院提供. BS631 型血小板聚集仪系北京生化仪器厂产;LS9800 液体闪烁计数器(LS9800 liquid scintillation counter)系 Beckman 产.

METHODS AND RESULTS

PD 对 AA 诱导兔血小板聚集和产生 TXB_2 的影响 1 测定血小板聚集 兔 10 只, 体重 2.4± SD 0.2 kg, ♀♂不拘, 心脏取血, 按常规法制备富含血小板血浆(PRP), 并调整 PRP 中的血小板数为 45×10^7／ml. 将 PRP 分成 6 组, 每组 1.5 ml, 加入比浊管内, 第 1 组加 IMC(终浓度 15.9 μmol／L), 作为空白组; 第 2 组加入与药物等容量的生理盐水(Normal saline, NS);第 3 组加入 IMC(终浓度 15.9 μmol／L); 第 4–6 组加入 PD (终浓度依次为 6.7, 26.8 和 107.2 μmol／L). 除第 1 组外, 其余各组都放入血小板聚集仪内, 温育 2 min 后, 在电磁搅拌条件下, 以 AA(终浓度 95.5 μmol／L)为诱导剂进行血小板聚集实验, 观察 5 min. 按文献[4]进行 t 检验.

2 TXB_2 提取和测定 将上述第 1 组和 2–6 组聚集后的 PRP 中立即加入 HCl(终浓度 0.09 mmol／L), 并放入冰水中终止反应. 于 4℃ 离心(1200× g) 15 min, 取出 1 ml 上清液, 用重蒸馏乙酸乙酯提取 TXB_2, 放–20℃ 下保存待测. 取出样品, 加 phosphate buffer solution (PBS) 1 ml 复溶后, 用重蒸馏石油醚提取一次, 取水相待测. 参照文献[5]和放射免疫药盒说明书操作. 在 LS9800 液体闪烁计数器上测定 cpm. 根据标准物 TXB_2 绘制的标准曲线查得样品 TXB_2 相应浓度值(pg／tube), 乘 10 除以 0.9 得每 ml PRP 所含 TXB_2 量(pg／ml). 2–6 组的 TXB_2 值减去第 1 组(空白组)的 TXB_2 值(血浆中原有的 TXB_2)得在诱导

Tab 1. Influences of 3,4,5-trihydroxystibene-3-β-mono-D-glucoside (PD), indomethacin (IMC) and aminophyllinum (AP) on ADP- and arachidonic acid (AA)-induced platelet aggregation and TXB_2 produced in rabbits. $n=10$. $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline.

Drug (μmol / L)		Pletlet aggregation				Tromboxane B_2			
		Curve of absorbance (mm)		Inhibition (%)		Amount (ng / ml)		Inhibition (%)	
		ADP	AA	ADP	AA	ADP	AA	ADP	AA
Saline		41 ± 7	58 ± 11			0.6 ± 0.3	6.9 ± 2.1		
PD	6.7	24 ± 13	30 ± 13	43 ***	48 **	0.4 ± 0.3	3.5 ± 1.7	43 **	50 ***
	26.8	20 ± 6	20 ± 10	51 ***	67 ***	0.3 ± 0.3	2.4 ± 0.9	53 ***	65 ***
	107.2	13 ± 6	6 ± 7	69 ***	90 ***	0.2 ± 0.3	0.9 ± 0.8	68 ***	87 ***
AP	456.3	18 ± 10		57 ***		0.3 ± 0.4		52 **	
IMC	15.9		13 ± 13		78 ***		2.0 ± 0.9		72 ***

剂 AA 作用下血小板聚集时产生的 TXB_2 值. 以各药物组血小板聚集时产生的 TXB_2 值与 NS 组比较进行 t 检验，并以下式计算各药物组抑制血小板产生 TXB_2 的抑制率:

抑制率 = NS 组 TXB_2 值 − 药物组 TXB_2 值 / NS 组 TXB_2 值 × %

通过相关处理检查 PD 抑制血小板聚集时的抑制率与抑制 TXB_2 产生的抑制率的相关性.

3 结果 与 NS 组比较，PD 6.7–107.2 μmol / L 非常显著地抑制 AA 诱导的兔血小板聚集和 TXB_2 的产生，前者的抑制率为 48–90%，后者的抑制率为 50–87%，$r=0.9988$，$P<0.05$ (Tab 1).

PD 对 ADP 诱导兔血小板聚集和产生 TXB_2 的影响 兔 10 只，体重 2.0 ± 0.3 kg，♀♂不拘. 除第 3 组加氨茶碱(终浓度 456.3 μmol / L)和以 ADP(终浓度 8 μmol / L)为诱导剂外，其它同前.结果:与 NS 组比，PD 6.7–107.2 μmol / L 明显地抑制 ADP 诱导的兔血小板聚集和 TXB_2 的产生，前者的抑制率为 43–69%，后者的抑制率为 43–68%，$r=0.9976$，$P<0.05$ (Tab 1).

DISCUSSION

血栓素 A_2(thromboxane A_2，TXA_2)主要产生于血小板. TXA_2 很不稳定，在水溶液中半衰期 30 秒，它的代谢产物 TXB_2 很稳定，所以通过测定 TXB_2 产量来反映 TXA_2 的活性. TXA_2 是一种很强的血管收缩和血小板聚集的活性物质. 所以，抑制血小板 TXA_2 产生的药物具有舒张血管和抗血栓形成作用. 实验证明 PD 能显著地抑制 AA 和 ADP 诱导兔血小板聚集作用和 TXB_2 产生. AA 和 ADP 诱导血小板聚集作用是通过各自的血小板受体而发挥作用的，但是，二者引起的聚集过程都可以产生 TXA_2，所以，抗血小板聚集的药物不管通过任何环节发挥作用都会抑制 TXA_2 的产生. 本文中阳性对照组 IMC 是直接抑制血小板中环氧化酶使 TXA_2 产生减少，而氨茶碱是通过抑制磷酸二酯酶使 cAMP 增加而发挥作用的，但都有非常显著地抑制血小板聚集和 TXA_2 产生的作用. PD 对 AA 诱导的血小板聚集和 TXB_2 产生的 ID_{50} 是 11.2 和 11.4mg，而对 ADP 诱导的血小板聚集和 TXB_2 产生的 ID_{50} 是 23.3 和 23.0mg，说明 PD 抑制 AA 作用比抑制 ADP 的作用强，所以，以抑制环氧化酶作用为主.

REFERENCES

1 Zhu ZJ, Zao KS, Wu KY, Huang XL. The improvement of microcirculation in rat with irreversible hemorrhagic shock by crystal No 4 of polygonum cuspidatum. *Acad J First Med Coll PLA* 1987; 7 : 113

2 Luo SF, Yu CL, Zhang PW. Influences of 3,4,5-trihydroxystibene-3-β-mono-D-glucoside on beat rate and injury of cultured newborn rat myocardial cells. *Acta Pharmacol Sin* 1990; II :

147
3 Shan CW. Effects of polydatin on platelet aggregation of rabbits. *Acta Pharm Sin* 1988; **23** : 394
4 Shan CW, Liang QZ. Effects of procainamide on blood platelet aggregation in rabbits. *Acta Pharmacol Sin* 1988; **9** : 338
5 Granstrom E, Kindahl H, Samuelesson B. Radioimmunoassay for thromboxane B_2. *Anal Lett* 1976; **9** : 611

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山莨菪碱和蝙蝠葛碱对牛脑动脉平滑肌细胞产生前列腺素的作用[1]

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Inhibitory effects of dauricine and anisodamine on production of prostaglandins on bovine cerebral arterial smooth muscle cells[1]

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ABSTRACT The effects of dauricine (Dau), anisodamine (Ani), platelet activating factor (PAF), leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) on the production of TXB_2 and 6-keto-$PGF_{1\alpha}$ (the stable metabolites of TXA_2 and PGI_2, respectively) in bovine anterior cerebral arterial smooth muscle cells were studied.

The normal quantities of TXB_2 and 6-keto-$PGF_{1\alpha}$ produced by bovine anterior cerebral arterial smooth muscle cells were 16 ± 5 and 464 ± 24 pg / 10^5 cells, respectively, when measured by radioimmunoassay (RIA). The levels of TXB_2 and 6-keto-$PGF_{1\alpha}$ in bovine anterior cerebral arterial smooth muscle cells decreased significantly when the cells were treated with Dau or Ani over 20 min. Both drugs inhibited the production of TXB_2 and 6-keto-$PGF_{1\alpha}$ in dose (1-100 μmol / L) dependent manner. The bovine anterior cerebral arterial smooth muscle cells were stimulated markedly by LTC_4 and LTD_4 to produce TXB_2 and 6-keto-$PGF_{1\alpha}$ on the same condition even at 0.01 μmol / L. When the cells were treated with PAF over 20 min, TXB_2 increased significantly, but 6-keto-$PGF_{1\alpha}$ remained unchanged. If the cells were preincubated with Dau or Ani 20 min before PAF, LTC_4 or LTD_4 stimulation, the prodution of TXB_2 and 6-keto-$PGF_{1\alpha}$ especially TXB_2 were inhibited significantly compared with that of PAF, LTC_4 or LTD_4 group, respectively.

The results indicated that PAF, LTC_4 and LTD_4 can enhance TXA_2 and PGI_2, especialy TXA_2, biosynthesis in bovine anterior cerebral arterial smooth muscle cells, and that Dau and Ani normalized the balance of TXA_2 / PGI_2 in the cells changed by PAF, LTC_4 or LTD_4, and that both drugs may have significance in the prevention and treatment of cerebral vascular diseases.

KEY WORDS vascular smooth muscle; dauricine; amisodamine; platelet activating factor; leukotrienes; thromboxane B_2; 6-ketoprostaglandin F1 alpha

提要 用放射免疫分析法测定了培养的牛脑动脉平滑肌细胞中 TXB_2 及 6-keto-$PGF_{1\alpha}$ 含量分别为 16 ± 5 及 464 ± 24 pg / 10^5 细胞. LTC_4 及 LTD_4 依剂量性刺激该细胞产生 TXB_2 及 6-keto-$PGF_{1\alpha}$, 但对 TXB_2 刺激产生作用强. PAF 只刺激 TXB_2 产生. 山莨菪碱和蝙蝠葛碱均能显著抑制该细胞及 PAF, LTC_4, LTD_4 刺激的该细胞产生 TXB_2 和 6-keto-$PGF_{1\alpha}$, 且对 TXB_2 的抑制作用更明显.

关键词 血管平滑肌; 蝙蝠葛碱; 山莨菪碱; 血小板活化因子; 白三烯; 血栓素 B_2; 6-酮前列腺素 $F_{1\alpha}$

血小板活化因子 (platelet activating factor, PAF)和白三烯 C_4(leukotriene C_4, LTC_4)、白三烯 D_4(leukotriene D_4, LTD_4)是

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